

**African Natural Plant Products:
New Discoveries and Challenges
in Chemistry and Quality**

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Foreword

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Before agreeing to publish a book, the proposed table of contents is reviewed for appropriate and comprehensive coverage and for interest to the audience. Some papers may be excluded to better focus the book; others may be added to provide comprehensiveness. When appropriate, overview or introductory chapters are added. Drafts of chapters are peer-reviewed prior to final acceptance or rejection, and manuscripts are prepared in camera-ready format.

As a rule, only original research papers and original review papers are included in the volumes. Verbatim reproductions of previous published papers are not accepted.

ACS Books Department

Preface

Africa's wealth of biodiversity and indigenous knowledge of plants and their products represents a treasure still largely untapped and under-recognized. Many valuable African natural plant products and botanicals have been used for centuries in traditional medicine for the prevention and treatment of diseases, improvement of human and animal health and nutrition. While we have begun to understand that their judicious use and scientific study can yield new chemistries, drugs, medicines, foods and flavors, the richness in the African environment is in the integral role plants play in multitude of African societies and cultures. The ability to protect these natural resources in many regions is closely tied to the complex issue of economic viability for those communities living and depending upon their indigenous resources, public policy and competing demands on limited resources.

Natural products can serve as a driver in economic development, providing governments with rationale and economically-based arguments to protect the environment while permitting the sustainable harvesting and judicious use of the indigenous resources. The increased interest and trade in natural products is becoming more important to rural African communities as income generating activity. Most studies focus on the global market in natural products that is estimated to experience continued growth in the U.S. alone, with global sales in herbs and botanicals reaching \$17.5 billion (2000). In this context, the entire African continent, as a source of raw processed and finished products, contributes to less than 1% of this total market, and is contributing largely only those plant and products which are only found in Africa.

These figures raise many questions and debate as to the underlying reasons. To us, it symbolizes opportunities and responsibilities. Such numbers also do not reflect the importance and true economic value and trade of the African natural products sector for it does not take into account, local and regional trade which likely far exceeds export values. Nor does it take into account that the vast majority of Africans that use their own plants and plant products to improve their health and nutrition. An economic model that could show the value that these plants contribute to African health care systems relative to just lowering costs that otherwise would be needed to cover medical doctor and hospital visits and treatments and the cost of purchasing prescription drugs, would show a far greater value that these medicinal plants contribute to African nations and would not even account for the reduction in pressures and issues surrounding accessibility and affordability, already so strained in much of the continent.

Relative to the still highly informal nature of the natural products trade, there many factors responsible for this low international market share. Some of these include: (1) The lack of ethnobotanical information on the uses, attributes and commercial value of African plants and their products; (2) The lack of quality standards, weak control and product standardization practices; (3)

Domestic perceptions that locally produced products are of inferior quality relative to imports; and (4) the lack of public and private sector investment in the infrastructure that facilities such export readiness, value-addition processing opportunities and thus international trade; and (5) The need for real public policy discussion and changes that could better integrate formally the use of botanicals in health care while assuring safety.

This book “African Natural Plant Products: New Discoveries and Challenges in Chemistry and Quality” is a truly international effort to address and highlight the remarkable chemical diversity, and range of African plants and products that are used for a wide variety of applications in foods, flavorings, medicine, health and nutrition. We are honored that so many African researchers, as well as scientists from other regions of the world were so willing to share their expertise. This book with 29 scientific articles and reviews was written by over 80 scientific authors, with the majority of them African (50%).

The book is divided into five sections. The overview provides both a foreword and an introduction on the economic value of African Plants and their Products with a focus on Africa. The section “Traditional Medicines from Africa” provide reviews on the latest uses and medicinal applications of plants mostly from South and West African countries. The section “Chemistry, Pharmacognosy and Validation of Traditional Medicines” provides recent advances on science-based validation of traditional uses of African Plants and case studies of African medicinal plants in pharmaceutical preparations and recent new discoveries in natural plant products chemistry, biology and medicine. The section “Quality Control of African Natural Plant Products” provides practical information on the quality, chemistry and proximate analysis, health and nutritional value of a wide array of African plants and products focusing on the development of quality standards. Finally, the last section “Applications and Commercialization of African Natural Plant Products” highlights different uses and applications of plants. The book closes with chapters on economic perspectives and models of benefit sharing in Africa.

As the editors, we thank all the authors from Africa, Europe, Asia and the USA for their contributions. We hope the publication of this volume will serve to strengthen ties within the higher education institutions and the public, government, and private sectors in Africa and the rest of the world. The Editors also hope the publication of this book will stimulate more scientific research, the sharing of information, and the respect and recognition of the knowledge that comes from the Traditional Medicinal Practitioners and Healers that carry with them centuries of knowledge passed down from prior generations.

We are particular thankful to the United States Agency for International Development whose funding of the Partnership for Food Industry Development in Natural Plant Products (www.pfidnp.org) made this work possible as has the USAID-Regional Center for Southern Africa and the American Chemical Society. We are indebted to our colleagues and friends in the Agri-Business in Sustainable Natural African Plant Products organization and networks (www.asnapp.org) whose dedication in the promotion of sustainable development of African natural products for the benefit of Africans and rural community development is appreciated and acknowledged. In this project, we are honored to participate. We recognize the emerging scientific network arising from the Global Institute for Bioexploration-Africa (www.gibex.org) that promotes ethical, natural product-based pharmacological bioexploration

between universities to benefit human health and the environment in developing countries. Each of these programs represents paradigms that foster and protect indigenous knowledge while making new discoveries and commercialization opportunities available to all.

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Chapter 1

African Natural Plant Products: A Foreword to the Science and Challenges

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Africa has been and continues to be a significant source of medicinal and aromatic plants to the world's food, drug, herb and dietary supplement market, and in the past decade numerous African plant materials have established a strong international market presence. This book provides an excellent opportunity to delve into the current and future contributions that African plants can and will continue to make both internal to Africa and on the global stage.

This book expertly covers various medicinal plants of African origin and the some of the latest basic and clinical research supporting their ongoing and potential uses in self-care and healthcare. This work also examines various issues and trends in medicinal plants from their uses in Traditional Medicine and ethnobotany, to our modern understanding of the plants' chemistry and pharmacognosy, natural products chemistry and applications of medicinal plants, quality control, and models of benefit sharing.

People around the world enjoy Africa's culinary contributions. These include the peanut (*Arachis hypogaea*, *Fabaceae*), yam (*Dioscorea* spp., *Dioscoreaceae*), watermelon (*Citrullus lanatus*, *Cucurbitaceae*), okra (*Abelmoschus esculentus*, *Malvaceae*) and many other foods and flavors. In North America and in many other parts of the world there is little recognition of the many contributions that Africa has made to modern culture, i.e., perhaps beyond the domain of ethnobotanists and pharmacognosists.

From the medicinal and beverage perspective any checklist of economically important medicinal plants from sub-Saharan Africa would have to include at

least two key plants that have become household words around in America and the entire world: The caffeine-containing extract of the seeds of the West African evergreen kola tree (*Cola nitida* and *C. acuminata*; *Sterculiaceae*) was employed by Atlanta pharmacist John Stith Pemberton as a significant ingredient in a refreshing fountain syrup used as a stimulant beverage in the late 1800s. That beverage, Coca-Cola®, became one of the most recognized brands in the world, and spawned an entire class of non-alcoholic beverages or “soft” drinks, i.e., colas. For those that prefer another caffeine containing beverage, the world must again honor sub-Sahara Africa for bringing to us coffee. While the origins of coffee may be shrouded in mystery, it is clear that *Coffea arabica* L. and its ancestors originate in East Africa.

Unlike many cultures in Asia, particularly India and China, where written records document the use of medicinal plants at least 3500 years ago, the ethnobotany of sub-Saharan Africa is a discipline that has been relatively difficult to adequately chronicle and describe, as most of the traditional African cultures are based on oral tradition, much of which had not begun to be documented until the arrival of Arabic, and later European, botanists during in the past millennium (although Graeco-Roman medical botany also included African plants).

Previous publications have documented much of the traditional ethnobotany of Africa, a continent with a wide range of plant species and cultures. Favorites in my library include the ambitious volume, *African Ethnobotany-Poisons and Drugs: Chemistry, Pharmacology, Toxicology* by Hans Dieter Neuwinger (1996) which covers much of the pharmacology of some 240 poisonous and medicinal plants of sub-Saharan Africa; Neuwingers’ other book, *African Traditional Medicine: a Dictionary of Plant Use and Applications* (2000); Edward Ayensu’s *Medicinal Plants of West Africa* (1978) and Ben-Erik Van Wyk and Nigel Gericke’s focus on the ethnobotany of South Africa, *People’s Plants: a Guide to Useful Plants of Southern Africa* (2000); and Ben-Erik van Wyk, B. Van Oudtschoorn and C.F. .Hansmann’s *Medicinal Plants of Southern Africa* (1997).

Africa has been and continues to be a significant source of medicinal plants to the world’s food, drug, and herb and dietary supplement market, and in the past decade numerous African plant materials have established a strong market presence. These include the increasingly popular beverage rooibos (*Aspalathus linearis*, *Fabaceae*), the prostate remedy pygeum (aka African prune, *Prunus africana* syn. *Pygeum africanum*, *Rosaceae*), the recently popular appetite suppressant dietary supplement hoodia (*Hoodia gordonii*, *Asclepiadaceae*), the increasingly popular cosmetic ingredient shea butter (*Vitellaria paradoxa*, *Sapotaceae*), and the classic aphrodisiac herb used in psychiatry, yohimbe (*Pausinystalia johimbe*, *Rubiaceae*). Further, recent clinical trials document the benefits of extracts of the roots of the South African *Pelargonium* (umckalaoba, *Pelargonium sidoides*, *Geraniaceae*) for use in bronchitis, tonsillitis, and other upper respiratory tract infections. Detailed reviews of rooibos and another on umckalaoba are presented in this work.

The cover story of the American Botanical Council’s journal *HerbalGram* (number 79) reviews the nutritional and other uses of the oil from the kernels of the “Tree of Life”, the marula tree (*Sclerocarya birrea*, *Andacardiaceae*). A

previous *HerbalGram* article reviewed the potential promise of a Ghanaian medicinal plant in the constant war against malaria; basic science and several clinical trials demonstrate the potential anti-malarial benefit of *Cryptolepis sanguinolenta* (*Asclepiadaceae*) roots in an oral infusion and again you will find an updated review of this promising medicinal plant, *Cryptolepis*, or golden root in this work as well. Why more attention is not being paid to this potential life-saving plant is a mystery.

Increased use of African medicinal plants on the continent and in international trade has stimulated new efforts to monitor the quality of these botanical materials with the formation of the African Herbal Pharmacopoeia project. The first monographs to establish identity and criteria for quality control were introduced in 2009. This effort will no doubt lead to a greater sense of confidence in many of the leading botanical raw materials of African origin in the medicinal plant trade.

The editors of this volume have spent an extensive period of time working with African medicinal and aromatic plant scientists and producers through their integral affiliation for over a decade with ASNAPP (Agribusiness in Sustainable African Natural Plant Products, www.asnapp.org), an international effort dedicated to stimulating and improving production of raw materials and value-added agricultural products for African farmers, including medicinal and aromatic plants using world-class science and market-first driven models while ensuring the environmental sustainability of those resources and the economic development of Africa.

As noted in numerous market reports in North America and elsewhere, there is a bright future for the appropriate development, marketing, and use of medicinal plants and related products in foods, dietary supplements, “natural health products” (the regulatory term in Canada), over-the-counter and prescription drugs, and cosmetics. This book provides an excellent opportunity to delve into the current and future contributions that African plants can and will continue to make to this expanded worldwide market.

Chapter 2

The Natural Products Industry: A Global and African Economic Perspective

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Plant-based natural products that are collected from the wild, grown as cultivated crops or in agroforestry systems are used widely in number of different industries for their pharmaceutical, nutraceutical, cosmetic and other industrial and health applications. The natural products subsector remains an area of economic growth around the world especially among the developing countries. Most importantly, gathering, processing and commercialization of these natural products are carried out largely by the poor and most vulnerable members of societies, and is usually conducted by women. Thus, the use and commercialization of natural products has a strong linkage to poverty reduction and income generating particularly in the rural areas of Africa, Asia and Latin America. The global natural products industry, including the key sub-sectors of food and beverages, cosmetics, herbal medicines and pharmaceuticals, has been estimated to be valued at about US\$ 65 billion per annum and continues to grow. While this suggests many potential opportunities for increased trade in natural products, the lack of international standard classification of natural products creates problems in trade, tracking and particularly in measuring trade volumes and tariff analysis. Natural products are widely dispersed, production is highly variable and is often collected in small quantities, is often seasonally dependent and some products are highly perishable. Therefore, the emergence of new

voluntary trade standards, quality assurance schemes, codes of practice and certification schemes and the development of appropriate post-harvest handling and storage or value-addition processing presents key market access challenges for natural products.

Natural Products

The natural products subsector remains an area of economic growth around the world. In some regions, where the raw materials are collected from the wild or cultivated, these plants and specialty crops provide an important source of income and provide economic opportunity in areas where such options are limited or not available. According to the PhytoTrade Africa (the Southern African Natural Products Trade Association), natural products are considered to be of any product that is harvested from the wild or grown in the wild. Bennett (*1*) defined natural products as; being plant derived, occurring naturally, wild harvested or cultivated in situ, sustainably harvested and pro-poor. He shows that natural products have the following characteristics; multiple products, multiple end uses, multiple potential target markets and multiple embedded values.

These natural products could include an array of medicinal and aromatic plant species, oil seeds, flowers, teas, fruits and nuts and the availability of these plant species varies from country to country. Some of these natural products have also been classified as non-timber forest products (NTFP) where NTFP is defined as any product obtained from plants of forest origin and host plant species yielding products in association with insects and animals or their parts and items of mineral origin except timber (*2*). According to the FAO (*3*), plant-based NTFP include edible plants (fruits, nuts, mushrooms and wild vegetables), exudates (resins, gums and oleoresins), medicinal and aromatic plants, perfumes and cosmetics (including essential oils and incenses), tans and dyes, honey and beeswax, fibre and floss-producing plants, fodder, rattan and bamboo for utensils, handicrafts and construction material. We suggest an even broader term for natural products to be more inclusive of that described by FAO to include all herbal plants as well as foods (fruits and vegetables) whose extracts and by-products are used to improve health, nutrition, personal hygiene and industrial uses as this reflects the uses of botanicals now marketed and traded internationally within the natural products industry (*4*). The trend in the natural products industry appears to be moving to include not only wild fruit and vegetables but traditionally cultivated ones from citrus and pome fruits to small fruits such as grape and wine-derived products to other horticultural and food and non-food agronomic plant extracts and byproducts.

Since the ancient past many plant-based products have been used in meeting food, medicine, shelter and cultural needs. In many countries, traditional people believed and based their health-care and medicinal needs on plants (as well as insects, animals) found in their environment to cure specific ailments, enhance health and beauty, improve health and nutrition and for religious and spiritual

aspects. The integral role that plants had then and continues to play in many societies, including African societies, do not differentiate foods from medicines per se. Given these beliefs and practices, and the continued use and dependence that the world's peoples have on plants for health and medicine and as that knowledge spreads to other societies and markets, so does the interest and potential demand for these and other natural products.

The phytopharmaceutical, nutraceutical or functional foods and cosmetic markets have gained commercial attention as these natural plant species are so important in the making of beverages, foods, cosmetics, oils, health care products, herbal teas, nutritional supplements and medicinal products. Today, there appears to be a renaissance in all things natural and that indeed new cures and therapeutic benefits may come from a better understanding of the chemistry and biological activity of our foods, beverages and plants.

This has stimulated increased scientific study on plants and increased the interest in the validation of traditional medicines and the role that they could play in modern health care systems. The increased public sector pressures to address poverty, illness and disease may give rise to governments recognition that public health care policies and research need to be more inclusive of plants and natural products as vehicles to provide affordable health and nutrition to their citizens in ways that still assure safety and efficacy.

Whether the strategy will be to use plants and extracts directly to improve health and nutrition or indirectly for income generation so that families and communities can use the additional income to purchase food, medicines, school fees and clothes can both be pursued. In both cases, a better understanding of the markets for these natural products and the role(s) that this sector can play in local and regional economic development is key regardless of whether internationally trade is the objective, as the economic impact will be felt locally for each of the intended markets. Furthermore, many parts of the plant species are also used in making different products such as handicrafts, mats, brooms and other household utensils in addition to the more health and medicinal applications.

Significance of Natural Products

Natural products derived from plants are diversified and could be classified under different categories based on their uses. Figure 1 illustrates natural products based on their most common uses and the industries that demand such raw and processed materials. A given plant could produce multiple benefits and could fall into more than one of those categories. This highlights that natural products have extensive uses in various industries and great potential to contribute to a diverse range of products.

However, given the limited statistical data on natural product extraction and its uses in various industries, it has become difficult to determine the actual contribution of natural products to an economy. The greater diversity in natural products within and across countries and the unorganized and informal nature of extraction is the primary reason that there are problems in monitoring and evaluation of natural product extraction and marketing in any country. The other

is the specialized nature and the low volumes and/or economic significance for any one natural product relative to the relative ease in tracking and economic significance of the major food and agricultural commodities for which better statistics are available.

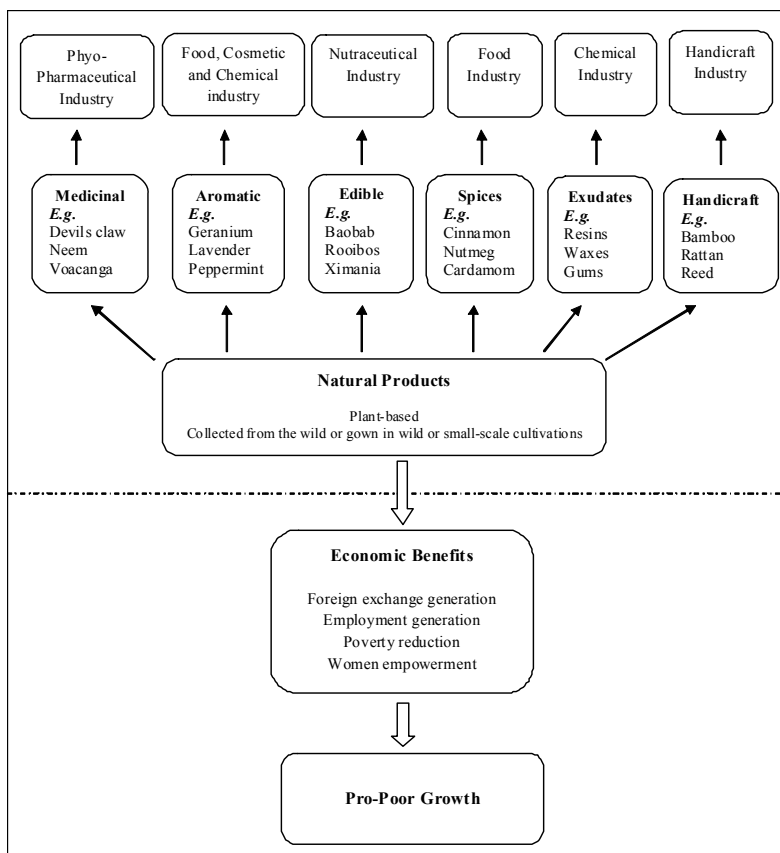


Figure 1. Natural Products and their Uses. Modified from Bennett (1).

The most significant contribution, in gross economic terms, of natural products to an economy is the foreign exchange generation through exports. However, given the complexity of natural product use it is difficult to obtain a comprehensive list of natural products that are exported from countries. Table I shows the total world export values of only 12 natural product groups at the 4-digit level. In 2007, these 12 natural product groups have contributed over US\$ 11 billion through exports (5). This highlights the economic significance of natural products to the exporting countries.

Table I. Natural Product Exports in 2007

<i>Product Code</i>	<i>Product Label</i>	<i>Value in US\$1000</i>
1302	Vegetable saps and extracts	3,200,411
1301	Lac; natural gums, resins, gum-resins and balsams	558,271
3301	Essential oils; resinoids; terpenic by-products etc	2,432,331
0903	Maté	72,975
0904	Pepper, peppers and capsicum	1,862,352
0905	Vanilla	124,090
0906	Cinnamon and cinnamon-tree flowers	191,946
0907	Cloves	155,389
0908	Nutmeg, mace and cardamoms	307,680
0909	Seeds of anise, badian, fennel, coriander, cumin, etc.	370,450
0910	Ginger, saffron, turmeric, thyme, bay leaves & curry	1,024,449
1211	Medicinal plants	1,694,419
Total Value of Exports		11,994,763

SOURCE: ITC (5).

Using southern Africa as an example of economic importance of natural products, Bennett (*1*) studied the marketplace of 10 Southern African developing countries showed that natural products have a trade potential of US\$ 3,428,962,767 per year. However, current trade statistics reveal that only US\$ 12,121,491 has been captured by these countries (Table II). Similarly, statistics show that natural products industry has a high potential in creating employment opportunities especially in gathering. Most importantly, gathering, processing and commercialization of these natural products are carried out by poor and vulnerable, particularly women (*6, 7*). Therefore, the natural products industry has a great potential in empowering women and raising their status within the household.

Table II. Current and potential by Product from ten Southern African Developing Countries (SADC countries)

<i>Product</i>	<i>Current trade (US\$/Yr)</i>	<i>Potential trade (US\$/Yr)</i>	<i>Current households employed (gathering only)</i>	<i>Potential households employed (gathering only)</i>
<i>Adansonia digitata</i>				
Baobab	11,203,928	961,358,568	1,165,965	2,640,333
<i>Kigelia africana</i>				
Kigelia	375,563	1,588,050,000	441,125	1,764,500
<i>Sclerocarya birrea</i>				
Marula	425,000	263,001,008	3,475,250	2,436,667
<i>Ximenia spp.</i>				
Ximenia	58,500	37,566,884	303,933	1,514,667
<i>Trichilia emetica</i>				
Trichelia	-	501,665,967	1,144,833	2,289,667
<i>Citrullus lanatus</i>				
Kalahari Melon	58,500	21,126,226	745,083	1,483,167
<i>Schinziophyton rautanenii</i>				
Manketti	-	19,677,684	197,208	42,597
<i>Parinari curatellifolia</i>				
Parinari	-	36,516,431	1,774,250	2,365,667
TOTAL	12,121,491	3,428,962,767	9,247,649	14,537,264

SOURCE: Bennett (1).

These and other natural products have a strong link with the poverty reduction particularly in the rural areas of Africa, Asia and Latin America (3, 8, 9, 10, 11). Further, Ndoye (6) argues that these natural products are essential for the livelihoods of forest dependent people and they have social, cultural and spiritual importance. Most importantly, these products enable rural households to diversify their income sources which intern contributes towards food security. The income generated from these natural products is a major constituent of the livelihoods of the rural poor particularly it acts as a safety net for the poorest of poor. According to Shackleton, Shackleton and Cousins (12), natural resources play an important role in the total economic value of rural households in South Africa where wild fruits, wild herbs and medicinal plants contribute 72-100%, 93-100% and 50-100% respectively to the total economic value.

Shackleton et al. (8) by conducting a study in northeast of South Africa showed that the most marginalized sectors of the community obtain a range of financial and non-financial benefits from the trade in natural products. However according to them, often only the poorest households most entirely depend on natural products and those that are doing well, the production and sale of natural products act as a livelihood diversification strategy. Similarly, a study done by Osemeobo (13) based on three states in Nigeria showed that the average annual

value of harvested wild plant products from the forest (including items consumed, sold, given out to neighbors and damaged after harvest) per household worth US\$ 11,956.54.

The household net income from wild plants was US\$ 6,742.61 per annum. Number of other studies done in Southern Africa has revealed a similar importance of natural products to the rural livelihoods (7, 14, 15, 16, 17). Shanley, Luz and Swingland (18), by taking Capim river basin in Amazonia, Brazil pointed out that during the distinct fruit seasons; sales of these regionally popular fruits provide work and income to substantial number of rural households. Based on Cambodia CFI (19) also shows that bamboo and rattan, resins and gums, aromatic and medicinal plants, fruits and nuts contribute significantly to the incomes of the rural poor. Similarly, Intercooperation (20) and FAO (3) also highlighted the economic importance of NTFP for rural livelihoods of Asia. Therefore, given the significance of natural products to the livelihoods of rural poor, there is an increasing concern on capturing value and developing markets for these underexploited products in the developing world (9).

Demand for Natural Products

The need, demand and ability to capture the economic trade for natural products are different for local, regional, and international trade. In the industrialized countries, consumers are more informed and economically able to address sophisticated concerns on the role of diet and proper nutrition to maintain health and beauty. Consumers, in general, are willing-to-pay premiums for labels like “natural, organic, eco-friendly, fair trade” and value-added terms like “fortified, enriched”. This has opened up many niche market opportunities for natural products. Based on a market report by Key note publications in 2005, Welford and Breton (21), shows that the global, natural products industry, including the key sub-sectors of food and beverages, cosmetics, herbal medicines and pharmaceuticals, is valued at US\$ 65 billion per annum and is booming with a 15–20% annual growth rate.

In the food industry, these natural products have now been classified under a new class of food called “nutraceuticals or functional” foods, because these products provide a health benefit beyond basic nutrition (22, 23). According to Wilkinson (24), most of the nutraceuticals used in the food industry are plant derived. Further, Addae-Mensah (25), showed that the world trade in medicinal plants accounts for about 30% of the total drug market and was estimated excluding plants used as raw material sources for the essential oils required to manufacture cosmetics, food additives and other non-medicinal purposes. The increased recognition of the value of natural products in the pharmaceutical, nutraceutical, cosmetic and other industries have created a huge demand for raw natural products. By taking Rooibos tea as an example from South Africa, Wilson (26) in detail describes the demand for Rooibos from different countries. However, for many natural products estimation of the actual demand is very difficult and the data sparse given the diversity of natural products used across industries and production variation within and across countries and the lack of

export and import statistics to track such trade for specific minor products not even considered as commodities.

According to IFORM and FiBL (27), consumer demand for organic products is increasing across the globe with retail sales estimated at 33 billion US-Dollars in 2005. Europe and North American demand are the key drivers of growth. In Europe, organic food sales have increased by 15% in 2007 than in 2006, with Germany accounting for one quarter of sales. Accordingly, German and British markets are the fastest growing markets in Europe. The Central Market and Price Agency (ZMP) of Germany have also estimated that organic food consumption in Germany doubles in every 6 years (28). Similarly, the Food and Health Survey of 2008 on consumer attitudes towards food, nutrition and health, commissioned by the International Food Information Council Foundation also showed that 60% or more of Americans either somewhat or strongly believe that certain foods and beverages can provide multiple health benefits (29).

More than 80% of all Americans say they are currently consuming or would be interested in consuming foods and/or beverages for such benefits (29). This is clearly evident by the 2008 consumer report of the Natural Foods Merchandiser, which has shown that the natural products industry in the USA grew by 9.8% in 2007 to more than US\$ 62 billion in sales (30). The survey revealed that food, supplements and natural personal care products have contributed 57.9, 28.8 and 13.3% respectively to the total sales of natural products. In terms of sales growth, carbonated, functional, ready-to-drink tea and coffee beverages have made the highest contribution of 29.4%.

Supply of Natural Products

The increasing demand for natural products has created a lucrative market for producers and manufacturers, yet not all producers have always fared well as the profitability along most commodity chains are not uniformly proportional in that the collectors, gatherers, and farmers, particularly those with small farms and land holdings. Prices to producers and to processors, manufacturers and exporters can be generally characterized as volatile, with those lower down the commodity chain often receiving the lowest relative profit margin, and are often the 'price-takers' not the 'price-setters' or the market drivers. This price fluctuation comes at the traditional costs in reducing the local inventory and supply of natural products and the incentive for collectors or growers to gather and cultivate a particular natural product, thus further decreasing the product availability and influencing the product price. From a global perspective, both Asia and Africa which are already in the global marketplace have a good potential to capture an increasing share of this global natural products market as each contain a great share of the world's biodiversity and also have agricultural production and agro/forestry costs that can be globally competitive and still rely heavily on agriculturally based economies. Based on the ITC (31), the most common medicinal plants exported by Africa, Asia, North America and South America and Europe are shown in the Table III. The plant species listed clearly reveal that natural products of both Asia and Africa are diversified.

Intercooperation (20), shows that only in India, of the 2,500 plant species that is used by traditional healers nearly 500 are used by the pharmaceutical industry.

Welford and Breton (21) reported that in Africa at least 1,000 out of 30,000 plant species have important medicinal properties. However, only around 50 species are currently traded in formal markets. Based on 10 Southern African countries, Bennett (1) reported that only eight natural products; baobab, kigelia, marula, ximenia, trichelia, Kalahari melon, manketti and parinari have a significant commercial potential (Table II). However, while his list focused on savannah and dryland edible oils and fruit, it does not consider all natural products from the larger southern African region such as the African aloe (*Aloe ferox*), devils claw (*Harpagophytum procumbens* and *H. spp.*), pygium (*Prunus africana*), rooibos (*Aspalathus linearis*), honeybush (*Cyclopia spp.*), buchu (*Agathosma betulina* and *A. spp.*), wild geranium (*Pelargonium sidoides*) and other natural product medicinals and herbal teas for which southern Africa is recognized as the leading region of production and export (32, 33, 34). Although there are number of plant species that have been used traditionally many applications - foods, medicines and more, their economic significance is yet to be recognized. This is primarily due to the less commercialized nature of the industry. The natural products industry of Africa is informal, less developed and characterized by low input and output; small scale with many bottlenecks including information scarcity on quality, standards, markets (32, 35, 36).

Table III. A List of Selected Common Natural Products by region (Africa, Asia, the Americas and Europe).

Africa	
<i>Product</i>	<i>Botanical name</i>
Baobab tree	<i>Adansonia digitata</i>
Buchu leaf	<i>Agathosma betulina</i>
Clove flower bud	<i>Syzygium aromaticum</i>
Cinnamon bark	<i>Cinnamomum verum</i>
Devil's claw	<i>Harpagophytum procumbens and H. zeyheri</i>
Fennel fruit	<i>Foeniculum vulgare</i>
Ginger rizome	<i>Zingiber officinale</i>
Gum arabic	<i>Acacia senegal</i>
Hibiscus	<i>Hibiscus sabdariffa</i>
Honeybush tea	<i>Cyclopia spp</i>
Kalahari melon	<i>Citrullus lanatus</i>
Kigelia	<i>Kigelia africana</i>
Lemon grass	<i>Cymbopogon citratus</i>
Manketti	<i>Schinziophyton rautanenii</i>
Marula	<i>Sclerocarya birrea</i>
Mongongo tree	<i>Schinziophyton rautanenii</i>
Mobola Plum	<i>Parinari curatellifolia</i>
Myrrh gum powder	<i>Commiphora spp.</i>
Parinari	<i>Parinari curatellifolia</i>
Pygeum bark	<i>Prunus africana</i>
Rooibos herb	<i>Aspalathus linearis</i>
Rosehip	<i>Rosa spp</i>
Sausage tree	<i>Kigelia pinnata</i>
Trichilia	<i>Trichilia emetica</i>
Vanilla fruit	<i>Vanilla planifolia</i>
Ximenia	<i>Ximenia spp.</i>

Table III. Continued.

<i>Asia</i>	
<i>Product</i>	<i>Botanical name</i>
Asian ginseng	<i>Panax ginseng</i>
Amala fruit	<i>Phyllanthus emblica</i>
Andrographis herb	<i>Andrographis paniculata</i>
Ashwagandha root	<i>Withania someifera</i>
Bacopa herb	<i>Bacopa monnieri</i>
Bitter orange fruit	<i>Citrus aurantium</i>
Black tea leaf	<i>Camellia sinensis</i>
Cassia bark	<i>Cinnamomum aromaticum</i>
Cardomom seed	<i>Elettaria cardamomum</i>
Chirata herb	<i>Swertia chirayita</i>
Clove flower bud	<i>Syzygium aromaticum</i>
Costus root	<i>Saussurea costus</i>
Epimedium herb	<i>Epimedium spp.</i>
Fennel fruit	<i>Foeniculum vulgare</i>
Fenugreek seed	<i>Trigonella foenumgraecum</i>
Garlic bulb	<i>Allium sativum</i>
Ginger rhizome	<i>Zingiber officinale</i>
Ginko leaf	<i>Ginkgo biloba</i>
Guggal oleo-gum-resin	<i>Commiphora wightii</i>
Gymnema leaf	<i>Gymnema sylvestre</i>
Henna leaf	<i>Lawsonia intermis</i>
Holy basil leaf	<i>Ocimum tenuiflorum</i>
Indian tinospora	<i>Tinospora cordifolia</i>
Lycium fruit	<i>Lycium barbarum</i>
Neem leaf	<i>Azadirachta indica</i>
Schisandra fruit	<i>Schisandra chinensis</i>
Senna leaf	<i>Cassia angustifolia</i>
Rhodiola root	<i>Rhodiola rosea</i>

Table III. Continued.

North and South America	
<i>Product</i>	<i>Botanical name</i>
Black cohosh rhizome	<i>Actaea racemosa</i>
Blackpepper fruit	<i>Piper nigrum</i>
Cardomom seed	<i>Elettaria cardamomum</i>
Cascara sagrada bark	<i>Frangula purshiana</i>
Coriander fruit	<i>Coriandrum sativum</i>
Cat's claw stem bark	<i>Uncaria tomentosa</i>
Echinacea herb and root	<i>Echinacea purpurea</i>
Maca hypocotyle	<i>Lepidium meyenii</i>
Mate' leaf	<i>Ilex paraguariensis</i>
Mexican wild yam	<i>Discorea composita</i>
Pau d'arco bark	<i>Tabebuia impetiginosa</i>
Pleurisy root	<i>Asclepias tuberosa</i>
Slippery elm bark	<i>Ulmus rubra</i>
Stevia leaf	<i>Stevia rebaudiana</i>
Wild cherry bark	<i>Prunus serotina</i>
Witch hazel leaf	<i>Hamamelis virginiana</i>
Europe	
Bayberry root bark	<i>Morella cerifera</i>
Black cohosh rhizome	<i>Actaea racemosa</i>
Bilberry fruit	<i>Vaccinium myrtillus</i>
Caraway fruit	<i>Carum carvi</i>
Fenugreek seed	<i>Trigonella foenum-graecum</i>
Ginkgo leaf	<i>Ginkgo biloba</i>
Narrow-leaved coneflower root	<i>Echinacea angustifolia</i>
Purple coneflower root	<i>Echinacea purpurea</i>
St. John's wort herb	<i>Hypericum perforatum</i>

Based on the UN COMTARDE database, FAO (3) shows that Asia is the world's largest producer and consumer of non-wood forest products (NWFP) where China and India has the biggest shares (Table IV). Accordingly, China producers and possesses more wild products than any other country in the world.

Table IV. Total Global Exports of Botanical Raw Materials, Extracts and Oils in 2006

<i>Country</i>	<i>Export Value (‘000 US\$)</i>	<i>% Share of World Exports</i>
China	1, 585, 001	14.2
India	1, 297, 498	11.6
Germany	765, 944	6.9
USA	751, 418	6.7
Kenya	672, 923	6.0
Other countries	6, 072, 779	54.5

NOTE: HS Codes: 0902–0910, 1210, 1211, 121220, 130120, 130190, 130219, 140410, 140490, 3301 and 400130

SOURCE: FAO, 2002

By default these natural products are organic as they are collected or grown in wild without any chemical or fertilizer additions. However, only some countries market these products with proper organic certifications. As reported by IFORM and FiBL (27), the ITC global survey on organic wild collection in 2006 has found out that 62 million hectares of land are registered to provide organic wild collections with a total of 979 organic wild collection projects.

Although Africa has the largest registered area of around 27 million hectares, its total harvested quantity is relatively low compared to the registered extent (Table V). Bamboo shoots (36%), fruits and berries (21%), nuts (19%) and medicinal and aromatic plants (9%) are the most widely collected natural products from the wild. In terms of Africa, only a small range of products are collected of which more commonest products are sheabutter, rosehip, gum arabic, argan oil and honeybush.

Classification of Natural Products

A standard classification of natural products does not exist yet. Given the greater diversity of uses, the myriad of plants, plant products and extracts and application categories for the same material (food, oil, cosmetic, etc.) the same natural product can be classified under different Harmonized System of (HS) Codes. According to the FAO (3), a similar problem exists for NTFPs. Therefore, based on number of research studies, the FAO compiled a possible classification of NTFP in accordance with the HS coding system. The FAO classification of plant products is based on their end use which could be used as criteria in classifying natural products (Table VI).

Table V. Identified Quantities, Registered Areas and Number of Wild Collection Projects Worldwide in 2005

<i>Continent</i>	<i>No. of Certified Wild Collection Projects</i>	<i>Registered Area (Ha)</i>	<i>Total Harvested Quantity (T)</i>
Europe	127	26, 715, 956	33, 365
Africa	25	27, 439, 963	4, 785
Asia	145	6, 261, 176	138, 426
Latin America	25	1, 346, 420	26, 876
North America	648	180, 000	102
Australia and Oceania	9	16, 090	20, 200
Total	979	61, 959, 605	223, 754

SOURCE: IFORM and FiBL (27)

Table VI. Main Categories of NTFP – Plant products

<i>Category</i>	<i>Description</i>
Food	Vegetable, food stuff and beverages provided by fruits, nuts, seeds, roots, mushrooms, etc.
Fodder	Animal and bee fodder provided by leaves, fruits etc.
Medicines	Medicinal plants (e.g. leaves, barks, roots) used in traditional medicine or by pharmaceutical companies
Perfumes and cosmetics	Aromatic plants providing essential (volatile) oils and other products used for cosmetic purposes
Dying and tanning	Plant material (mainly bark and leaves) providing tannins and other plant parts (especially leaves and fruits) used as dyes
Utensils, handicrafts and construction materials	Heterogeneous group of products including thatch, bamboo, rattan, wrapping leaves and fibres
Ornamentals	Entire plants (e.g. Orchids) and parts of the plants (e.g. Pots made from roots) used for ornamental purposes
Exudates	Substances such as gums (water soluble), resins (water soluble) and latex (milky or clear juice) released from plants by exudation
Other	For example, insecticides, fungicides

SOURCE: FAO (3)

Lack of a proper classification system creates problems in trade, tracking and particularly in measuring trade volumes and tariff analysis. Given this problem, the Natural Futures of IUCN has begun to address this issue and attempted to classify a few Southern African natural products under HS coding system. Accordingly, they have analyzed the end uses of Marula, Ximenia,

Kigelia, Baobab and Trichilia in detail and identified possible HS codes that fit well for each of the above natural products. A detailed description of product classification based on their end use is presented in Table VII. This shows that classifying a given natural product into a particular HS coding system needs thorough research in identifying its composition and a careful understanding of potential demand for these by trading partners.

Table VII. A Detailed Classification of Southern African Natural Products

<i>Product</i>	<i>Uses</i>	<i>HS Code</i>	<i>Market</i>
Marula	Fresh fruit	08109095	For processing into pulp and seed extract
	Frozen fruit	081190	For processing into pulp and seed extract
	Fruit skins	08129098	Flavor and fragrance ingredient
	Dried fruit	081340	Snack food and food ingredient
	Seed oil	16159040	Cosmetic and food use
	Fruit juice	20098088	Beverage and food ingredient
	Essential oil	33019090	General cosmetic ingredient
Ximения	Fresh fruit pulp	081090 080990	For processing into pulp and seed extract
	Dried fruit	0813.40	Finished food or ingredient
	Seed oil	120999	Pharmaceutical or cosmetic ingredient
		121190.80	
		121190.90	
		12190.30	
		121190.20	
	Seed oil	15159040	Cosmetic, pharmaceutical or cosmetic ingredient
		330190.50	
	Jam, jelly	20071091	Finished food
	Food supplement	29369019	Food fortifier
	Skin cream	33049950	Personal care
Soap	340119	Personal care	
Shampoo	34022090	Personal care	

Table VII. Continued.

<i>Product</i>	<i>Uses</i>	<i>HS Code</i>	<i>Market</i>
Kigelia	Seed oil	120999	Pharmaceutical or cosmetic ingredient
		121190	
		330190	
	Seed oil	15159040	Cosmetic ingredient
	Food supplement	29369019	Food fortifier
Body cream, body butter	330499	Personal care	
	340120	Personal care	
Baobab	Fruit pulp-fresh	08109095	For processing into pulp and seed extraction
		90	
	Seed oil-crude	15159040	Cosmetic ingredient
	Jam	20071091	Finished food
	Fruit pulp processed	20089293	For pulp processing as a food or pharmaceutical ingredient
	Juice concentrate	20098039	Food or beverage ingredient
	Liqueur	220890	Alcoholic beverage
	Food supplement	29369019	Food fortifier
	Skin cream	33049950	Personal care
	Soap	340119	Personal care
	Shampoo	34022090	Personal care
	Excipient	unknown	Pharmaceutical ingredient
Trichilia	Seed oil	120999	Pharmaceutical ingredient
		121190	
	Seed oil	15159040	Cosmetic ingredient
	Seed oil	330190	Pharmaceutical or cosmetic ingredient
	Food supplement	29369019	Food fortifier
	Body cream, body butter	330490	Personal care
	Soap	340120	Personal care

SOURCE: Natural Futures (37)

Marketing Channels of Natural Products

Natural products are widely dispersed and production is highly variable. Several natural products are highly perishable. Therefore, many of the wild collected natural products are collected generally in small quantities and are sold to local village traders. Marketing practices adopted by farmers and or collectors are diverse and are difficult to be generalized. In some countries, farmers or

collectors bring the products to a central place where processors or brokers purchase the products from farmers. Sometimes this central place is owned and/or operated by the communities in an association, cooperative and/or non-legally registered manner. These market channels are sometimes referred to as centralized market channels and are more effective when the product is more scattered. In contrast, in decentralized market channels, farmers or collectors sell their products to the traders who come to the village. In Africa, both systems may be operating along side each other.

Generally, the number involved in wild collection is high and is primarily performed by poor, rural women. During the peak season, these wild collectors sell their collection along the road-sides as unprocessed or processed. Shanley, Luz and Swingland (18), showed that in Belém during the fruit season, there will be an increasing number vendors who sell processed items like juice, ice-cream, pulp etc. Accordingly, this has attracted many consumers from the urban areas and resulted in an increased year round market demand for native fruits. These natural products cross the borders after passing through several intermediaries but most of the time the level of value addition along the channel is minimal.

However, Das (38) by taking an Indian NTFP as an example pointed out that there are number of collectors along the marketing channel where secondary collectors sell NTFP to tertiary collectors with a minimum of 20-30% profits from the price given to the primary collectors. They also pointed out that tertiary collectors sell the NTFPs at 3 to 4 times more than that of the secondary collectors. Finally, the wholesale buyers sell these natural products that are unprocessed or part processed as bulk to processors or distributors. Standards required for quality could vary depending on the buyer. Reiner (39) shows that with only a few exceptions, international markets for NTFP are niche markets because extensive harvesting for mass markets will threaten the sustainability of resources. Reiner argues that there is a significant potential for fair trade and organic markets, eco-markets and specialty markets as the volumes traded are small and trade chains are short.

In some instances, the natural products are or could be sold as processed, value added products from the place of origin as ready to be consumed items. These products could be sold directly to consumers, brought into a neighboring village, or sold via mail order/internet, natural product stores, grocery chains and restaurants. In this process, individual companies or association could take a lead in carrying out the marketing aspect or facilitating the marketing. For example, Phyto Trade Africa (The Southern African Natural Products Trade Association) focuses in developing the products, supply chain as well as the natural products industry of Southern Africa. They collect raw material from the rural producers and market a wide range of products as ethical, sustainable and organic natural products.

According to the Natural Foods Merchandiser (30) in developed marketplaces, there are primarily six channels of distribution at the retail level. These include the natural products retailers/health food stores, mass market retailers (drug, grocery and discount stores), multi-level marketers, mail order, health care practitioners and the internet. The recent survey pointed out that nearly half of the natural products are sold through natural products

retailers/health food stores and only and only around 15% use internet to buy natural, organic and health products.

Challenges Faced by the Natural Products Industry

Given the increasing demand, the natural products industry is becoming a lucrative industry for many developing countries. However, according to the FAO (3), this growing demand has led to overharvesting of certain plant species and hence the Convention on International Trade in Endangered Species (CITES) have declared some species as endangered species. This is primarily because three quarters of the total production is still wild harvested. According to the Intercooperation (20), pointed out that 25% of the modern medicine is made from plants first used traditionally. The same report reveals that in India, 90% of the plant species used by the pharmaceutical industry are collected from the wild. Along with this, excessive deforestation has also threatened the existence of natural products in the wild. Therefore, the sustainability issue must be addressed by promoting sustainable harvesting practices and cultivation of important species rather than over harvesting of natural products from the wild. To address this, the WHO has published guidelines on good agricultural and collection practices (GACP) for medicinal plants which are used for all NTFPs and natural products (40).

Countries that are blessed with high biodiversity possess many plant species that been used for centuries for traditional medicines, foods and other uses. Therefore, it is important to both preserve the species, the knowledge about the species and their diverse uses and applications while still developing pragmatic paths for their continued local use, which will continue and creating economic opportunities for more regional and international trade in an environmental and culturally sustainable and appropriate manner. These economic opportunities could be leveraged to assist in the conservation and preservation of the indigenous resources while creating income generating opportunities for vulnerable populations that could benefit. However, the hindering factor for many developing countries is the lack of capital by the private sector to invest in the infrastructural needs relative to storage, processing and value-added; the lack of scientific programs to identify potential uses and increase quality. Govindasamy et al (35, 36) studied the African natural products marketplace and found that each was characterized by low input and low output and informal; primarily consisting of small-scale farmers (suppliers) with low levels of formal education and agricultural production knowledge.

Traders lacked regular supplies of good quality products and that the scale of natural products operations may be a bottleneck as were the lack of information, lack of capital, low product quality and assurance mechanisms, difficulty in accessing financial credit and loans at reasonable rates, and poor facilities and processing equipment, and little historical investment into this sector. Furthermore, they reported from their survey of the traders themselves that the domestic markets of wholesalers and retailers are largely at low levels of commercialization; in general traders have limited technical knowledge about natural products, and limited capital to expand their businesses and exploit

available foreign markets. On the demand side, there may be a corresponding lack of consumer information as to the range of products available, where to find them, what remedies they offer, and information on quality and safety.

Another issue that is ever present in the natural products industry when referring to medicinal plants is in the complex arena of intellectual property rights. Addae-Mensah (25), by discussing the intellectual property right issues on herbal medicine pointed out that development of medicinal plants relies very heavily on the knowledge carried by indigenous peoples and rural societies and hence raises the equitable sharing of the benefits of such knowledge and the intellectual property rights of these indigenous rural communities and countries.

As discussed, the natural products industry is operating at a subsistence level with less commercialization. Rural, poor communities who are involved in harvesting of products have minimal knowledge on appropriate techniques of harvesting or processing. Producers have poor linkages with traders and generally, information flow across the market channel is weak. Therefore, most of the benefits are captured by the retailers (35, 36). This shows that it is important to strengthen the supply chains and develop appropriate mechanism like fair trading to pass real benefits to the rural poor.

Most importantly, the emergence of new voluntary trade standards, quality assurance schemes, codes of practice and certification schemes represent a key market access challenge for natural products. Although, at the initial level these could act as barriers to trade once they are implemented they would lead to gain premiums from trade and hence could consider these as double edged swords. Welford and Breton (21), by discussing the Phytotrader Africa's experience in certification in natural products also showed that development of ethical and environmental standards are more applicable to natural products industry. However, they also highlight the possible difficulties in attaining stringent standards required for certification.

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Chapter 3

Herbal Medicine in Swaziland: An Overview

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Herbal medicine is very popular in Swaziland with about 85% of the population relying on it for their primary health care. The widespread use of medicinal plants in the country has inspired research in herbal medicine which has led to some documentation of the heritage and provided scientific insights into the practice. Although the herbal medical practitioners have no scientific way of diagnosing their patients and they have no documentation or proof for the efficacy of their products, they have many herbal remedies that have been used for generations for symptom management of various diseases. This review provides examples of medicinal plants used for preparing herbal remedies and the various diseases for which they are used. Indigenous medicinal plants have tremendous potential to contribute to the health care systems in Africa and in the search for novel therapeutic compounds because they possess genetic materials still to be explored.

Swaziland is a small land-locked country located in southern Africa approximately between latitudes 310 and 320, and longitude 260 and 280 east. It is almost surrounded by South Africa in the north, south, southeast and west while bordered by Mozambique in the east. Swaziland is the smallest country in the southern hemisphere with an area of 17,363 km². It has a mono ethnic population of about 1.2 million people and the language of the people is siSwati.

The country is very rich in culture and tradition which is highly treasured and prized with a pride and recognition that seeks to preserve its cultural heritage in a rapidly changing surrounding world. As a homogenous ethnic entity, the country has only one culture and anything done which is not in line with the traditional norms is often resisted and branded as “unSwazi”. Any infringement of the customs and tradition by anyone is believed to evoke divine wrath (1). The practice of herbal medicine is part of the tradition of the people.

Herbal medicine is the art of restoring or preserving the health of humans physically, mentally, socially, spiritually and otherwise through the use of plant remedies. The term is often used interchangeably with traditional medicine or traditional remedies because it has evolved through the ages from the traditional and cultural practices of the indigenous people. In the industrialised countries of the West where biomedicine is conventional, herbal medicine is usually referred to as complementary or alternative medicine. Some people in the industrialised nations will even term elements of herbal remedies as health supplements.

The practice of herbal medicine can only be understood within the context of the culture of the herbal medical practitioners and their beliefs on the causes of diseases (2,3). Whereas orthodox medicine centres around rigours of scientific experiments and diseases are known to be caused by physio-pathological agents, herbal medicine posits that the human being is both somatic and spiritual and that disease can be due to supernatural causes arising from anger of ancestral spirits, effects of witchcraft, evil spirits, disharmony of an individual with the environment or the entry of a foreign object into the body (2,4,5). It is therefore not only the symptoms of the disease that are taken into cognisance in herbal medical practice but even the psychological and sociological factors for which indigenous knowledge provides a clue. This is why the practice of herbal medicine is said to be holistic.

Swazis like the Shona and Ndebele people from Zimbabwe have strong belief in ancestral spirits. They believe in life after death and that people do not die but just pass on (bayendlula) to join the world of spirits (5,6). It is because of this belief that Swazis don't usually say that a person has died but rather that a person has “passed on”. In Swazi traditional belief, it is these individuals that have passed into the spirit world who are said to impart the indigenous knowledge and the art involved in herbal medical practice to their living relatives (5).

Patronage of Herbal Medicine

Most indigenous cultures in developing countries rely on herbal remedies for their medical care. The World Health Organisation has estimated that 80% of people in Africa rely on herbal medicine for their primary health care (7). In

Swaziland, herbal medicine has been the main vehicle for delivering health to the majority of people from time immemorial. The practice has been flourishing in the country as far back as 1894 to the extent that it caught the attention of the colonial administration which made efforts to outlaw it (8). It is currently estimated that about 85% of the population patronise herbal medicine and rely on it for their primary health care (9). The popularity of traditional medical practice in the country is because of the following socio-cultural reasons (3):

Firstly, the practice is anchored in the cultural and religious beliefs of the people; it is closely intertwined with beliefs of the causes of illness. The culture of any person has great influence on the person's perception of life. Secondly, traditional medicine has a holistic approach to the healing and prevention of diseases such that the whole person is treated. The body, mind and soul are viewed as indivisible whole connected to the social, physical and spiritual world. Thirdly, conventional drugs are very expensive and unaffordable by many in the society, especially in rural communities where most of the populace reside.

The Herbal Medical Practitioners provide an environment that their patients are used to and with which and with whom they relate in a humane manner within the cultural setup where the patients feel very much at home. They have patronage from every strata of the society. They are consulted not only for medical needs but for almost any need and perplexities of life. They are the first port of call in cases of ill health or any spiritual, moral, psychological and social problems. The patients are assured of attention, understanding, sympathy and solution to whatever their problems may be. Herbal medical Practice is also more accessible to the people as the practitioners are in thousands. The ratio of the herbal medical practitioners to the populace in 1985 was 1:110 while that of the orthodox medical practitioners to the population was 1:10,000 (8). Increase in the patronage of herbal medicine is likely to continue because of global economic downturn and as bodies like the World Health Organisation continue to advocate for its promotion and its integration in the national health systems of developing countries (10).

Although a majority of the population embraces herbal medical practice in Swaziland, there is no policy nor legal instrument for its' practice in the country. There is also no regulatory body for the control of the practice. The Herbal Medical Practitioners have been operating through the King's Order-in-Council of 1954 which enabled them to practice.

There is inadequate documentation about herbal medicine in the country. The plant remedies have not been scientifically validated for safety and efficacy for them to be included in list of essential drugs for national health systems (11). Herbal medical practice is also still shrouded in secrecy and perceptions of metaphysical powers. It is essential that research should explode the myths and superstitions associated with herbal remedies by establishing the real basis of the therapeutic properties of the medicinal plants used in herbal remedies (12).

Ethnopharmacology (Medicinal Plants for Treating Specific Diseases)

Swaziland is one of the African countries where the overall plant diversity is at the highest level in terms of the number of species present (13). The rich and diverse flora has been greatly exploited in herbal medicine for symptomatic treatment of various ailments. The Herbal Medical Practitioners possess vast indigenous knowledge on almost any type of plant in the flora. Their knowledge on medicinal plants and skill in preparing remedies for various types of diseases is quite commendable.

In an ethnobotanical survey of a 25 ha of land in Swaziland, two herbalists gave information on thirty five herbal remedies for twenty three diseases. Some of the diseases could be classified as gynaecological, obstetric and sexual related diseases, while others are haematological, orthopaedic, gastro-intestinal, congenital, dental and cardiovascular diseases, to mention a few. Equally worthy of note is the fact that the remedies were based on different organs of thirty seven medicinal plants from twenty seven families all from just the flora of the 25 ha of land (14). Much of the vast indigenous knowledge of the herbal medical practitioners is untapped, underutilized and under-valued. The knowledge may be useful in the search for novel therapeutic substances. The following are examples of plants used for preparing herbal remedies for treating specific diseases. Plant names are also listed in parentheses, in both the local tribal language common english name after the scientific name.

Diseases of the Cardiovascular System

Aloe marlothii Berger [*inhlaba lenkhulu*, mountain aloe] (Asphodelaceae) leaves are for cardiac problems. The leaf contains alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids and tannins (15). Concoction of the leaves of *Aloe arborescens* Mill [*inhlaba* lencane, krans aloe] and *Aloe saponaria* Haw. [*lihala*, soap aloe] is also used for cardiac problems (12).

Clausena anisata (Willd.) Hook.f.ex Benth. [*umnukelambiba*, horsewood] (Rutaceae) stem bark concoction is for cardiac problems. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols, saponins and steroids (15). Extracts of the roots are molluscicidal and the root contains alkaloids and coumarins (16,17).

Euclea natalensis A. DC. [*umdlelanyamatane*, Natal ebony] (Ebenaceae) root decoction is for oedema (11).

Momordica balsamina L. [*inkakha*, bitter gourd] (Cucurbitaceae) aerial parts are eaten as a vegetable for hypertension and diabetes (18). The leaf contains alkaloids, flavonoids, glycosides and polyphenols (18). The seed oil contains two conjugated octadecatrienoic acids (19). The plant also possesses antiplasmodic, analgesic and sedative properties which have been demonstrated in rodents (20).

Persea americana Mill. var. *americana* [avocado] (Lauraceae) stem bark decoction is for hypertension and palpitation. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids and tannins (11,15).

Psidium guajava [umgwava, guava] (Myritaceae) leaf infusion is used for hypertension, palpitation, diarrhoea and menorrhagia. The leaf contains alkaloids, flavonoids, glycosides, polyphenols, saponins and steroids (11,14,15). The leaf also has hypoglycaemic, anagelsic and anti-inflammatory properties (21,22).

Syzygium cordatum Hochst. [umcozi, waterberry] (Myrtaceae) stem bark and leaf decoction is for oedema (11).

Diseases of the Digestive System

Agapanthus caulescens Spreng. [hlakahla, agapanthus] (Liliaceae) root decoction is used for constipation and as an emetic. The root contains alkaloids, flavonoids, saponins and steroids (18,23).

Cussonia natalensis Sond. [umsenge, rock cabbage tree] (Araliaceae) stem bark concoction, and *Gardenia spatulifolia* Stapf & Hutch [umvalansangweni, Transvaal gardenia] (Rubiaceae) root concoction are also used as emetics to stop stomachache (12).

Bidens pilosa L. [chuchuzza, black jack] (Asteraceae) leaves are for stomachache. The leaf contains alkaloids, flavonoids, glycosides, polyphenols and steroids (15). The leaves are also eaten as a vegetable (23).

Bolusanthus speciosus (Bol.) Harms. [umhhohlo, tree wisteria] (Papionaceae) root concoction is used for abdominal pains (12).

Capparis tomentosa Lam. [indodebovu, woolly caper bush] (Capparidaceae) stem bark concoction is used as an emetic. The stem bark contains alkaloids, anthranoids, polyphenols, saponins and steroids (15). Extracts of the aerial parts have antimicrobial properties (24).

Carica papaya L. [umphopho, pawpaw] (Caricaceae) leaf infusion is used for dysentery. The leaf contains flavonoids, glycosides, polyphenols, steroids and tannins (15).

Cassine transvaalensis Burt Davy [ngcotfo, Transvaal saffronwood] (Celastraceae) stem bark infusion is used for stomachache. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols, steroids and tannins (15). Extracts of the roots has exhibited strong antioxidant properties (25).

Cassine aethiopica Thunb. [macundza, forest saffron] (Celastraceae) stem bark concoction is used for peptic ulcers and chest pain (26).

Catharanthus roseus (L.) G. Don. [imbali, periwinkle] (Apocynaceae) root decoction is used for stomachache (26).

Clerodendrum glabrum E. Mey. [umphehlacwtsi, Natal glorybower] (Verbenaceae) leaf infusion is used for stomachache. The leaf contains glycosides, polyphenols, saponins and steroids (15).

Combretum zeyheri Sond. [imbondo/lemhlophe, largefruited bush willow] (Combretaceae) root infusion is used for diarrhoea. Roots contain flavonoids, glycosides, polyphenols, saponins, steroids and tannins (15).

Crassula alba Forssk. [mgazini, bride's bouquet] (Craussulaceae) root is used in the treatment of diarrhoea (18).

Croton gratissimus Butch. var. *gratissimus* [*mhuluka*, lavender feverberry] (Euphorbiaceae) stem bark infusion is used for stomachache. The stem bark contains alkaloids, glycosides, polyphenols and saponins (15).

Diospyros galpini (Hiern) De Winter [*umcafutane*, dwarf hairy jackalberry] (Ebenaceae) root decoction is for anus problems. Root contain flavonoids, polyphenols, saponins, steroids and tannins (15).

Ekebergia capensis Sparrm. [*umnyamatsi*, Cape ash] (Meliaceae) bark decoction is used for heartburn (as an anti-acid). Roots contain polyphenols, saponins and terpenoids (18). The roots are also used in a remedy for dysentery (17).

Elaeodendron transvaalensis (Burt Davy) [*ingwavuma*, bushveld saffron] (Celastraceae) stem bark concoction is used as an emetic. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols, saponins and tannins (15). The plant is also used to treat sexually transmitted diseases and its extract exhibits antimicrobial activity (27).

Elephantorrhiza elephantina (Burch.) Skeels [*intfolwane*, elephant root] (Fabaceae) rhizome infusion is used for diarrhoea (15). Rhizomes are also used for stomach disorders and skin diseases (28,29). The root contains alkaloids, anthranoids, polyphenols and steroids (15). Extracts of the plant have some antibacterial properties (30). The antibacterial activity provides a scientific rationale for the use of the plant in herbal medicine for diarrhoea.

Euclea divinorum Hiern [*umgwali*, magic gwarra] (Ebenaceae) stem bark decoction is used for constipation. The stem bark contains alkaloids, glycosides, saponins and tannins (15). The plant also contains flavonoids (31). From a root decoction a laxative remedy is made and the plant has exhibited some cytotoxic properties (18,32).

Gardenia cornuta Hemsl. [*umvalasangweni*, Natal gardenia] (Rubiaceae) stem bark concoction is used as an emetic. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols and steroids (15).

Gnidia kraussiana Meisn. var. *kraussiana* [*umsilawengwe*, yellow heads] (Thymelaeaceae) root decoction is for stomachache. Roots contain anthranoids, flavonoids, polyphenols, saponins and tannins (15). The root is also used for remedies for burns and snakebites (28).

Helichrysum odoratissimum (L.) Sweet [*imphepho yentsaba*, everlasting plant] (Asteraceae) leaf infusion is used for constipation (18). Young branches are used for conjunctivitis (33). The leaf contains alkaloids, polyphenols steroids and tannins (18). The flowers contain flavonoids and a chalcone (34).

Hypoxis gerrardii Bak. [*inkhofe*, Silver-leaved star flower] (Hypoxidaceae) corm infusion is used for abdominal cramps. The corm contains flavonoids, polyphenols, saponins, steroids and tannins (15).

Hypoxis hemerocallidea. Fisch. & C. A. Mey. [*lilabatseka*, African potato] (Hypoxidaceae) corm infusion or concoction is used in the treatment of ulcers. The plant is also used as all purpose remedy for any illness including HIV/AIDS (15). The corm contains glycosides, polyphenols, saponins, steroids and tannins (15). The corm is used for urinary infection and prostate hypertrophy (28). A major constituent of the corm is a pentenyne glycoside hypoxoside (35). The corm also has molluscicidal property (36).

Laggera crispata (Vahl) Hepper & J. R. I. Wood [nsukumbili lomdvuna, African beech] (Asteraceae) stem decoction is used in the treatment of ulcers (11).

Lansea edulis (Sond.) Engl. [umfokolovu, wild grape] (Anacardiaceae) root bark decoction is used for constipation (12). The stem bark and root decoction is also used in the treatment of dysentery. The stem bark contains flavonoids, glycosides, polyphenols, steroids and tannins (15).

Melia azedarach L. [umsilinga, Persian lilac] (Meliaceae) root bark infusion is for anal prolapse and diarrhoea. The root bark contains flavonoids, glycosides, polyphenols, saponins, steroids and tannins (14,15).

Mimusops zeyheri Sond. [umkhamamasi, Transvaal red milkwood] (Sapotaceae) stem bark decoction is used for ulcers and wounds (12). Its fruit is edible (23).

Olea capensis L. subsp. *enervis* (Harv. Ex C. H. Wr.) Verdoorn [sephulo, black ironwood] (Oleaceae) stem bark decoction is used for peptic ulcers (12).

Pittosporum viridiflorum Sims. [mfusamvu, cheesewood] (Crassulaceae) bark powder is used for toothache (12).

Schkuhria pinnata (Lam.) Cabrera [silindzamatala, yellow tumbleweed] (Asteraceae) stem and leaves are used for stomachache (11).

Sclerocarya birrea (A. Rich.) Hochst. subsp. *caffra* [umganu, marula tree] (Sond.) Kokwaro (Anacardiaceae) stem bark decoction is used for diarrhoea. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols, steroids and tannins (15). Its anti-diarrhoeic activity is due to the tannin content (37). The plant is also used for childhood convulsion and epilepsy, and its anticonvulsant activity has been demonstrated in experimental animal model (38).

Spirostachys africana Sond. [umtfolo wesintfu, African sandalwood] (Euphorbiaceae) stem bark decoction is for constipation (12).

Strychnos henningsii Gilg [umnono, red bitterberry] (Strychnaceae) stem bark is for stomachache (11).

Syzygium guineense (Willd.) DC. [umcozi, woodland waterberry] (Myrtaceae) stem bark decoction are used in the treatment of diarrhoea (12).

Talinum caffrum (Thunb.) Eckl. & Zeyh. [umphunyuka, ox bush] (Portulacaceae) leaf infusion is used for stomachache. The leaf contains alkaloids, polyphenols, saponins and tannins (15).

Teucrium riparium Hochst. [umnunu] (Lamiaceae) root bark infusion is used in the treatment of diarrhoea. The root bark contains alkaloids, flavonoids, polyphenols and tannins (15). The plant is also used for snakebites (28).

Vernonia oligocephala (DC.) Sch. Bip. Ex Walp. [lihlunguhlungu, wiletee] (Asteraceae) root bark decoction is used for diarrhoea. The root bark contains flavonoids, glycosides, polyphenols, saponins and steroids (15). The plant is also used for diabetes and stomachache (28). The plant has weak antiplasmodial activity (39).

Xysmalobium undulatum (L.) Ait. F. [lishogwe, milk bush] (Asclepiadaceae) tuber infusion is used for diarrhoea and headaches. The tuber contains flavonoids, glycosides, polyphenols, saponins and steroids (15). The steroids contained in the tuber are cardenolides (40).

Diseases of the Ear

Priva meyeri Jaub. & Spach [*sanama*] (Verbenaceae) leaves are used for otitis media and earache with sores in children (11).

Sansevieria hyacinthoides (L.) Druce [*indlebe yembongolo*, mother-in-law's tongue] (Dracaenaceae) leaf juice and root decoction are used for otitis media (14,11).

Senecio oxyriifolius DC. [*mzimbomubi*, false nasturtium] (Asteraceae) bulb decoction is used for ear infection with discharging pus (26).

Diseases of the female genital system

Acalypha villicaulis Hoschst. [*vuma lobovu*, brooms and brushes] (Euphorbiaceae) root decoction is used for infertility in women (11). The root concoction of *Carissa bispinosa* (L.) Desf. Ex Brenan [*umvusankunzi*, num-num shrub] (Apocynaceae), powdered roots of *Bauhinia galpinii* N. E. Br. [*lusololo*, pride of the Kaap] (Fabaceae) and *Cryptolepis oblongifolia* (Meisn.) Schltr. [*luphondvongoti*, red-stemmed cryptolepis] (Asclepiadaceae), powdered stem bark of *Heteropyxis canescens* Oliv. [*inkunzi*, bastard lavender tree] (Heteropyxidaceae), root decoction of *Ximenia caffra* Sond. [*umthunduluka*, sour plum] (Olacaceae) and root concoctions of *Heteromorpha trifoliata* (Wendl.) Eckl. & Zeyh. [*libangalala lemashangane*, stinkbos] (Apiaceae) are also used for impotence in men (11,12,26).

Albizia adianthifolia (Schumach.) W. F. Wight. [*ilnhlangushiyane*, flat crown] (Fabaceae) aqueous concoction of the roots is used in the treatment of uterine problems. The bark infusion is used for skin problems. The bark contains flavonoids, glycosides, polyphenols, saponins, steroids and tannins (14,15).

Cardiospermum halicacabum L. [*likhambilemamba*, balloon vine] (Sapindaceae) decoction of the root bark is taken for menorrhagia. The stem is for venereal diseases and gonorrhoea. The root bark contains flavonoids, glycosides, polyphenols, saponins and steroids (15).

Cephalantus natalensis Oliv. [*umfomfo*, tree strawberry] (Rubiaceae) root bark decoction is used for infertility in women and to increase libido. The root bark contains alkaloids, cardenolides, glycosides, polyphenols and tannins (18). The fresh berry is eaten for malaria fever (28). The root bark contains alkaloids, cardenolides, glycosides, polyphenols and tannins (18).

Cyperus fastigiatus Rottb. [*insikane*, sedge] (Cyperaceae) root bark decoction is used for excessive uterine bleeding and the leaf infusion is for lower abdominal pains (26).

Grewia caffra Meisn. [*liklolo*, climbing raisin] (Tiliaceae) root bark is used in parturition as oxytocic (18). The root bark decoction is used for urino-genital problems. The root bark contains anthranoids, glycosides and saponins (15).

Gunnera perpensa L. [*gobho*, river pumpkin] (Gunneraceae) root bark decoction is used for excessive uterine bleeding (26).

Indigofera sanguinea N. E. Br. [*cubhujeye*, true indigo] (Papilionaceae) leaves and roots are used to treat threatened abortion (12).

Maytenus senegalensis (Lam.) Excell. [*sibhubhu*, red spike thorn] (Celastraceae) stem bark decoction is taken by women for infertility. The stem bark contains flavonoids, polyphenols, steroids and tannins (15). Leaves of the plant are also used for treating nausea (11). The plant also has antidiabetic and antimalarial properties (41,42).

Oxygonum dregeanum Meisn. [*tinkhobe tagogo*, starstalk] (Polygonaceae) decoction of the bulb is used for infertility in women (26).

Parinari capensis Harv. [*umkhuna/umvalandlebe*, jacket plum] (Rosaceae) root decoction is used as tonic during pregnancy (26).

Peltophorum africanum Sond. [*sikhabamkhombo*, African wattle] (Fabaceae) stem bark decoction is used for menorrhagia and obstructed labour. The stem bark contains alkaloids, flavonoids, glycosides and steroids (11,15).

Rhoicissus tridentata (L. f.) Wild & Drum. [*sinwati*, bushman's grape] (Vitaceae) root bark decoction is for menorrhagia and infertility in women. The root bark contains glycosides, polyphenols, saponins and tannins (15,26).

Rubia cordifolia L. [*intilalubombo*, sticky-leaved rubia] (Rubiaceae) stem or root bark powder is used for uterine problems (12).

Scilla natalensis Planch. [*lukhovu*, slangkop] (Hyacinthaceae) bulb decoction is used for medical curethage and waist or back pain (18,26). The bulb contains alkaloids, flavonoids, polyphenols, saponins and terpenoids (18). Homoiso flavanones, 5,7-dihydroxy-6-methoxy-3-(4-hydroxybenzyl)chroman-4-one and 5,7-dihydroxy-3-(3-hydroxy-4-methoxybenzyl)chroman-4-one have also been isolated from the bulb (43).

Ximenia americana L. [*umfundvuluka*, blue sourplum] (Olacaceae) stem and root bark decoction is used for threatened abortion. The root bark is also used for eye problems (12).

Diseases of the Genito-Urinary System

Acrotome hispida Benth. [*sisefo*, white cat's paws] (Lamiaceae) leaf infusion is used in the treatment of gonorrhoea. The leaf contains alkaloids, flavonoids, polyphenols and steroids (15,26).

Ageratum conyzoides L. [*matfodlwana*, blue weed] (Asteraceae) leaf decoction is used for urinary tract infection and infertility in women (11). The leaves have antibacterial properties and are used for wound healing (44,45). The leaves contain terpenoids and alkaloids (46,47).

Aster bakerianus Burt Davy ex C. A. Sm. [*ludlutjana*, Michaelmas daisy] (Asteraceae) root decoction is used for internal sores in the lower abdomen (11).

Berkheya setifera DC [*lulwimi iwenkhomo*, buffalo tongue] (Asteraceae) root bark decoction is used in the treatment of infertility in women and for stomachache. The root bark contains cardenolides, polyphenols and terpenoids (18,23). The root bark and leaf are also used in the treatment of toothaches (12).

Canthium gueinzii Sond. [*sinmati*, climbing turkey-berry] (Rubiaceae) root bark is used for dymenorrhoea, discharge and burning on micturition (18).

Cissus quadrangularis L. [*mhlahlampetfo*, climbing cactus] (Vitaceae) stem is used for gonorrhoea. It is also used for arthritis and stomach ulcers. The plant contains ascorbic acid, carotenoids and triterpenoids (11,48). The plants' anti-

ulcer properties are thought to be partly due to its antioxidant effects (48). The plant also exhibits antibacterial property (49) which may be the scientific basis of its use for treating gonorrhoea in herbal medicine in Swaziland.

Cyathea dregei Kunze [*licimamlilo*, grassland tree fern] (Cyatheaaceae) bark decoction is used for chancroid. The bark contains cardenolides, glycosides, polyphenols and terpenoids (18).

Diospyros lycioides Desf. [*mvuthuza*, Eastern bluebush] (Ebenaceae) leaf decoction is for pubic lice (26).

Eriosema angustifolium Burtt Davy. [*umvusankunzi/libangalala*] (Papilionaceae) root bark concoction is for increasing libido and penile erection. The root contains alkaloids, polyphenols, saponins and tannins (18).

Faurea galpinii Phillips [*sicalaba*, bush beechwood] (Proteaceae) stem bark decoction is used for sores in the genitalia and candidiasis (18,24). The bark contains flavonoids, polyphenols and saponins (18).

Grewia caffra Meisn. [*liklolo*, climbing raisin] (Tiliaceae) root bark infusion is used for urino-genital problems. The root bark contains anthranoids, glycosides and saponins (15).

Grewia haxamita Burret [*umsiphane*, giant raisin] (Tiliaceae) root bark infusion is used for urino-genital problems. The root bark contains alkaloids, glycosides, polyphenols, steroids and tannins (15).

Pterocarpus angolensis DC. [*umvangati*, wild teak] (Papilionaceae) stem bark decoction is used for gonorrhoea. The stem bark contains glycosides, polyphenols, saponins and tannins (50).

Scilla nervosa [*ndwendwendwe*, wild squill] (Burch.) Jessop. (Hyacinthaceae) bulb decoction is used for lower abdominal pains in women (26). The bulb contains alkaloids, cardenolides, flavonoids, polyphenols, saponins, tannins and terpenoids (18). The bulb is also mixed with the roots of *Crossandra fruticulosa* Lindau [*likhambilebantfwana*, shade crossandra] (Acanthaceae) and then together used in treating peptic ulcers in children (12).

Vernonia oligocephala (DC) Sch. Bip. Ex Walp. [*lihlunguhlungu*, bicolored-leaved vernonia] (Asteraceae) root decoction is used for reducing abdominal pains in women (26).

Diseases of the Musculo-Skeletal System

Agapanthus caulescens Speng. [*hlakahla*, African lily] (Liliaceae) bulb concoction is used for bone fractures (11). The bulb is also used as an emetic (23).

Catunaregam spinosa (Thunb.) Tirvengadam [*mazuba*, thorny bone-apple] (Rubiaceae) root is used for bone fractures (11).

Clutia monticola S. Moore [*mbumbumbu*, branching clutia] (Euphorbiaceae) root powder is for sores and wound healing (26).

Dichrostachys cinerea (L.) Wight & Arn. [*lusekwane*, large-leaved sickle bush] (Fabaceae) root powder is used for bone fractures (11).

Ochna arborea Burch. ex DC. var. *arborea* [*mahlanganisa*, Cape plane tree] (Ochnaceae) stem bark decoction is used for bone fractures. The stem bark contains alkaloids, anthranoids, glycosides, polyphenols and saponins (15).

Ozoroa sphaerocarpa R. & A. Fernands [*imfuce*, bastard currant tree] (Anacardiaceae) and *Athrixia phyllicoides* DC. [*liphephetse*, Zulu tea] (Asteraceae) stem barks are used together for wound healing (12).

Pentanisia angustitifolia Hochst. [*licimamlilo lelibovu*, wild verbena] (Rubiaceae) root decoction is used for sores (26).

Ricinus communis L. [*mhlafutfo/umhlafulfo*, castor oil plant] (Euphorbiaceae) leaves are for arthritis and boils (11).

Solanum incanum L. [*intfuma*, bitter apple] (Solanaceae) root bark infusion is used in the treatment of backaches. The root bark contains flavonoids, glycosides, polyphenols, saponins and steroids (15).

Trichillia emetica Vahl. [*umkhulu*, Natal mahogany] (Meliaceae) stem bark decoction is also used for backache. The stem bark contains alkaloids, flavonoids, glycosides, polyphenols and steroids (12,15). The plant exhibits antimicrobial properties against some bacterial and fungal strains (51). The stem bark of the plant is also used in combination with the stem bark of *Spirostachys africana* Sond. (Euphorbiaceae) in the treatment of constipation (12).

Urginea delagoensis Bak. [*mahlanganisa*, sea squill] (Hyacinthaceae) bulb decoction is used for bone fractures and also as a laxative (11,26).

Diseases of the Respiratory System

Artemisia afra Jacq. ex Willd. [*umhlonyane*, wild wormwood] (Asteraceae) leaf infusion is used for cough and the leaf decoction is for stomachache (26,11). The leaf contains alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids and tannins (15) and volatile terpenes. The essential oil is recognized to have antimicrobial properties (52). The essential oil is responsible for the effectiveness in cough.

Asclepias albens (E. Mey.) Schltr. [*umdzayi*, cartwheels] (Asclepiadaceae) leaf infusion is used for coughs. The leaf contains anthranoids, glycosides, polyphenols, saponins, steroids and tannins (15).

Asclepias fruticosa L. [*lubetjane*, milkweed] (Asclepiadaceae) stem bark decoction is used in the treatment of asthma. The stem bark contains alkaloids, flavonoids, polyphenols, saponins and steroids (15). The aerial parts contain cardenolides (53). The powdered leaf is used as a snuff for the treatment of pulmonary tuberculosis, producing violent and prolonged sneezing in the process (28).

Barleria elegans S. Moore ex C. B. Clarke [*umwungu*, white bushveld barleria] (Acanthaceae) is used for chest pain (11).

Conyza ulmifolia (Burm. F.) Kuntze [*madacaza*, goldenrod] (Asteraceae). Leaf decoction is used for coughs and catarrh (15,28). The leaf contains flavonoids, glycosides, polyphenols, saponins, steroids and tannins (15).

Dichrostachys cinerea (L.) Wight & Arn. [*umzilazembe*, large-leaved sickle bush] (Mimosaceae) root decoction is used for coughs (12).

Dicoma anomala Sond. [*imboziso*, gryshout] (Asteraceae) root decoction is used for chest pain (26). The plant contains volatile oil (28).

Ekebergia capensis Sparrm. [*umnyamatsi*, Cape ash] (Meliaceae) stem bark decoction is used for chest pain. The stem bark contains flavonoids, glycosides,

polyphenols, saponins and tannins (15). Limonoids and triterpenoids have also been isolated from the stem bark of the plant (54,55).

Lantana camara Linn. [*bukhwebeletane*, lantana] (Verbenaceae) leaf infusion is used for coughs and also against malaria, where it reduces the fever. The leaf contains anthranoids, flavonoids, polyphenols, saponins and steroids (14,15). A glycoside of pentacyclic triterpenoid oleanenoic acid isolated from the plant has molluscicidal property (56). The essential oil from its flowers and leaves contain mainly sesquiterpenes (57).

Opuntia imbricata (Haw.) DC. [*umdodlofiya*, prickly pear] (Cataceae) root bark infusion is used for chest pain. The root bark contains anthranoids, flavonoids, glycosides, saponins and steroids (15).

Syzigium cordatum Hochst. [*umncozi*, waterberry] (Myrtaceae) stem bark infusion is used for cough and chest tightness (26). A cold infusion of the leaf is used for stomach troubles and diarrhea (28).

Vangueria infausta [*umntulu*, wild medlar] (Mimosaceae) root bark decoction is used for chest pain. The root bark contains alkaloids, anthranoids, flavonoids, glycosides, polyphenols, saponins and steroids (15). A biflavonoid with radical scavenging activity and flavonoids with antimicrobial properties have been isolated from the aerial part of the plant (58).

Skin and Subcutaneous Tissue

Albizzia adiantifolia (Schumach.) W. F. Wight [*inhlangushiyane*, flat crown] (Fabaceae) stem bark infusion is used for skin problems. The stem bark contains flavonoids, glycosides, polyphenols, saponins, steroids and tannins (15). The saponin triterpenoids are biologically active (59).

Aloe marlothii A. Berger [*inhlaba lenkhulu*, flat flowered aloe] (Asphodelaceae) leaf juice is used for sores (11).

Gymnosporia heterophylla (Eckl. & Zeyh.) Loes. [*sihlangu*, angular-stemmed spike thorn] (Celastraceae) root decoction is used for skin cracks (11).

Ledebouria ovatifolia (Bak.) Jessop [*umhlabelo*, Cooper's squill] (Hyacinthaceae) bulb decoction is used for abscess (15). The plant is also used as pain relief during the treatment of bone fracture and as a post operative analgesic (60). The bulb contains alkaloids, flavonoids, glycosides, polyphenols and saponins (15). The spasmolytic and analgic activities have been demonstrated in rats (60). The bulb contains bufadienolides and exhibits strong antibacterial activity against Gram-positive bacteria (61).

Rhus pentheri Zahlbr. [*inhlangushiyane*, common crowberry] (Anacardiaceae) stem bark decoction is used for skin cracks (11).

Tragia sonderi Prain [*imbabatane*, African dogwood] (Euphorbiaceae) root decoction is used for itchy skin (11).

Urginea sanguinea Schinz. [*gibizisila*, giftulp] (Hyacinthaceae) fresh bulb is used for scabies. The bulb contains flavonoids, glycosides and polyphenols (15). The plant is used for asthma but it contains cardiac glycosides and is cytotoxic in chick embryos (62). The plant also contains bufadienolides (63). The bulbs of *Bowiea volubilis* Harv. Ex Hook. F. [*gibizisila*, climbing potato] (Hyacinthaceae) and *Boophane disticha* (L.f.) Herb. [*siphaluka*, Cape poison

bulb] (Amaryllidaceae) are also used for making concoction used for scabies (12).

Eye Diseases

Abrus precatorius L. [*umphitsi*, lucky bean] (Fabaceae) seed is used in the treatment of cataracts (11).

Dombeya pulchra N. E. Br. [*libuka*, silver dombeya](Sterculiaceae) leaf or stem concoction is used for glaucoma (14).

Headache

Margaritaria discoidea (Baill.) Webster [*madlozini*, Pheasant berry] (Euphorbiaceae) root bark decoction is used for headache. The root bark contains flavonoids, glycosides, polyphenols and tannins (15). The plant also has strong anti-*Candida* activity (64).

Morella pilulifera (Rendle) Killick [*umlulama/umbhemiso*, broad-leaved wax berry] (Myrtaceae) root powder is used for headache (11). The root bark decoction is also used for headache. The root bark contains alkaloids, flavonoids, polyphenols and tannins (15).

Ptaeroxylon obliquum (Thunb.) Radlk [*umtsatse*, sneezewood] (Ptaeroxylaceae) stem is used for headache (11).

Stylochiton natalense Schott [*umfanakamacetjane*, bushveld arum] (Araceae) root bark is also used in the treatment of headache (12).

Kidney Diseases

Albizia versicolor Welw. Ex Oliv. [*sivangatani*, largeleaved false thorn] (Fabaceae) stem bark decoction is the prime one in Swaziland used in the treatment of kidney problems (11).

Nervous System and Mental Disorders

Cheilanthes calomendos Swartz [*mphasetje*, lip fern] (Pteridaceae) root powder and concoction are used in the treatment of epilepsy (12).

Cheilanthes multifida Sw. [*umgciko*, lip fern] (Pteridaceae) leaf decoction is used for dizziness (11).

Dioscorea dregeana (Kunth) T. Durand & Schinz [*ndiyaza*, wild yam] (Dioscoreaceae) bulb decoction is used for psychosis (11).

Lippia javanica Spreng. [*umsutane*, fever tree] (Verbenaceae) leaves are used for psychotropic behaviour. The leaf contains alkaloids, polyphenols, saponins and tannins (18) and essential oils (28,65). The root decoction is also used for kidney problems (12). Its essential oil is antibacterial and

antiplasmodic. The essential oil contains 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one and is (65). The root also has strong antiplasmodial property (73). Its aerial parts in combination with the stem bark of *Combretum molle* R. Br. Ex G. Don [*imbhondvo/lemhlophe*, velvet bush willow] (Combretaceae) are used in the treatment of asthma (12).

Lonchocarpus capassa Rolfe [*sihomuhomu*, apple-leaf tree] (Papilionaceae) bark decoction is used to reduce hallucinations (12).

Pellaea calomelanos (Swartz) Link [*umphasetje*, hard fern] (Adiantaceae) leaves are used for dizziness and fainting spells (18).

Prunus persica (L.) Batsch. [*lipentjisi*, peach] (Rosaceae) bulb and leaf decoction is used for psychosis (11).

Zanthoxylum capense (Thunb.) Harv. [*umnungwane*, small knobwood] (Rutaceae) is used to treat dizziness (12).

Parasitic Diseases

Acanthospermum australe Kuntze. [*sanama*, star bur] (Asteraceae). A leaf and stem decoction is used in the treatment of pubic lice. The stem contains alkaloids, flavonoids, glycosides, polyphenols, steroids and tannins (11,15).

Andrachne ovalis (E. Mey ex Sond.) Mull. Arg. [*mahlombohlanya*, false lightning bush] (Euphobiaceae), *Annona senegalensis* Pers. [*untelemba*, wild custard apple] (Annonaceae) and *Spathodea campanulata* P. Beauvais [*oruru*, African tulip tree] (Bignoniaceae) each exhibit antibacterial activity, and are used individually for treating boils, sores, wounds and ulcers (66). The methanolic extract of the stem bark of *Annona senegalensis*, and the leaves of *Andrachne ovalis* and *Spathodea campanulata* each exhibit a strong antibacterial activity, likely due to the phenolic content of the extracts (66).

Anthospermum rigidum Eckl. & Zeyh. [*sambulela*] (Rubiaceae) leaf infusion is also used for pubic lice. The leaf contains flavonoids, polyphenols and tannins (15). Studies have also shown that apart from the antibacterial properties of these plants, they also have additional medicinal values. The root bark of *Andrachne ovalis* shows insecticidal activity and is a remedy for snake bites and epilepsy (16,28). The alcoholic extract of the leaves is strongly molluscicidal and can be used in the vector control of bilharzia (67). The leaf decoction is used for persistent dizziness (11). The stem bark contains alkaloids, anthranoids, cardenolides and polyphenols. The root bark contains alkaloids, cardenolides, polyphenols and saponins (50).

Spathodea campanulata decoction of the leaves and stem bark is used for treating malaria. The leaf and stem bark extracts each demonstrated antiplasmodium activity in the early infection but less effective once infection is established (68,69,70). The antimalarial active principles in the stem bark are 3 β -20-hydroxyurs-12-en-28-oic acid and two of its derivatives; 3 β -hydroxyurs-12,19-dien-28-oic acid (tomentosolic acid) and 3 β ,20 β -dihydroxyurs-12-en-28-oic acid. The active principles each had significant blood schizontocidal actions in both the early and established infections (71). The stem bark also contains β -sitosterol (72). Other uses of *Spathodea campanulata* in traditional Swazi medicine which has been documented include the use of the bark, leaves and

flowers for dressing ulcers, skin diseases and wounds. A decoction of the stem bark is also taken to treat constipation and gastro-intestinal troubles. A cold infusion of the leaves is used for urethral inflammation (73,74).

Annona senegalensis stem bark is used for the treatment of hysteria and a constituent of its root bark has been found effective for treating cancer using sarcoma 180 ascites cells (75). The bark and root are used in the treatment of sexually transmitted diseases (18). The root bark contains cardenolides, glycosides and saponins. The stem bark contains anthranoids, cardenolides, glycosides saponins and terpenoids (50,18). Its fruits are edible and are taken for diarrhoea, dysentery and vomiting (23,33).

Snakebite

Strychnos madagascariensis Spreng. ex. Baker. [umkhwakhwa, blak monkey orange] (Loganiaceae) leaf decoction is the only Swazi plant documented that is used for snakebite. The leaf contains alkaloids, flavonoids, glycosides, polyphenols, saponins and steroids (15). The fruit is edible (23). The stem bark is for stomachache (11).

Conclusions

Herbal medical practice in Swaziland provides recipes for various types of diseases. The diseases highlighted in this review are mostly examples of the ones documented in the ongoing ethnomedical surveys of our country. One can conclude from the examples that the indigenous knowledge of the herbalists is very vast. The Herbal Medical Practitioners are knowledgeable and highly skilled in the art of identifying plants and in preparing remedies with materials from the local biodiversity for various ailments. Research and technological exploitation of the indigenous knowledge of the herbalists on plant resources could be useful in bio-prospecting for new drugs and in the primary health delivery system of the country especially because most of the indigenous plants have not been explored. Although the practice of herbal medicine is not based on scientific principles and some of the practices cannot be explained in current scientific terms, the biological and chemical screenings of some of the medicinal plants used have shown that there is some scientific basis for the use of plants in therapy. An example is the use of plants with antibacterial properties for dressing wounds (66). Another example is the use of medicinal plants for the treatment of diarrhoea. Many studies have validated the use of some of the plants. They have shown that the flavonoid and tannin content of the plants are thought to be responsible for anti-diarrhoeal activity of the plants by increasing colonic water and electrolyte reabsorption (76). Research on medicinal plants should explode the myths surrounding herbal medicine by identifying the active principles in the plants (12). Ethnobotanical information has been found very useful in the past for the development of potent drugs which are in use in orthodox medical practice (77,78).

Herbalists and other indigenous peoples who are sources of this ethnomedical knowledge that is exploited should be acknowledged and adequately compensated. They should be accorded the intellectual property rights (IPRs) over the ethnobotanical information exploited. This will afford them the freedom to use the IPRs as trade-able commodities to enhance their socio-economic status. Major strategies available for the indigenous communities to receive such IPRs include intellectual property systems; developing a sui generis system of intellectual property protection; entering bilateral contractual arrangements; or creating a new system combining various elements of each of these (79). The ability to use traditional knowledge in a modern scientific manner can also foster and promote the judicious use of Swaziland's natural resources and encourage the conservation and sustainable use of the countries genetic resources as a vehicle to not only further improve the health and welfare of Swaziland and other countries but also to economically improve those in Swaziland involved in the collection and trade of these genetic resources.

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Chapter 4

Baobab (*Adansonia digitata* L.): A Review of Traditional Uses, Phytochemistry and Pharmacology

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Baobab (*Adansonia digitata* L., Malvaceae) is a multi-purpose tree species native to Africa. Its fruit pulp has very high vitamin C content (~ ten times that of orange), and can be used in seasoning, as an appetizer and to make juices. Seeds contain appreciable quantities of crude protein, digestible carbohydrates and oil, whereas they have high levels of lysine, thiamine, Ca and Fe. They can be eaten fresh or dried, ground into flour and thus added to soups and stews. Processing eliminates a number of anti-nutritional factors present in the seed. Baobab leaves are superior in nutritional quality to fruit pulp, and contain significant levels of vitamin A. The leaves are a staple for many populations in Africa, and are eaten fresh or dried. Several plant parts have interesting anti-oxidant and anti-inflammatory properties, and baobab has been used extensively since ancient times in traditional medicine.

Baobab or *Adansonia digitata* L. belongs to the Malvaceae family (1) and is a deciduous tree native to arid Central Africa (2). Its distribution area is large and this species can be found in most of Sub-Sahara Africa's semi-arid and sub-humid regions as well as in western Madagascar (29). It has been introduced to areas outside Africa and grown successfully (11). Baobab is a very long-lived tree with multipurpose uses. The different plant parts are widely used as foods, medicines and the bark fibers are also used (11). The tree provides food, shelter, clothing and medicine as well as material for hunting and fishing (Venter and Venter, 1996, cited in (4)). Every part of the baobab tree is reported to be useful (Owen, 1970, cited in (4) and (14)).

Food Uses

Fruits

The baobab fruit pulp is probably the most important foodstuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce for food, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking (11). The pulp has recently become a popular ingredient in ice products in urban areas ((37, Sidibe *et al.*, 1998b, cited in (4)). The pulp is never cooked as the hot drinks are being prepared, rather it is added at the end of the preparation process after the drinks are allowed to cool (9). Fruit pulp is important in local diets as a seasoning component and appetizer (Burkill, 1985, cited in (25)). When the pulp is soaked in water, it produces a milky solution, which can be consumed as a milk substitute (Burkill, 1985, cited in (25)). The baobab fruit pods are also good for burning and a potash-rich vegetable salt may be obtained from this ash for making soap (Burkill, 1985, cited in (25)).

Seeds

Baobab seeds can be eaten fresh, or they may be dried and ground into a flour which can either be added to soups and stews as a thickener, or roasted and ground into a paste, or boiled for a long time, fermented and then dried for use ((11), FAO, 1988, cited in (32)).

Leaves

The leaves of the baobab tree are a staple for many populations in Africa, especially the central region of the continent ((2) cited in (4)). During the rainy season when the baobab leaves are tender, people harvest the leaves fresh. During the last month of the rainy season, leaves are harvested in great abundance and are dried for domestic use and for marketing during the dry season. The leaves are typically sun-dried and either stored as whole leaved or pounded and sieved into a fine powder (Sidibe *et al.*, 1998b, cited in (4)).

Young leaves are widely used, cooked as spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice (11).

Phytochemistry

A variety of chemicals have been isolated and characterised from *A. digitata*. They belong to the classes of terpenoids, flavonoids, sterols, vitamins, amino acids, carbohydrates and lipids (8).

Fruits

The baobab fruit is composed of an outer shell (epicarp) (45%), fruit pulp (15%) and seeds (40%) (8). The woody epicarp or pod contains the internal fruit pulp (endocarp) which is split in small floury, dehydrated and powdery slides that enclose multiple seeds and filaments, the red fibres, that subdivide the pulp in segments (Nour *et al.*, 1980, cited in (20)) (Figure 1).



Figure 1. Baobab Fruit (SOURCE: Emmy De Caluwé, Benin, 2004)

Fruit Pulp

The baobab fruit pulp is dry, acidulous and mealy, and rich in mucilage, pectins, tartarate and free tartaric acids. The presence of the tartarate gives rise to the name 'cream of tartar tree' ((11), (3) cited in (25)). Pulp sweetness is provided by fructose, saccharose and glucose contents. Fruit pulp is also acidic and this is due to the presence of organic acids including citric, tartaric, malic, succinic as well as ascorbic acid (Airan and Desai, 1954, cited in (11)).

When eaten raw, the pulp is a rich source of calcium and vitamins B and C (Burkill, 1985, cited in (25)). It contains sugars but no starch, and is rich in pectins. The fruit pulp has a very high vitamin C content, almost ten times that of oranges. However, the vitamin C content of the bulk fruit pulp reportedly varies from 1623 mg/kg in one tree to 4991 mg/kg in another ((9), Sidibe *et al.*, 1998a, cited in (4)).

Chemical Composition

The pulp is acidic, due to the presence of the organic acids citric, tartaric, malic, succinic and ascorbic, with pH 3.3 (33). The latter source also shows that the pulp is rich in pectin (average 56.2%). The pectin is mainly water soluble and has a low degree of esterification and a low intrinsic viscosity. This suggests that it will probably not yield a high quality jelly of high solids content, because it tends to precipitate rapidly in acid media to form irregular gels. It is of lower quality than commercial apple pectin and citrus waste pectin (Mubarak *et al.*, 1977, cited in (33)).

The fruit pulp contains a high amount of carbohydrate, low protein, and extremely low fat (35). Analysis of ripe fruit points to an average of 8.7% moisture with 2.7% protein, 0.2% fat, 73.7% carbohydrate, 8.9% fibers and 5.8% ash (Arnold *et al.*, 1985, cited in (11)). Proximate composition of the fruit pulp of baobab differs according to different literature sources (8, 12, 18, 31, 33, 34, 35) (Table 1). Green (1932, cited in (33)) reported the absence of starch in the pulp, which was confirmed by (33).

Table I. Proximate Composition of Baobab Fruit Pulp

Constituent	A	B	C	D	E	F	G
Moisture	17.9-33.8	-	-	4.7	6.7 ± 0.03	6.21 ± 0.02	10.4 ± 0.4
Dry matter	-	89.45	86.8	-	-	-	-
Protein	1.44-2.15	2.19	3.1	2.5	2.6 ± 0.3 ^a	10.9 ± 0.30	3.2 ± 0.1
Fat	0.06-0.69	0.37	4.3	0.7	0.2 ± 0.01	4.28 ± 0.13	0.3 ± 0.0
Ash	3.23-3.38	5.71	5.0	5.1	5.3 ± 0.02	1.98 ± 0.64	4.5 ± 0.2
Crude fiber	-	11.15	8.3	45.1	5.7 ± 0.02	6.21 ± 0.41	5.4 ± 0.3
Carbohydrate	-	70.03	79.4	35.6 ^b	23.2 ± 0.2 ^c	45.21 ± 0.17	76.2 ± 1.0
Metabolizable energy (kcal/100g)	-	-	355.8	203	-	182.8	320.3 ± 4.4

NOTE: A, D: units are g/100 g; B, C, E, F, G: units are percent (%); ^a: %N x 6.25; ^b: simple sugars; ^c: sugars

SOURCE: A: (8), B: (12), C: (18), D: (31), E: (33), F: (34), G: (35)

Amino Acid Profile

Protein accounts for about one-fifth of dry matter in baobab fruit pulp (17%) (38), thus can be considered a rich source of amino acids (Table II) compared to the WHO 'ideal' standards (38) (Table III). The baobab fruit pulp is particularly high in valine, tryptophan and phenylalanine + tyrosine. Comparing to baobab leaves, baobab fruit pulp is inferior in terms of overall protein quality (38).

Table II. Amino Acid Composition of Baobab Fruit Pulp

<i>Amino Acid</i>		<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Crude protein (Total protein)		27	-	-	170
Aspartic acid	ASP	2.96	-	6.4	15.9
Glutamic acid	GLU	3.94	4.02	6.5	38.8
Serine	SER	1.18	-	3.2	8.8
Glycine	GLY	1.21	-	2.9	7.0
Histidine	HIS	0.42	2.71	1.2	3.80
Arginine	ARG	2.28	6.04	7.6	19.3
Threonine	THR	0.65	2.96	2.8	5.7
Alanine	ALA	2.21	-	3.3	7.2
Proline	PRO	2.35	0.92	2.2	7.5
Tyrosine	TYR	1.06	4.21	20.6	4.7
Valine	VAL	1.62	0.43	4.8	9.1
Methionine	MET	0.14	4.92	0.2	1.6
Isoleucine	ILE	1.37	10.73	2.2	6.5
Leucine	LEU	2.06	8.41	4.3	10.9
Phenylalanine	PHE	1.09	4.11	4.4	8.4
Lysine	LYS	1.63	14.62	1.7	8.7
Cysteine	CYS	1.37	11.23	1.0	3.6
Tryptophan	TRP	0.18	1.49	-	2.8

NOTE: A, D: units are mg/g dry weight; B: units are g/100 g; C: units are mg/100 g protein

SOURCE: A: (7), B: (34), C: (35), D: (38)

Table III. Essential Amino Acid Content of Baobab Fruit Pulp Compared to WHO 'Ideal' Standards

<i>Amino Acid</i>	<i>Baobab Fruit/Ideal x 100%</i>
Threonine	83
Valine	106
Methionine + cystine	86
Isoleucine	95
Leucine	91
Phenylalanine + tyrosine	128
Lysine	93
Tryptophan	170

SOURCE: (38)

Fatty Acid Profile

For fatty acids, (7) recorded total lipid content of 155 mg/g dry weight, and stated that significant linoleic acid is present. (38) also points baobab fruit out as a rich source of linoleic acid, 27 mg/g dry weight, whereas it contains a very low amount of α -linolenic acid (<1 mg/g) (Table IV). The two fatty acids which are essential for human nutrition are linoleic acid (18:2n-6) and α -linolenic acid (18:3n-3) (38).

Table IV. Fatty Acid Content of Baobab Fruit

<i>Fatty Acid</i>	<i>A</i>	<i>B</i>
Total fatty acid content	-	84.1
C8:0	-	ND
C10:0	-	ND
C12:0	-	ND
C14:0 Myristic	Tr	0.18
C16:0 Palmitic	0.15	27.0
C16:1 Palmitoleic	ND	ND
C18:0 Stearic	Tr	3.30
C18:1n-9 Oleic	ND	25.0
C18:2n-6 Linoleic	0.023	27.0
C18:3n-3 α -Linolenic	0.15	0.86
C20:0 Arachidic	ND	0.69
C20:1 Gadoleic	-	0.042
Total lipid content	155	127

NOTE: A, B: units are mg/g dry weight; ND: not detected; Tr: trace

SOURCE: A: (7), B: (38)

Mineral Composition

Several reports have studied the mineral composition of baobab fruit pulp (7, 12, 33, 35, 38) (Table V). Baobab fruit pulp contains very little iron (29.9 µg/g) and is a relatively poor source of manganese (< 15 µg/g), but contains appreciable amounts of calcium ranging from 2000 to 9000 µg/g dry weight (35, 38). The high calcium contents of the fruit pulp make baobab fruits attractive as a natural source of calcium supplementation for pregnant and lactating women, as well as for children and the elderly (35).

Table V. Mineral Content of Baobab Fruit Pulp

Minerals	A	B	C	D	E	F	G	H
Al	-	-	-	-	-	-	-	-
Ba	-	-	-	-	-	-	-	-
Ca	3410	211	1156	-	655	0.06	295	2160
Cu	ND	0.55	-	4.3	-	7.14 10 ⁻³	1.6	11.5
Cr	-	-	-	-	-	-	-	<5.0
Fe	17	4.23	58	12	8.6	3.47	9.3	29.2
K	-	-	28,364	-	-	2.31	1240	7260
Mg	2090	123	2090	781	-	0.10	90	3000
Mn	ND	0.39	-	5.9	-	2.7 10 ⁻³	-	9.8
Mo	ND	-	-	-	-	-	-	<5.0
Na	54.6	-	188	-	-	1.86	27.9	7.9
Ni	-	-	-	-	-	-	-	<5.0
P	733	49.79	450	-	50.8	3.18 10 ⁻³	-	4520
Se	-	-	-	-	-	-	-	<5.0
Zn	10.4	0.47	-	4.7	-	0.64	1.8	31.8

NOTE: A, C, D, H: units are µg/g dry weight; B, E, F, G: units are mg/100 g

SOURCE: A: (7), B: (12), C: (18), D: (30), E: (33), F: (34), G: (35), H: (38)

Vitamins

Baobab fruit pulp has among the highest vitamin C or ascorbic acid content found in any fruit. Umoh (cited in (32)) reports 373 mg/100 g wet weight, which is more than six times the level of vitamin C in citrus fruits (30-50 mg/100 g wet weight). Besco *et al.* (20) documented an ascorbic acid content in baobab fruit ranging from 150-499 mg/100 g of product. Scheuring *et al.* (37) revealed a remarkable baobab tree-to-tree variability for vitamin C content in the fruit pulp, ranging from 1500-5000 mg/kg and reported that it was quite stable from one year to the next. The exact vitamin C content depends on the individual tree (9). With baobab fruit powder, a drink with a vitamin C content equal to that of orange juice is easily obtained. However, to retain vitamin C in soft drinks it is important not to boil the pulp but rather to add the powder to previously boiled water (9).

Baobab fruit contains detectable levels of α -carotene (0.17 $\mu\text{g/g}$) and lutein (1.53 $\mu\text{g/g}$ dry weight) (38). Toury *et al.*, 1957 and Oliviera, 1974, both cited in (33), point out baobab pulp as a valuable source of thiamine.

Baobab Milk

In some areas the use of baobab milk is very common. The dried pulp is scraped from baobab fruits and made into a solution. The milk is a highly nutritious drink ((5) cited in (4)). Baobab milk can be made from a mixture of baobab and acha flour, or from baobab pulp alone. Acha (*Digitaria exilis*) and baobab (*Adansonia digitata*) grains are cleaned, fermented for 24 to 120 hours, dried and hammer-milled into fine flours. The mixtures can be composed of 70% acha and 30% baobab flours (70:30 protein basis) (5).

Baobab milk contains more protein (1.5%) and minerals (Fe: 17.8 mg; Ca: 134.2 mg) than human milk (protein: 1.3%; Fe: 0.2 mg; Ca: 30 mg) or cow milk (Fe: 0.1 mg; Ca: 1.20 mg) and most leading national commercial infant milk powder formulas e.g. Cerelac (Fe: 10.0 mg). The composite flours contain more nutrients than the baobab or the acha flour alone (5).

Seeds

The vernacular name for *Adansonia digitata*, baobab, means 'fruit with many seeds' (25) (Figure 2). Murray *et al.* (31) reported that baobab seed flour is an important source of energy and protein. The baobab seed contains appreciable quantities of some nutrients like crude protein (28.4%), digestible carbohydrates (24.10%), and oil (29.7%) all expressed on a dry weight basis (Addy and Eka, 1984, cited in (32)). Besides, baobab seeds have high levels of lysine, thiamin, calcium, and iron (FAO, 1988, cited in (32)). Baobab seed can be classified as both protein- and oil-rich. It is also a very rich source of energy and has a relatively low fat value (14).



Figure 2. Baobab Seeds (SOURCE: Emmy De Caluwé, Benin, 2004)

Fermentation of baobab seeds decreases protein and carbohydrate but increases fat levels. Fermentation has varied effects on the mineral concentrations of the baobab seeds (32).

Chemical Composition

Baobab seed kernels have an energy value of 1803 kJ/100 g (Arnold *et al.*, 1985, cited in (11)). Arnold *et al.*, cited in (11), provided data on chemical composition: moisture 8.1%, protein 33.7%, fat 30.6%, carbohydrates 4.8%, fibre 16.9% and ash 5.9%. However, higher levels of carbohydrates have been recorded (Palmer and Pitman, 1972, cited in (11)). According to (35), the seed contains relatively high amounts of protein, crude fat, and crude fibre, and low levels of carbohydrates. The baobab seeds have been subjected to extensive research, a the proximate analysis of the seeds are provided (Table VI).

Table VI. Proximate Composition of Baobab Seeds

Constituent	A	B	C	D	E	F	G	H	I
Moisture	7.4	-	6.38 ± 0.05	7.80	4.78-5.31 ± 0.29	4.8 %	-	6.12 ± 0.14	4.3 ± 0.1
Dry matter	-	8.19	-	-	-	-	-	-	-
Protein	35.1	15.12	26.70 ± 0.53	30.00	12.85-13.75 ± 0.45	36.6	25.45 ± 0.04	21.42 ± 0.34	18.4 ± 0.5
Fat	29.1	11.56	23.50 ± 0.71	29.60	32.69-33.31 ± 0.31	29.3	18.87 ± 0.12	17.51 ± 0.13	12.2 ± 0.1
Ash	8.4	5.76	5.50 ± 0.06	7.90	7.35-7.65 ± 0.15	9.1	7.61 ± 0.09	-	3.8 ± 0.1
Crude fibre	-	49.72	-	-	19.86-20.42 ± 0.28	14.1	-	7.15 ± 0.01	16.2 ± 0.9
Carbohydrate	-	17.84	37.92	24.92	20.66-21.30 ± 0.32	11.2 ^a	48.07 ± 0.06	37.16 ± 0.70	45.1 ± 1.7
Metabolizable energy(kcal/100 g)	-	-	469.98	452.00	-	454	-	182.8	363.8 ± 9.7

NOTE: A, F, G: units are g/100 g dry weight; B, C, D, E, H, I: units are percent (%); ^a: simple sugars

SOURCE: A: (8), B: (12), C: (14), D: FAO analysis cited in (14), E: (25), F: (31), G: (32), H: (34), I: (35)

Amino Acid Profile

The seed contains a relatively high amount of essential amino acids (35) (Table VII). In contrast to other plant seed protein profiles, baobab seed protein contains a high amount of lysine. Because lysine is limited in most cereal plants, it may be possible to use baobab seed protein to improve cereal protein quality, especially in weaning food mixtures (35). The high protein solubility at acidic and alkaline pH suggests that the baobab seed protein could be an adequate food ingredient (35).

Table VII. Amino Acid Composition of Baobab Seeds

<i>Amino Acid</i>	<i>A</i>	<i>B</i>	<i>C</i>
Crude protein (Total protein) *	196	-	-
Aspartic acid ASP	21.1	-	10.3
Glutamic acid GLU	48.9	2.10	23.7
Serine SER	11.4	-	6.1
Glycine GLY	10.4	-	8.6
Histidine HIS	5.05	1.43	2.2
Arginine ARG	2.21	8.62	8.0
Threonine THR	6.98	1.64	3.8
Alanine ALA	10.6	-	7.1
Proline PRO	9.55	0.62	6.9
Tyrosine TYR	5.59	3.62	1.5
Valine VAL	11.6	0.76	5.9
Methionine MET	2.29	5.94	1.0
Isoleucine ILE	8.27	7.10	3.6
Leucine LEU	14.0	7.48	7.0
Phenylalanine PHE	10.3	5.18	4.0
Lysine LYS	11.2	17.36	5.0
Cysteine CYS	3.6	12.63	1.5
Tryptophan TRP	2.81	2.64	-

NOTE: A, B, C: units are mg/g dry weight

SOURCE: A: (7), B: (34), C: (35)

Table VIII provides a list of the essential amino acid content of baobab fruit pulp compared to WHO's 'ideal' standards (7). Baobab seeds are rated "good" in that they scored as well or better than the WHO standard protein in 5 of 8 categories.

Table VIII. Essential Amino Acid Content of Baobab Seeds Compared to WHO's 'Ideal' Standards

<i>Amino Acid</i>	<i>A</i>	<i>B</i>
Threonine	90	102.5
Valine	118	120
Methionine + cystine	85.7	51.4
Isoleucine	105	92.5
Leucine	101.4	108.6
Phenylalanine + tyrosine	135	23.3
Lysine	100	120
Tryptophan	140	140

NOTE: A, B: units are baobab leaves/ideal x 100%

SOURCE: A: (7), B: Addy and Eteshola, 1984, cited in (11)

Fatty Acid Profile

The oil of baobab seeds contained high proportions of linoleic and oleic acid as well as palmitic and α -linolenic acid (7, 13) (Table IX). According to Osman (35), baobab seed oil is an excellent source of mono- and polyunsaturated fatty acids. Of the total fatty acids 73.11% is unsaturated while 26.89% is saturated. The more that polyunsaturated fatty acids play an important role in modulating human metabolism, the high linoleic acid content then is of nutritive significance. The ability of some unsaturated vegetable oils to reduce serum cholesterol level may focus attention on the seed oil of baobab (25). This high content of mono- and polyunsaturated fatty acids suggests that baobab seed oil would be useful as a food oil (35).

Table IX. Fatty Acid Content of Baobab Seeds (Seed Oil)

<i>Fatty Acid</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>
Total fatty acid content	-	-	67.79	-	-	-
C8:0	-	-	-	-	-	-
C10:0	-	-	-	-	-	-
C12:0 Lauric	-	0-0.3	-	-	0.34	-
C14:0 Myristic	Tr	0.3-1.5	0.19	-	1.46	0.2
C16:0 Palmitic	1.43	25-46	15.50	4.43	2.22	24.2
C16:1n-7 Palmitoleic	0.018	0.3-1.7	0.20	-	1.65	-
C18:0 Stearic	0.16	0.4	3.12	3.98	-	4.6
C18:1n-9 Oleic	2.14	21-59	24.69	26.07	58.71	35.8
C18:2n-6 Linoleic	1.38	12-29	19.11	39.42	23.25	30.7
C18:3n-3 α -Linolenic	1.016	0-8	1.58	-	8.17	1.0
C20:0 Arachidic	Tr	0.5-1	0.74	2.26	-	1.3
C20:1 Gadoleic	-	0-3.6	0.19	4.01	3.64	0.9
Total lipid content	90	-	-	-	-	-

NOTE: A: units are mg/g dry weight; B, D, E, F: units are percent (%); C: units are g fatty acid/100 g oil; Tr: trace

SOURCE: A: (7), B: Baobab Fruit Company, 2002, cited in (11), C: (13), D: (25), E: (34), F: (35)

Mineral Composition

The most important minerals in baobab seeds are calcium and magnesium (Table X). At the same time, the seeds are a poor source of iron, zinc and copper (7, 12, 35).

Table X. Mineral Content of Baobab Seeds

<i>Minerals</i>		<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Al	Aluminium	-	-	-	-	-
Ba	Barium	-	-	-	-	-
Ca	Calcium	3950	264	4.34 ± 1.00	0.53	410
Cu	Copper	ND	1.19	0.27 ± 0;03	5.36 x 10 ⁻³	2.6
Cr	Chromium	-	-	-	-	-
Fe	Iron	18.3	4.35	6.70 ± 0.11	1.89 x 10 ⁻⁴	6.4
K	Potassium	-	-	173.64 ± 15.20	1.74	910
Mg	Magnesium	3520	278	11.04 ± 2.50	0.20	270
Mn	Manganese	10.6	1.01	1.52 ± 0.01	2.84 x 10 ⁻³	-
Mo	Molybdenum	ND	-	-	-	-
Na	Sodium	19.4	-	1.71 ± 0.05	1.64	28.3
Ni	Nickel	-	-	-	-	-
P	Phosphorus	6140	678	-	1.49 x 10 ⁻³	-
Se	Selenium	-	-	-	-	-
Zn	Zinc	25.7	4.29	1.03 ± 0.01	0.91	5.2

NOTE: A: units are µg/g dry weight; B, D, E: units are mg/100 g; C: units are ppm; ND: not detected

SOURCE: A: (7), B: (12), C: (25), D: (34), E: (35)

Anti-nutritional Factors

The acceptability and optimal utilization of baobab seed as a protein source is limited by the presence of antinutrients such as protease inhibitors, tannins, phytic acid and amylase inhibitors. While processing techniques may 'rob' a food item of some nutrients, processing systems may also enhance food nutritional quality by reducing or destroying the antinutrients present. Some of the commonly used processing techniques include soaking in water, boiling in alkaline or acidic solutions. A decrease in tannin contents of baobab seeds was observed after a cold-water, hot-water and hot-alkali treatment. This can be explained by several possibilities: tannins are water soluble, tannin molecules are degraded when heated, heating may cause the formation of water-soluble complexes with other macromolecules of the seeds or reaction between tannic acid and the base. Dehulling and cold-water treatment of seeds greatly reduced the activity of amylase inhibitors (14).

Sun drying, roasting and fermentation are traditional processing techniques that were tested by Obizoba and Amaechi (15) to improve the chemical composition of both baobab pulp and seed. They showed that fermentation of

the seeds for 6 days offered advantages over roasting as shown by crude protein, moisture and minerals contents. A 6-day fermentation appeared to be the most promising method for producing nutritious food from baobab seed (15).

Nnam and Obiakor (32) reported that fermentation decreased the tannin levels in baobab seed. Tannins are known to reduce the availability of proteins, carbohydrates, and minerals by forming indigestible complexes with nutrients. The reduced tannin level due to fermentation could improve the availability of nutrients in the seed.

When comparing the different processing methods such as boiling in water, acid or alkali or fermentation, the alkali treatment exhibited the best results (24). Alkali treatment appeared to be the most effective method for reducing trypsin inhibitor and tannin contents, and had the additional advantage of improving protein digestibility. However, such alkali treatments may also cause the production of harmful compounds (De Groot and Slump, 1969, cited in (24)).

Leaves

Depending on the season, families use either fresh and/or dried leaves, which can serve as a significant protein and mineral source for those populations for whom it is a staple food (11). In general, baobab leaves are nutritionally superior to the fruit of the tree (38).

Chemical Composition

According to Becker, 1983, (2, 6), cited in (11), the leaves contain (expressed on dry weight basis): 13–15% protein, 60–70% carbohydrate, 4–10% fat and around 11% fibre and 16% ash (Table XII). Energy value varies between 1180-1900 kJ/100g of which 80% is metabolizable energy.

Table XI. Proximate Composition of Baobab Leaves

<i>Constituent</i>	<i>A</i>	<i>B</i>
Moisture	-	-
Dry matter	93.6	8.23
Protein	14	10.14
Fat	4.3	6.25
Ash	10.8	15.90
Crude fiber	-	27.49
Carbohydrate	64.6	40.21
Metabolizable energy (kcal/100 g)	353	-

NOTE: A: units are g/100 g; B: units are percent (%)

SOURCE: A: (6), B: (12)

The leaves also contain an important amount of mucilage (Gaiwe *et al.*, 1989, cited in (3)). Ten percent of the dry matter of leaves consists of mucilage (29). Woolfe *et al.*, 1997, cited in (8), found that the mucilage is an acidic polysaccharide with associated proteins and minerals.

Amino Acid Profile

On a qualitative and quantitative basis, baobab leaf appears to be a good source of protein for those populations for whom this plant material is a staple (2). The amino acid profile has been previously reviewed (2, 6, 7, 38). Because Nordeide *et al.* (6) showed results in units (mg of amino acid/g N) that differ from those found by other researchers (2, 7) (mg of amino acid/g dry weight), these data are not compared to each other in this review. Nordeide *et al.* (6) concluded that baobab leaves are potentially protein sources to be used to complement the amino acid profile to improve the overall protein quality of the local diet. Baobab leaf contains 10.6% protein on a dry weight basis and significant amounts of all the common amino acids (Table XII) (2).

Table XII. Amino Acid Composition of Baobab Leaves

<i>Amino Acid</i>	<i>A</i>	<i>B</i>	<i>C</i>
Crude protein (Total protein)	106.3	103	112
Aspartic acid ASP	10.3	12.9	12.4
Glutamic acid GLU	13.4	11.4	14.4
Serine SER	4.7	4.55	4.5
Glycine GLY	6.0	5.57	5.4
Histidine HIS	2.1	2.18	2.4
Arginine ARG	8.5	7.07	8.3
Threonine THR	4.1	3.65	4.3
Alanine ALA	6.9	6.58	6.8
Proline PRO	5.6	6.76	6.4
Tyrosine TYR	4.5	4.15	4.3
Valine VAL	6.3	6.55	7.0
Methionine MET	2.4	1.04	1.5
Isoleucine ILE	6.7	5.46	6.1
Leucine LEU	8.7	8.75	9.8
Phenylalanine PHE	5.7	6.02	6.7
Lysine LYS	6.1	6.11	6.4
Cysteine CYS	2.7	2.11	2.4
Tryptophan TRP	1.6	2.05	3.4

NOTE: A, B, C: units are mg/g dry weight

SOURCE: A: (2), B: (7), C: (38)

To provide a reference point, Yazzie and collaborators (2) compared the amino acid composition of baobab leaf to the 'ideal' protein as defined by the World Health Organization which is based on the amino acid needs of a preschool child. Baobab leaves contain the same amount or more of the following essential amino acids as contained in the 'ideal' protein: lysine (5.7%), arginine (8.0%), threonine (3.9%), valine (5.9%), tyrosine + phenylalanine (9.6 %), tryptophan (1.5%) and methionine + cysteine (4.8%) (2). A calculation of the 'chemical' score of protein quality using tryptophan, the most limiting of the essential amino acids, shows that the leaves contain significant amounts of all the essential amino acids (2). In terms of protein content and WHO standards, leaves of baobab can be rated "good" in that they score well for 5 of the 8 essential amino acids (Table XIII). For each of the eight essential amino acid categories baobab leaves score close to or above the 100% mark.

Table XIII. Essential Amino Acid Content of Baobab Leaves Compared to the WHO 'Ideal' Standards

<i>Amino Acid</i>	<i>A</i>	<i>B</i>	<i>C</i>
Threonine	93	87.5	95
Valine	124	128	124
Methionine + cystine	139	85.7	100
Isoleucine	130	132.5	138
Leucine	136	121.4	124
Phenylalanine + tyrosine	162	165	163
Lysine	105	107.2	104
Tryptophan	310	200	300

NOTE: A, B, C: units are baobab leaves/ideal x 100%

SOURCE: A: (2), B: (7), C: (38)

The fact that the leaves contain significant amounts of tryptophan has at least two implications. First, for those people for whom baobab leaves are a staple, this food source may provide significant amounts of tryptophan. Second, since a part of the niacin requirement in humans can be satisfied by the conversion of tryptophan to niacin (Satyanaryana and Fao, 1983, cited in (2)), baobab leaf may also serve as a niacin source.

Fatty Acid Profile

Total lipid content of baobab leaves was reported to be 55 mg/g of dry weight, though the fatty acid composition revealed that the leaves did not provide significant sources of linoleic acid (7) (Table XIV). This observation was also confirmed by Sena *et al.* (38).

Table XIV. Fatty Acid Content of Baobab Leaves

<i>Fatty Acid</i>	<i>A</i>	<i>B</i>
Total fatty acid content	-	9.9
C8:0	-	0.014
C10:0	-	ND
C12:0	-	0.09
C14:0 Myristic	Tr	0.37
C16:0 Palmitic	0.24	3.2
C16:1 Palmitoleic	0.011	0.21
C18:0 Stearic	0.035	0.35
C18:1 Oleic	0.058	0.39
C18:2 Linoleic	0.10	1.0
C18:3 Linolenic	0.081	4.1
C20:0 Arachidic	Tr	0.15
C20:1 Gadoleic	-	ND
Total lipid content	55	153

NOTE: A, B: units are mg/g dry weight; ND: not detected; Tr: trace

SOURCE: A: (7), B: (38)

Mineral Composition

Baobab leaves are also significant sources of minerals (2, 6, 7, 11, 12, 19, 21, 30, 38) as shown in Table XV. Some studies (7, 12, 21) reported that baobab leaves have a higher content of iron compared to numerous other wild-gathered foods, and are a rich source of calcium. Comparisons between published data on the minerals iron, calcium, zinc and phosphorous show wide variations in content (11). The plant state of maturation, genetic variances, and environmental factors are all possible explanations for the reported discrepancies ((6), cited in (19)). Moreover, mineral composition in food may vary greatly depending on where the food was grown (19), on seasonal variations (2), and on the used analytical method. (2) highlight baobab leaves as a substantial source of calcium, iron, potassium, magnesium, manganese, molybdenum, phosphorus, and zinc. Since iron-deficiency anaemia is common in regions of Africa where baobabs grow, the leaves may represent an important source of iron (2, 11).

Table XV. Mineral Content of Baobab Leaves

<i>Minerals</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>	<i>H</i>
Ca	26,400						-	
	3070	1810	20,000	2526	2240 ± 196	1824.6 ± 184.8	-	14,700
	3150						-	
Fe	1000						278 ^a	
	321	41	155	93.10	-	30.6 ± 3.4	212 ^b	986
	2540						117 ^c	
K	10,800						-	
	1400	-	-	-	-	-	-	5830
	3210						-	
Mg	3120						936 ^a	
	4360	-	5490	246	-	400.6 ± 118.6	1217 ^b	4230
	5350						2742 ^c	
Mn	43.8						98.4 ^a	
	79.5	-	31.0	6.82	-	6.6 ± 0.1	18.5 ^b	87.6
	89.3						20.1 ^c	
Mo	9.1						-	
	19.8	-	ND	-	-	-	-	5.6
	17.6						-	
P	1480						-	
	2880	-	3020	115	-	875.6 ± 108.6	-	1530
	1200						-	

Table XV. Mineral Content of Baobab Leaves (Continued)

Minerals	A	B	C	D	E	F	G	H
Zn	40.3	2.5	18.7	1.24	-	22.4 ± 2.2	19.7 ^a	17.5
	35.1						7.4 ^b	
	35.4						12.1 ^c	
Cu	-	-	11.6	1.02	-	1.6 ± 0.1	5.3 ^a	<5.0
	-	-					2.9 ^b	
	-	-					4.9 ^c	
Na	-	-	1630	-	-	-	-	38.2
	-	-					-	
Al	1230	-	-	-	-	-	-	-
	228	-	-	-	-	-	-	-
	2870	-	-	-	-	-	-	-
Ba	187	-	-	-	-	-	-	-
	182	-	-	-	-	-	-	-
	454	-	-	-	-	-	-	-
La	12.1	-	-	-	-	-	-	-
	15.8	-	-	-	-	-	-	-
	17.3	-	-	-	-	-	-	-

NOTE: A, C, G, H: units are µg/g dry weight; B, D, E, F: units are mg/100 g dry weight; ND: not detected; ^a: dark leaf; ^b: fine light leaf; ^c: rough light leaf

SOURCE: A: (2), B: (6), C: (7), D: (12), E: (19), F: (21), G: (30), H: (38)

Vitamins

Baobab leaves contain an interesting level of vitamin A (6, 9). Vitamin A in the human diet is usually estimated in terms of Retinol Equivalent: $1\ \mu\text{g RE} = 6\ \mu\text{g } \beta\text{-carotene}$ or $12\ \mu\text{g } \alpha\text{-carotene}$ (37). The amount of carotenoids in baobab leaves depends on the tree and the method of leaf drying. The simple practice of drying leaves in the shade doubles the provitamin A content of the leaf powder (37). The choice of small leaves, which is tree-specific, further increased provitamin A by 20% (9, 37). Higher levels of pro-vitamin A can be retained in dried leaves by ensuring the leaves are dried in the shade and not in full sun. β -carotene is also very sensitive to sunlight (9, 37). To maintain the highest vitamin content, storage of baobab leaves as whole leaves rather than ground leaf powder has been recommended (11). The use of leaves harvested from old or young trees does not seem to have any effect on the level of pro-vitamin A (37). One report (11) illustrated how different kind of leaves and drying techniques yield different carotenoid levels. (38) shows also high content of lutein ($50.9\ \mu\text{g/g}$ dry weight).

Bark

Baobab bark is mainly used for medicinal properties. Secondly, the bark is well-known for its fibers used to make ropes, sacks, clothes, baskets and mats. (11). The alkaloid 'adansonin' in the bark is thought to be the active principle for treatment of malaria and other fevers (11). Baobab bark which is often given to infants to promote weight gain was found to be high in fat, calcium, copper, iron, and zinc (12).

Friedelin, lupeol and baurenol (all three terpenoids) were identified in the leaf bark of baobab. In addition, betulinic acid was isolated from the bark whereas the leaf exclusively yielded taraxerone and acetate of lupeol and baurenol (Odetokur, 1996, cited in (8)).

Biological Activity

Anti-oxidant Activity

Dietary antioxidants, including polyphenolic compounds, vitamins E and C, and carotenoids, are believed to be effective nutrients in the prevention of oxidative stress related diseases (Kaur and Kapoor, 2001, cited in (20)), such as inflammation, cardiovascular disease, cancer and aging related disorders (Willet, 2001, cited in (20)). The high antioxidant capacity of products deriving from *Adansonia digitata* show their therapeutical, nutraceutical and cosmoceutical potential. Moreover, in view of the very high antioxidant capacity, some authors (20, 23) have proposed the red fiber as a new value-added ingredient for food preparation and/or nutraceutical application in the promotion of health. Research studies have (20, 23) compared the overall antioxidant capacity (IAC), corresponding to the sum of the corresponding water- and lipid-soluble antioxidants capacity, of baobab plant products with those of orange and kiwi. The IAC value for the examined products resulted as follows: baobab red fiber (1617.3) >>> baobab fruit pulp (240.5) >> baobab fresh leaves (89) > baobab seeds (51.4) > orange fresh fruit pulp (24.3) > kiwi fruit pulp (2.4).

Vitamin C Healing Effect

Vitamin C is a powerful antioxidant and extremely important in human nutrition. Vitamin C has been shown to be related to low blood pressure, enhanced immunity against many tropical maladies, lower incidence of cataract development and lower incidence of coronary disease. The daily recommended intake for healthy, non-smoking adults is 65 mg; smokers need more vitamin C than non-smokers. While 65 mg/day is the minimum recommended intake, a full saturation of the total pool of vitamin C in the body is about 140 mg/day. Convalescents recovering from infectious diseases or nursing mothers benefit significantly from daily intakes exceeding 250 mg. Using the average vitamin C content of baobab fruit, 2800 mg/kg, the recommendations can be converted into amounts of baobab powder. The daily recommended dose of vitamin C can be obtained from 23 g of baobab powder. The daily saturation of the vitamin C pool in the body requires 50 g of baobab powder; the special dosage for convalescents is 90 g (9).

Anti-viral Activity

Adansonia digitata root-bark and leaf methanol extracts have shown high antiviral activity (against *Herpes simplex*, Sindbis and Polio), together with viricidal (direct inactivation of virus particles) and also intracellular antiviral activity, which could indicate the presence of multiple antiviral compounds, or a single compound with multiple actions (17, 27). Whether such studies will show

as effective results in humans is unknown but these couple of preliminary reports may provide rationale behind some of the medicinal uses of this plant.

Anti-inflammatory and Anti-pyretic Activity

According to Ramadan and coworkers (36), aqueous extract of the baobab fruit pulp produces a marked anti-inflammation activity. The effect was comparable to that induced by standard phenylbutazone (15 mg/kg). This anti-inflammatory effect may be due to the presence of sterols, saponins and triterpenes in the aqueous extract. The extract also shows a marked antipyretic activity (36). The antipyretic activity of the extract resembles that normally induced by standard dose of administered acetylsalicylic acid (ASA) in hyperthermic rats. Leaves are applied locally for a variety of inflammatory conditions, insect bites and guinea worm sores (8). Anti-pyretic activity has been reported (36).

Anti-microbial Activity

There was some antibiotic activity against *Staphylococcus aureus*, *Streptococcus jecalis*, *Bacillus subtilis*, *Escherichia coli* and *Mycobacterium phlei* (28).

Anti-trypanosoma Activity

Extracts of baobab root are trypanocidal to either *Trypanosoma brucei brucei* and *T. congolense*. *T. brucei brucei* and *T. congolense* are unicellular parasites transmitted by the bites of tsetse fly and is the causative agent of sleeping sickness in humans and related diseases in animals (27).

Uses in Traditional Medicine

Baobab leaves, bark, pulp and seeds are used as food and for multiple medicinal purposes in many parts of Africa ((29), Etkin and Foss, 1982, cited in (2)). Ethnomedicine has been an intensive area of research, with several authors discussing the main ethnomedicinal uses of baobab products (Table XVI).

Fruit

Baobab is used in folk medicine as an antipyretic or febrifuge to overcome fevers. Both leaves and fruit pulp are used for this purpose. Fruit pulp and powdered seeds are used in cases of dysentery and to promote perspiration (*i.e.* a diaphoretic) (11). Baobab fruit pulp has traditionally been used as an

immunostimulant (El-Rawy *et al.*, 1997, cited in (26)), anti-inflammatory, analgesic ((36) cited in (26)), pesticide (Tuani *et al.*, 1994, cited in (26)), antipyretic, febrifuge, and astringent in the treatment of diarrhoea and dysentery ((36) cited in (26)). The fruit pulp has been evaluated as a substitute for improved western drugs (le Grand, 1985, cited in (26)). The aqueous extract of baobab fruit pulp exhibited significant hepatoprotective activity and, as a consequence, consumption of the pulp may play an important part in human resistance to liver damage in areas where baobab is consumed (26). Medicinally, baobab fruit pulp is used as a febrifuge and as an anti-dysenteric, and in the treatment of smallpox and measles as an eye instillation (Burkill, ined., cited in (10)). In Indian medicine, baobab pulp is used internally with buttermilk in cases of diarrhea and dysentery. Externally, use is made of young baobab leaves, crushed into a poultice, for painful swellings (11).

Leaves

Powdered leaves are used as an anti-asthmatic and known to have antihistamine and anti-tension properties. The leaves are also used to treat a wide variety of conditions including fatigue, as a tonic and for insect bites, Guinea worm and internal pains, and dysentery (11); and diseases of the urinary tract, ophthalmia and otitis (11). In Indian medicine, powdered leaves are similarly used to check excessive perspiration (11). Baobab leaves are used medicinally as a diaphoretic, an expectorant, and as a prophylactic against fever, to check excessive perspiration, and as an astringent (Watt and Breyer-Brandwijk, 1962; Carr, 1959; Woodruff, 1969; and Beltrame, 1974, cited in (10)). The leaves also have hyposensitive and antihistamine properties. Leaves are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, inflammations, insect bites and guinea worm (Burkill, ined., cited in (10)).

Seeds

Seeds are also used in cases of diarrhea, and hiccough. Oil extracted from seeds is used for inflamed gums and to ease diseased teeth (11). Since seed oil is used to also treat skin complaints, it can be considered to have cosmetic applications as well (11).

Bark

The widest use in tradition medicine comes from the baobab bark as a substitute for quinine in case of fever or as a prophylactic. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present. This process can be prevented by adding alcohol or a small quantity of sulphuric acid to the decoction (Kings, 2002, cited in (11)). Baobab bark is used in Europe as a febrifuge (antipyretic). In the Gold Coast (Ghana), the bark is used instead of quinine for curing fever (8). In Indian medicine, baobab bark is used internally

as a refrigerant, antipyretic and antiperiodic. It is used as a decoction, 30 g/l of water, boiled down to two thirds (11). The activity of baobab bark as a febrifuge, however, has not been detected in experimental malaria treatments, although it is both diaphoretic and antiperiodic (Burkill, ined., cited in (10)). The bark, however, is certainly used for the treatment of fever in Nigeria (Oliver, 1959 cited in (10)). Moreover, the bark contains a white, semi-fluid gum that can be obtained obtainable from bark wounds and is used for cleansing sores (Burkill, ined., cited in (10)). According to Loustalot and Paga (1949), cited in (10), there are no alkaloids present in the bark, and accounts from Nigeria are inconclusive (Burkill, ined., cited in (10)). However, according to Watt and Breyer-Brandwijk (1962), cited in (10), the bark contains the alkaloid 'adansonin', which has a strophanthus-like action. In East Africa, the bark is used as an antidote to strophanthus poisoning. In Congo Brazzaville, a bark decoction is used to bathe rickety children and in Tanzania as a mouthwash for toothache (Burkill, ined., cited in (10)). Furthermore, a new flavanonol glycoside was reported in the root bark (Chauhan *et al.*, 1984, cited in (36)).

Baobab bark, fruit pulp and seeds appear to contain an antidote to poisoning by a number of *Strophanthus* species. The juice of these species has been widely used as an arrow poison especially in East Africa. In Malawi, a baobab extract is poured onto the wound of an animal killed in this way to neutralize the poison before the meat is eaten (Wickens, 1982, cited in (11, 10)).

An infusion of roots is used in Zimbabwe to bathe babies to promote smooth skin (Wickens, 1982, cited in (11)).

Table XVI. Ethnomedical Uses of Baobab

<i>Continent/ Country¹</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
Africa	leaves	diaphoretic, expectorant, prophylactic against fever, excessive perspiration, astringent, treatment kidney and bladder diseases, asthma, general fatigue, diarrhoea, inflammations, insect bites, Guinea worm, hyposensitive, antihistamine properties, sources with poultices	(3, 10)
India		emollient, maturant, diuretic, diaphoretic, febrifuge	Kritikar, 1993, cited in (8)
-		anti-inflammatory, insect bites, Guinea worm sores	(8)
-		astringent, sudorific, tonic	(8)
-		earache, ophthalmia	(8)
-		prevent Guinea pigs from asthmatic crisis induced by histamine aerosols	(8)
-		anti-asthmatic, antihistaminic, antitension properties, treatment of fatigue, insect bites, Guinea worm, internal pains, treatment of dysentery	(11)
-		diseases of the urinary tract, ophthalmia, otitis	(11)
India		excessive perspiration	(11)
India		painful swellings	(11)
-	leaves, flowers	respiratory disorders	Addy <i>et al.</i> , 1995, cited in (8)
-	leaves, pulp	antipyretic, febrifuge	(11)

¹ When '-' is mentioned, no information about country, nor continent is mentioned in the reference.

Table XVI. Ethnomedical Uses of Baobab (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
West Africa	pulp	fever, dysentery	Burkill, ined., cited in (10) and FAO, 1988, cited in (4)
West Africa		eye-drops in cases of measles	Burkill, ined., cited in (10) and FAO, 1988, cited in (4)
-		excipient in tablet formulation, lubricant, glidant and diluent properties	Arama, <i>et al.</i> , 1989, cited in (8)
-		relief from bronchial asthma	(8)
-		diaphoretic in fever, diarrhoea, dysentery, haemopytosis	(8)
-		against itching in case of allergy	(8)
Konkan (India)		toothache, gingivitis	(8)
India		internally with buttermilk in cases of diarrhoea, dysentery	(11)
-		immunostimulant, anti-inflammatory, analgesic, pesticide, antipyretic, febrifuge, astringent in treatment of diarrhoea and dysentery	El-Rawy <i>et al.</i> , 1997, (36), Tuani <i>et al.</i> , 1994, cited in (26)
-		hepatoprotective activity	(26)

Table XVI. Ethnomedical Uses of Baobab (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
-	pulp, seeds	dysentery, perspiration (i.e. a diaphoretic)	(11)
-	seeds	diarrhoea, hiccough	(11)
-		inflamed gums, easing of sore teeth	(11)
-		skin complaints, cosmetics	(11)
India	inner fibrous part of fruit shell	emmenogogue	Kritikar, 1993, cited in (8)
-	bark, fruit shell	stimulating and promoting granulation of foul sores	Addy <i>et al.</i> , 1995, cited in (8)

Table XVI. Ethnomedical Uses of Baobab (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
Nigeria	bark	febrifuge	(3) cited in (11)
Malawi		neutralizes the arrow wound in flesh of animal killed by poisoned arrow, before meat is eaten	(3, 10)
Congo-Brazzaville		bathe rickety children	(3, 10)
Tanzania		mouthwash for toothache	(3, 10)
-		treatment of sores	FAO, 1988, cited in (4)
Gold Coast		substitute of quinine for curing fever	Kritikar, 1993, cited in (8)
Europe		febrifuge (antipyretics), substitute for cinchona bark	(8)
-		substitute for quinine in cases of fever, prophylactic	(11)
India		internally as a refrigerant, antipyretic, antiperiodic	(11)
Nigeria		increase weight gain of infants	(16)
Zambia	roots	bathe babies to promote smooth skin	(3, 10)
-		tonic for malaria patients	FAO, 1988, cited in (4)

Conclusions

Adansonia digitata L. is a multipurpose tree species widely used for food and medicine.

The baobab fruit pulp is probably the most important foodstuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking. The fruit pulp has a very high vitamin C content and is a rich source of calcium. The acidic pulp is rich in pectin, contains a high amount of carbohydrate, is low in protein, and extremely low in fat. Nevertheless, the fruit pulp can be considered as a rich source of amino acids and linoleic acid. It contains a very low amount of α -linolenic acid and iron.

Baobab seeds can be eaten fresh, or may be dried and ground into a flour which can either be added to soups and stews as a thickener, or roasted and ground into a paste, or boiled for a long time, fermented and then dried for use. The seeds can be classified as both protein- and oil-rich. They contain appreciable quantities of crude protein, digestible carbohydrates and oil, whereas they have high levels of lysine, thiamine, Ca, Mg and Fe. Baobab seeds contain high proportions of linoleic and oleic acid as well as palmitic and α -linolenic acid. Processing eliminates a number of anti-nutritional factors present in the seeds.

The leaves of the baobab tree are a staple for many populations in Africa. Young leaves are widely used, cooked like spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice. Baobab leaves are superior to fruit pulp in nutritional quality, and contain interesting levels of vitamin A. They appear to be a good source of protein, and contain particularly significant amounts of the amino acid tryptophan. Baobab leaves are a significant source of Fe, Ca, K, Mg, Mn, P and Zn.

Baobab bark is mainly used for its medicinal properties and for its fibers. The alkaloid 'adansonin' in the bark is thought to be the active principle for treatment of malaria and other fevers, as a substitute for quinine.

Several plant parts have interesting anti-oxidant, anti-viral and anti-inflammatory properties, and baobab has been used extensively since ancient times in traditional medicine. However, for baobab, the nutritional and medicinal data are widely scattered and research is fragmentary.

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Chapter 5

Tamarind (*Tamarindus indica* L.): A Review of Traditional Uses, Phytochemistry and Pharmacology

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Tamarind (*Tamarindus indica*, Fabaceae), a tropical fruit found in Africa and Asia is highly valued for its pulp. Tamarind fruit pulp has a sweet acidic taste due to a combination of high contents of tartaric acid and reducing sugars. The pulp is used for seasoning, in prepared foods, to flavour confections, curries and sauces, and as a major ingredient in juices and other beverages. Commercial tamarind-based drinks are available from many countries. Vitamin B content is quite high; carotene and vitamin C contents are low. Presence of tannins and other dyeing matters in the seed testa make the whole seed unsuitable for consumption, but they become edible after soaking and boiling in water. Tamarind kernel powder is an important sizing material in textile, paper and jute industries. Seeds are gaining importance as an alternative source of proteins, and are besides rich in some essential minerals. Seed pectin can form gels over a wide pH range. Leaves and flowers can be eaten as vegetables, and are prepared in a variety of dishes. They are used to make curries, salads, stews and soups. Tamarind leaves are a fair source of vitamin C and β -carotene; mineral content is high, particularly P, K, Ca and Mg. Anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity has been documented from several plant parts. Tamarind is also extensively used in traditional medicine. The traditional uses, its phytochemistry and pharmacognosy is reviewed to

provided with a particular orientation to its value in sub-Saharan Africa.

Tamarind or *Tamarindus indica* L. of the Fabaceae, subfamily Caesalpinioideae, is an important food in the tropics. It is a multipurpose tree of which almost every part finds at least some use (17), either nutritional or medicinal. Tamarind is indigenous to tropical Africa but it has been introduced and naturalized worldwide in over 50 countries. The major production areas are in the Asian countries India and Thailand, but also in Bangladesh, Sri Lanka, Thailand and Indonesia. In America, Mexico and Costa Rica are the biggest producers. Africa on the whole does not produce tamarind on a commercial scale, though it is widely used by the local people. Minor producing countries in Africa are Senegal, Gambia, Kenya, Tanzania and Zambia (3, 17).

Food Uses

Fruits

Tamarind fruit pulp is used for seasoning, as a food component, to flavour confections, curries and sauces, and is a main component in juices and certain beverages. Tamarind fruit pulp is often made into a juice, infusion or brine (3). The drinks are popular in many countries around the world, though there are many different recipes. In some African countries, the juice obtained from the fruit pulp is mixed with wood ash to neutralize the sour taste of the tartaric acid. However, the most common method is to add sugar to make a pleasantly acid drink. In Ghana, the pulp is mixed with sugar and honey to make a sweet drink. Most of the producing countries manufacture drinks commercially. Sometimes pulp is fermented into an alcoholic beverage (FAO, 1988, cited in (3)).

Seeds

Tamarind seed is a by-product of the tamarind pulp industry. The presence of tannins and other dyeing matter in the testa make the whole seed unsuitable for direct consumption (Rao and Srivastava, 1974, cited in (17)). However, the seeds become edible after soaking and boiling in water, which removes the seed coat (3). In the past, and even today, seeds have been wasted (3) even though they could be ground to make a palatable livestock feed (NAS, 1979, cited in (3)).

The major industrial product of tamarind seed is the tamarind kernel powder (TKP) which is an important sizing material used in the textile, paper, and jute industries (17). Tamarind seed is also the raw material used in the manufacture of polysaccharide (jellose), adhesive and tannin. In 1942, two Indian scientists announced that decorticated kernels contained 46-48% of a gel-forming

substance. This polysaccharide (pectin) with carbohydrate character and gelly forming properties, named 'jellose' (Rao, 1948, 1956, cited in (3)), has been recommended for use as a stabiliser in ice cream, mayonnaise and cheese, and as an ingredient or agent in a number of pharmaceutical products (Morton, 1987, cited in (3)). According to Siddhuraju *et al.* (4), tamarind seeds may be adopted as an inexpensive alternative protein source to alleviate protein malnutrition among traditional people living in developing countries. Flour from the seed may be made into cake and bread. Roasted seeds are claimed to be superior to groundnuts in flavour (13).

Leaves and Flowers

Tamarind flowers, leaves and seeds can be eaten as vegetables and are prepared in a variety of dishes (13). They are used to make curries, salads, stews and soups in many countries, especially in times of scarcity (Benthall, 1933, cited in (3)). Before consumption, leaves are sometimes boiled in water and prepared together with tamarind fruits (5).

Phytochemistry

The chemical composition of amino acids, fatty acids, and minerals of tamarind plant parts have been reported. Differences in values found in the literature are likely to be due to differences in genetic strains, stages of maturity at which the plant parts were harvested, growing conditions (2), harvesting and handling techniques as well as to differences in analytical methodologies. Nevertheless, a review of the phytochemistry will provide insight into the relative value that this species provides when used.



Figure 1. Tamarind Fruit (SOURCE: Emmy De Caluwé, Senegal, 2007)

Fruits

The fruit (Figure 1) contains about 55% pulp, 34% seeds, and 11% shell (pod) and fibers (Rao and Srivastava, 1974, cited in (17)). Pods contain 1-10 seeds, which are irregularly shaped, flattened or rhomboid. Seeds are very hard, shiny, reddish, or purplish brown. They are embedded in the pulp, lined with a tough parchment resembling a membrane, and joined to each other with tough fibers (Purseglove, 1987, cited in (17)). There are great differences and variations in fruit size and flavour (17).

Fruit Pulp

Tamarind valued highly for its fruits, especially the pulp which is used for a wide variety of domestic and industrial purposes (Kulkarni *et al.*, 1993, cited in (3)). The pulp constitutes 30-50% of the ripe fruit (Purseglove, 1987 and Shankaracharya, 1998, cited in (3)), the shell and fiber account for 11-30% and the seed about 25-40% (Chapman, 1984 and Shankaracharya, 1998, both cited in (3)).

The most outstanding characteristic of tamarind is its sweet acidic taste, the acid due to mostly tartaric acid. The latter is synthesised in tamarind leaves in the light and translocated to the flowers and fruits (Lewis *et al.*, 1961 and Patnaik, 1974, both cited in (3)). Tartaric is an unusual plant acid formed from the primary carbohydrate products of photosynthesis, and once formed, it is not metabolically used by the plant (3). The content of tartaric acid does not

decrease during fruit ripening, suggesting it is not utilized in fruit development. At this same time of fruit development, reducing sugars increase to 30-40% giving the sour fruit a sweeter taste (3). As a result, tamarind is known to be simultaneously the most acidic and sweetest fruit (Lewi and Neelakantan, 1964a and Coronel, 1991, both cited in (3)). In Thailand, two species of tamarind are found, the so-called sweet and sour tamarind.

Tamarind fruit also contain minerals and exhibit high antioxidant capacity that appear to be associated with a high phenolic content, thus can be an important food source (3).

Chemical Composition

The proximate composition of the tamarind fruit depends on locality (3) (Table I). The pulp has a low water content and a high level of protein, carbohydrates and minerals. However, (9) reported that the fruit pulp is relatively poor in protein (87.9 g/kg) and oil (25.3 g/kg).

Table 1. Proximate Composition of Tamarind Fruit Pulp

<i>Constituent</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>	<i>G</i>
Dry matter	-	-	-	-	-	95.0	-
Water/moisture	20.60	15.00-47.00	22.60	33.89	62.50-69.20	-	189.3
Protein	3.10	14.00-4.40	3.10	3.28	1.40-3.30	8.2	87.9
Fat	0.40	0.90-1.00	0.40	0.50	0.71-0.81	2.4	25.3
Carbohydrate	70.80	62.50	71.80	59.76	21.40-30.85 ^a	80.8	668.7
Fiber	3.00	5.10	3.00	1.79	-	-	22
Ash	2.10	1.50-4.20	2.10	2.57	1.16-1.72	3.6	-
Gross energy kJ (kcal)	-	-	272.00	230.00	-	1780 (426)	-
Metabolizable energy kJ (kcal)	-	-	-	-	-	1580 (377)	-

NOTE: A, B, C, D, E: units are percent (%); F: units are g/100 g; G: units are g/kg; ^a: total sugars

SOURCE: A: Purseglove, 1987, cited in (3), B: FAO, 1998, cited in (3), C: Leung and Flores, 1961, cited in (3), D: Wenkam and Miller, 1965, cited in (3), E: Hasan and Ijas, 1972, cited in (3), F: (5), G: (9)

Amino Acid Profile

As indicated (9), the fruit pulp is relatively poor in protein (87.9 g/kg) though the fruit is rich in several amino acids (Table II). According to Glew and co-workers (2), essential amino acids of tamarind fruit pulp exceed those of the 'ideal' protein standard established by the World Health Organization, except for tryptophan (82%) (Table III). Although tamarind fruit contains potentially useful amounts of protein, they have been shown to be poorly digested and utilized by rats, as compared with proteins in coconut meat that are extensively digested and efficiently utilized by mammals (Grant *et al.*, 1995, cited in (2)).

Table II. Amino Acid Composition of Tamarind Fruit Pulp

<i>Amino Acid</i>		<i>A</i>	<i>B</i>
Crude protein (Total protein)		116.00	50.0
Aspartic acid	ASP	12.00	4.01
Glutamic acid	GLU	16.70	3.95
Serine	SER	6.88	2.19
Glycine	GLY	5.15	1.74
Histidine	HIS	3.37	1.35
Arginine	ARG	8.74	1.95
Threonine	THR	6.05	1.99
Alanine	ALA	6.20	2.03
Proline	PRO	7.61	15.30
Tyrosine	TYR	4.34	1.83
Valine	VAL	6.97	2.21
Methionine	MET	2.84	0.27
Isoleucine	ILE	5.20	2.00
Leucine	LEU	8.89	2.96
Phenylalanine	PHE	4.78	2.76
Lysine	LYS	8.22	1.75
Cysteine	CYS	1.35	1.20
Tryptophan	TRP	1.04	0.38

NOTE: A, B: units are mg/g dry weight

SOURCE: A: (2), B: (6)

Table III. Essential Amino Acid Content of Tamarind Fruit Pulp Compared to WHO's 'Ideal' Standard

<i>Amino Acid</i>	<i>Tamarind Fruit/Ideal x 100%</i>
Threonine	153
Valine	172
Methionine + cystine	132
Isoleucine	160
Leucine	115
Phenylalanine + tyrosine	125
Lysine	123
Tryptophan	82

SOURCE: (2)

Fatty Acid Profile

Tamarind fruit pulp is relatively poor in oil (25.3 g/kg of crude lipid), greenish yellow in color and liquid at room temperature. Saponification values of the oil are high, indicating that it contains a high proportion of low molecular weight fatty acids (9).

With regard to the two essential fatty acids, the fruit pulp contains very little linoleic acid (3.42 mg/g dry weight) and even lower amounts of α -linolenic acid (0.21 mg/g dry weight) (2) (Table IV).

Table IV. Fatty Acid Content of Tamarind Fruit Pulp

<i>Fatty Acid</i>	<i>A</i>	<i>B</i>
C10:0	ND	-
C12:0	0.01	-
C14:0 Myristic	0.01	Tr
C14:1	ND	-
C15:0	0.01	-
C16:0 Palmitic	1.80	0.095
C16:1n-7 Palmitoleic	0.12	ND
C18:0 Stearic	0.70	Tr
C18:1n-9 Oleic	2.29	0.110
C18:1n-7	0.55	-
C18:2n-6 Linoleic	3.42	0.014
C18:3n-6	ND	-
C18:3n-3 α -linolenic	0.21	0.061
C20:0 Arachidic	0.07	ND
C20:1 Gadoleic	0.02	-
C20:2n-6	0.01	-
C20:3n-6	ND	-
C20:4n-6	ND	-
C20:5n-3	ND	-
C22:0	0.03	-
C22:1	0.01	-
C24:0	0.03	-
C24:1	0.20	-
Total lipid content (mg/g dw)	9.4	460

NOTE: A: units are $\mu\text{g/g}$ dry weight; B: units are mg/g dry weight; ND: not detected; Tr: trace

SOURCE: A: (2), B: (6)

Mineral Composition

The fruit pulp is a rich source of several elements (2, 5, 6, 9), including relatively high amounts of copper (9.09 µg/g dry weight), manganese (215 µg/g dry weight) and zinc (13.2 µg/g dry weight). Fruit pulp is also a good source of calcium and phosphorus, but is unfortunately, extraordinarily low in iron (2) (Table V).

Table V. Mineral Content of Tamarind Fruit Pulp

<i>Minerals</i>		<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Al	Aluminium	-	18.4	-	-
Ba	Barium	-	2.0	-	-
Ca	Calcium	240	1899	1830	465.75
Cu	Copper	-	9.09	ND	21.83
Co	Cobalt	-	0.07	-	-
Cr	Chromium	-	2.94	-	-
Fe	Iron	14	31.7	13.9	8.49
K	Potassium	-	6547	-	62.00
Mg	Magnesium	-	1153	1580	72.03
Mn	Manganese	-	215	ND	-
Mo	Molybdenum	-	0.1	39.9	-
Na	Sodium	-	62.1	ND	76.66
Ni	Nickel	-	1.29	-	0.52
P	Phosphorus	-	1220	1000	91.00
Pb	Lead	-	0.1	-	-
Se	Selenium	-	0.03	-	-
St	Strontium	-	2.75	-	-
Ti	Titanium	-	0.154	-	-
Zn	Zinc	2.3	13.2	ND	1.06

NOTE: A, B: units are µg/g dry weight; C, D: units are mg/100 g dry weight; ND: not detected

SOURCE: A: (2), B: (5), C: (6), D: (9)

Vitamins

The ascorbic acid content in tamarind is very low and varies from 2-20 mg/100g (Lefevre, 1971 and Ishola *et al.*, 1990, both cited in (3)). Tamarind fruit pulp was reported to have a high content of vitamin B (thiamine, riboflavin and niacin) as well as a small amounts of carotene and vitamin C (13).

Seeds

Tamarind seed consists of the seed coat or testa (20-30%) and the kernel or endosperm (70-75%) (Coronel, 1991 and Shankaracharya, 1998, both cited in (3)). Tamarind seed is a typical underutilized material (17). Commercially, tamarind seed is a by-product of the commercial utilization of the fruit pulp; and has been reported to have several uses (3, 17). Tamarind seeds can be used for extracting tamarind gum, whereas the seed coats, a by-product of manufacturing tamarind gum, were found as a source of natural antioxidants (18). The seeds are presently gaining importance as an alternative source of proteins (3).

Pectin

As described above, tamarind seed kernels contain polysaccharides, when mixed with water form mucilaginous dispersions and possess the property of forming gels with sugar concentrates, like fruit pectins. However, unlike other fruit pectins, tamarind polysaccharide can form gels over a wide pH range, including neutral and basic conditions. Tamarind polysaccharides, contrary to fruit pectins, are not affected by boiling in neutral aqueous solutions, even if boiled for long periods. Therefore, it can be useful as a gel formation agent and substituted for fruit pectins. Tamarind polysaccharides do not contain galacturonic acid and methyluronate, and is therefore not regarded as a true pectin, being termed as 'jellose' (Rao, 1948, cited in (3)).

Chemical Composition

Tamarind seeds are rich sources of different components (4, 9, 14). The seed and kernels are high in proteins (13-20%), while the seed coat is rich in fiber (20%) and tannins (20%) (3) (Table VI). The high saponification value (221 mg KOH/g oil) of tamarind oil suggests that it could be also good for soap making and in the manufacturing of lather shaving creams (Eka, 1980, Hilditch, 1949, cited in (14)).

Table VI. Proximate Composition of Tamarind Seeds

<i>Constituent</i>	<i>A</i>	<i>B</i>	<i>C</i>
Moisture	8.0 ± 1.14	101.0	10.75 ± 0.53
Crude protein	13.0 ± 1.1 ^a	269.3	24.28 ± 0.50 ^a
Fat/crude lipid	7.1 ± 0.74	109.1	-
Ash	4.2 ± 0.53	20.1	1.50 ± 0.01
Crude fiber	14 ± 1.3	74.0	18.00 ± 1.10
Carbohydrate	61.7 ± 0.63	500.5	38.27 ± 0.41
Oil yield	-	-	7.2 ± 0.45
Metabolizable energy (kJ/100 g)	1520	-	-

NOTE: A, C: units are g/100 g dry matter, B: units are g/kg; ^a: % N x 6.25

SOURCE: A: (4), B: (9), C: (14)

Amino Acid Profile

Tamarind seeds contain 13% crude protein, which is comparable to a previous report (Balogun and Fetuga, 1986, cited in (4)). According to Ishola and collaborators (9), tamarind seeds are a good source of protein (269.3 g/kg). Amino acid profiles of tamarind reveal that the proteins contain fairly balanced essential amino acid levels (Table VII). All essential amino acid levels, except threonine, valine and tryptophan, are higher than the FAO/WHO reference pattern (Table VIII). Tamarind seeds could, therefore, be used as a less expensive source of protein to help alleviate protein malnutrition found so widespread in many developing countries ((4) cited in (3)).

The seeds also contain small amounts of anti-nutritional factors (tannins, phytic acid, hydrogen cyanide, trypsin inhibitor activity and phytohaemagglutination activity) (3).

Table VII. Amino Acid Composition of Tamarind Seed

<i>Amino Acid</i>	<i>A</i>	<i>B</i>
Total protein	7.2 ± 0.15	173.00
Aspartic acid ASP	0.97	18.00
Glutamic acid GLU	1.09	28.20
Serine SER	0.34	9.53
Glycine GLY	0.44	16.70
Histidine HIS	0.23	5.54
Arginine ARG	0.44	16.60
Threonine THR	0.20	5.20
Alanine ALA	0.31	6.52
Proline PRO	-	8.46
Tyrosine TYR	0.22	9.53
Valine VAL	0.45	7.14
Methionine MET	0.13	1.40
Isoleucine ILE	0.33	6.69
Leucine LEU	0.66	10.90
Phenylalanine PHE	0.34	7.11
Lysine LYS	0.48	10.50
Cysteic acid CYS	0.15	3.52
Tryptophan TRP	0.06	1.78

NOTE: A: units are g/100 g dry weight; B: units are mg/g dry weight

SOURCE: A: (4), B: (6)

Table VIII. Essential Amino Acid Content of Tamarind Seeds Compared to WHO's 'Ideal' Standard

<i>Amino Acid</i>	<i>Tamarind Seed/Ideal x 100%</i>
Threonine	75.0
Valine	82.0
Methionine + cystine	140.0
Isoleucine	97.5
Leucine	90.0
Phenylalanine + tyrosine	160.0
Lysine	110.9
Tryptophan	35.0

SOURCE: (6)

Fatty Acid Profile

According to Ishola *et al.* (9), the seed is a good source of fatty acids (109.1 g/kg) (Table IX). Tamarind seeds contained between 1 and 2 mg/g dry weight linoleic acid (6). Tamarind seeds have a higher percentage of unsaturated (55.6%) fatty acids than saturated (44.4%) fatty acids (14). Linoleic acid, present in tamarind seed oil, is undoubtedly one of the most important polyunsaturated acids in human food because of its association in the reduction or prevention of heart vascular diseases (Omode *et al.*, 1995, cited in (14)). Dietary fat rich in linoleic acid, apart from preventing cardiovascular disorders such as coronary heart diseases and atherosclerosis, also is associated with preventing high blood pressure (Vles and Gottenbos, 1989, cited in (14)). The presence of one of the three essential fatty acids in seed oil makes it nutritionally valuable. Palmitic, stearic and linoleic acids are present in high proportions in tamarind oil, which make the latter nutritionally valuable (14).

Tamarind seeds give an amber-colored oil, free of smell and sweet in taste, which resembles linseed oil. It is used for varnishes, paints and as burning lamp oil (Morton, 1987, cited in (3)), but it is also said to be palatable and of culinary quality (Watt, 1893, cited in (3)). Tamarind oil has iodine value below 100 mg/100 g which places it in the non-drying oil group. A report by Ekpa and Ekpe (1995) cited in (14) has shown that, unlike free fatty acid content, which is a measure of free fatty acids present in a fat or oil, acid value is a measure of total acidity of the lipid, involving contributions from all the constituent fatty acids that make up the glyceride molecule. The nutritional value of a fat depends, in some respect, on the amount of free fatty acids. In the tropics, where vegetable oils are the most common dietary lipids, it has been shown that it is desirable that the free fatty acid content of cooking oil lies within 0.0 – 3.0 % (Bassir, 1971, and Onyeike and Acheru, 2002, both cited in (14)). The low percent of free fatty acids in tamarind oil indicates that the oil may be a good edible oil with an extended shelf-life without spoilage via oxidative rancidity (14).

Table IX. Fatty Acid Content of Tamarind Seeds

<i>Fatty Acid</i>	<i>A</i>	<i>B</i>	<i>C</i>
Total fatty acid content (mg/g dw)	-	75.0	-
C14:0 Myristic	-	Tr	-
C16:0 Palmitic	15.20	0.540	27.41
C16:1 Palmitoleic	-	ND	-
C18:0 Stearic	4.19	0.170	13.86
C18:1 Oleic	24.50	1.070	24.13
C18:2 Linoleic	48.30	1.650	24.75
C18:3 Linolenic	2.50	0.014	-
C20:0 Arachidic	-	0.064	2.25
C20:1 Gadoleic	-	-	3.13
C22:0 Behenic	5.2	-	-
Total lipid content (%)	75.0	-	-

NOTE: A, C: units are percent (%); B: units are mg/ g dry weight; ND: not detected; Tr: trace

SOURCE: A: (4), B: (6), C: (14)

Mineral Composition

Tamarind seeds appear to be a good source of different mineral elements (4, 6, 9, 14), such as calcium, phosphorus, magnesium and potassium (3, 20) (Table X). Calcium content of tamarind seeds is quite high compared to that of some of the cultivated pulse crops (Kuzayli *et al.*, 1966, cited in (4)). Of all the minerals studied in (4), K is the element in highest concentration, with the values for the trace mineral copper also relatively high (6).

Table X. Mineral Content of Tamarind Seeds

<i>Minerals</i>	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>
Ca Calcium	172 ± 1.3	1850	786.86	36.6
Cu Copper	0.47 ± 0.04	11.6	18.97	2.1
Fe Iron	6.3 ± 0.50	26.7	ND	45.5
K Potassium	1340 ± 6.8	-	610.00	1308
Mg Magnesium	214 ± 2.0	1960	118.33	104
Mn Manganese	0.68 ± 0.04	ND	-	12.1
Mo Molybdenum	-	13.9	-	-
Na Sodium	21.3 ± 0.51	ND	19.17	8.9
Ni Nickel	-	-	ND	-
P Phosphorus	312 ± 4.2	2280	165.00	-
Zn Zinc	7.1 ± 0.63	26.3	3.00	7.0

NOTE: A, C, D: units are mg/100 g dry weight; B: units are µg/g dry weight; ND: not detected

SOURCE: A: (4), B: (6), C: (9), D: (14)

Anti-nutritional Factors

Tamarind seeds also contain small amounts of anti-nutritional factors such as tannins, phytic acid, hydrogen cyanide, trypsin inhibitor activity and phytohaemagglutination activity, (3). The presence of tannins and other coloring matter in the testa make the whole seeds unsuitable for direct human consumption. Therefore, the testa has to be separated from the kernels by boiling or roasting. Otherwise, side effects such as depression, constipation and gastrointestinal disorders may result (Anon, 1976, cited in (3)). Bhatta *et al.* (2001, cited in (3)), have considered that a natural source of tannin from tamarind seed husks can be used to depress gas production in rumen fermentation, particularly in crossbred dairy cows.

Even though food legumes are important sources of dietary protein in developing countries, their acceptability and utilization has been limited due to the presence of relatively high concentrations of certain antinutritional factors (Nowacki, 1980, cited in (4)). The antinutritional factors of the seeds has been reviewed, (4), showing that contents of total free phenolics and tannins in tamarind seem to be higher than in some of the commonly consumed legumes such as chickpea or cowpea (Khan *et al.*, 1979, and Rao and Deosthale, 1982, both cited in (4)). Since tannins and phenols are water-soluble compounds (Uzogara *et al.*, 1990, cited in (4)), they can be eliminated by decortication, soaking, heat treatment, or cooking (Singh, 1988, cited in (4)). These methods may enhance protein digestibility by reducing the levels of tannins in tamarind seeds.

However, tamarind seeds have low levels of phytic acid comparable that of lima bean (Egbe and Akinyele, 1990, cited in (4)). Phytic acid decreases bioavailability of certain minerals and may interfere with the utilization of proteins due to the formation of phytate-protein and phytate-mineral-protein complexes and also inhibits the digestive enzymes (Reddy *et al.*, 1982, cited in (4)). The phytate could, however, be substantially eliminated by processing methods such as soaking and autoclaving (Reddy *et al.*, 1982, cited in (4)).

Tamarind seeds contain 2.8 mg/100 g cyanogens, which is probably too low to cause any concern since cooking is known to reduce cyanogens content significantly (Sathe and Salunkhe, 1984, cited in (4)).

Trypsin inhibitor activity of tamarind is 26 Trypsin Inhibitor Unit mg⁻¹ and exhibits lower inhibitory activity than that of various edible legumes like *Cicer arietinum*, *Lens exculenta*, *Vigna unguiculata* (Al-Bakir *et al.*, 1982, cited in (4)) or soybean (Kanwar *et al.*, 1991, cited in (4)). In an earlier study with soybeans (Kanwar *et al.*, 1991, cited in (4)), it was reported that cooking eliminates more than 98% trypsin inhibitor activity.

Globulin proteins of tamarind exhibit weak agglutinating activity without any specificity against erythrocytes from human blood groups A, B, and O, respectively, as has been reported earlier for certain pulses like *Bauhinia purpurea*, *B. racemosa*, *B. malabarica* and *Vigna trilobata* (Rajaram and Janardhanan, 1991, Siddhuraju *et al.*, 1992, and Vijayakumari *et al.*, 1993a, all cited in (4)). Nonetheless, albumin proteins of *T. indica* specifically agglutinate erythrocytes from B blood group as in *Mucuna flagellipes* (Mabdiwe and Agogbua, 1978, cited in (4)). Hence, the hemagglutinating activity is very weak.

Lectins bind to specific receptor sites on the surface of epithelial cells in the intestines which leads to a nonspecific interference with the absorption of nutrients (Liener, 1980, cited in (4)). However, the phytohemagglutinating activity can be easily eliminated by dry or thermal treatments (Liener, 1980, cited in (4)). *In vitro* protein digestibility of *T. indica* is 71.6%, which is comparable to the levels reported for *Glycine max* (Gross, 1982, cited in (4)).

Leaves and Flowers

Tamarind flowers, leaves and seeds can be eaten as vegetables and are prepared in a variety of dishes (13). The foliage has a high forage value, though tamarind is rarely harvested for this purpose because it affects fruit yields. Tamarind trees growing in woodlands are often eaten by wild animals, such as elephants or giraffes, for which tamarind is a preferred plant, perhaps because of its high crude protein content (3). In the southern states of India, cooked seeds of tamarind are occasionally fed to draught animals (13).

Both leaves and bark are rich in tannins. Leaves yield a red dye, which is used to give a yellow tint to cloth previously dyed with indigo (13). Tamarind leaves are a fair source of vitamin C and β -carotene and the mineral content is high, particularly potassium, phosphorous, calcium and magnesium (3).

Chemical and Mineral Composition

The chemical composition of the dried leaves (5) (Table XI) shows that the nutritional value is comparable to those of baobab leaves, except amounts of Ca, which in baobab are about five times higher. As reported in (8), leaves and roots of tamarind contain a number of glycosides such as vitexin, isovitexin, orientin and isoorientin.

Table XI. Proximate Composition of Dried Tamarind Leaves

<i>Constituent</i>	
Dry matter (g/100 g)	96.1
Protein (g/100 g)	14.0
Fat (g/100 g)	3.9
Carbohydrate (g/100 g)	72.7
Ash (g/100 g)	5.5
Ca (mg/100 g)	330.0
Fe (mg/100 g)	91.0
Zn (mg/100 g)	2.7
Gross energy kJ (kcal)	1820 (435)
Metabol. Energy kJ (kcal)	1600 (382)

SOURCE: (5)

The amino acid profile of tamarind leaves showed that the leaves of *T. indica* were potentially acceptable protein sources that would complement the amino acid profile and thus improve the protein quality of local diets (5). These same authors (5) also report that tamarind leaves only contained traces of α and β -carotene.

Bark

Tamarind bark and leaves contain tannins. The bark is rich in tannins reaching up to 70%, and as such has found a place for use in the tanning industry. The bark is used for tanning hides and in dyeing (Morton, 1987, cited in (3)). In Zambia, bark tannins are used in the preparation of ink and for fixing dyes (Storss, 1995, cited in (3)). The bark is also burnt to make ink in many other African countries. Tamarind twigs are sometimes used as 'chewsticks' whereas the bark is used as a masticatory, alone or as a substitute of lime in betelnut (3). The bark contains the alkaloid hordenine (8).

Biological Activity

Anti-oxidant Activity

Four antioxidative compounds were isolated and identified from the seed coats: phenolic antioxidants, such as 2-hydroxy-3', 4'-dihydroxyacetophenone, methyl 3,4-dihydroxybenzoate, 3,4-dihydroxyphenyl acetate and epicatechin (15, 18). These antioxidants may be used for increasing shelf life of food products and improving the stability of lipids and lipid-containing foods by preventing loss of sensory and nutritional quality by preventing lipid peroxidation. These compounds may also find a place as food additives though studies are needed to evaluate their effectiveness within food matrices. Extracts exhibit antioxidant potential by reducing lipid peroxidation *in vitro* ((18), Tsuda *et al.*, 1993, cited in (15)). Raw and dry heated tamarind seed coats exhibit good antioxidant activity against the linoleic acid emulsion system and the values were lower and higher than the synthetic antioxidant, butylated-hydroxy-anisole (BHA), and ascorbic acid, respectively (12).

Phenolic plant compounds may have many biologic effects in terms of health promotion. An important protective effect is reduction of oxidative damage, mediated by lipid peroxidation, which in living systems is strongly associated with mutagenesis, carcinogenesis, ageing, and atherosclerosis (Yagi, 1987 and Cultar, 1984, 1992, all cited in (15, 18)). Pumthong (1999), cited in (15), described the antioxidant activity of extracts of tamarind pericarp, and reported the presence of mainly polymeric tannins and oligomeric procyanidins but the latter were not yet identified or quantified.

The antioxidative activity of tamarind seed was also investigated by Osawa *et al.* (1994), cited in (3). They found that ethanol and ethyl acetate extracts prepared from the seed coat exhibited antioxidative activity. This suggests that tamarind seed coats, a by-product of the tamarind gum industry, may have potential as a low cost source of antioxidants (18), but we note that so many plants and plant extracts show antioxidative activity (Ramos *et al.*, 2003, cited in (3)).

Leaf extracts exhibit antioxidant activity in the liver (3). Antioxidant activity of tamarind leaves reported by Perez *et al.* (2003) and Ramos *et al.* (2003), cited in (11), was similar to the antioxidant activity of tamarind flowers observed by Al-Fatimi *et al.* (11). The latter mechanism is probably caused by polyphenolic compounds which have already been isolated from the seeds (Luengthanaphol *et al.*, 2004 and Sudjaroen *et al.*, 2005, both cited in (11)).

Besides anti-oxidant activities, hypolipemic activity was observed from tamarind fruit extract in hypercholesterolemic hamsters (16). Treatment of hypercholesterolemic hamsters with tamarind fruit pulp extract (5%) led to a decrease in the levels of serum total cholesterol (50%), non-high-density lipoprotein cholesterol (73%) and triglyceride (60%), and to an increase of high-density lipoprotein cholesterol levels (61%).

Anti-inflammatory Activity

Proteinaceous compounds with high inhibitory activities against human neutrophil elastase (HNE) were found in tamarind seeds. A serine proteinase inhibitor, providing high activity against HNE was detected, isolated and purified from tamarind seeds (1). Proteinase inhibitors are widely distributed among bacteria, animals and plants. They are present in reproductive and storage organs, and vegetative tissues of most plant families (Ryan, 1990, and Shewry and Lucas, 1997, both cited in (1)). They have regulatory and defensive roles, and act as storage proteins (Xavier-Filho, 1993, cited in (1)). Among the various groups of proteinase inhibitors, serine proteinase inhibitors are the best studied and have been isolated from various leguminous seeds (Oliveira *et al.*, 2002, Macedo *et al.*, 2002, Mello *et al.*, 2002, and Oliva *et al.*, 2000, all cited in (1)).

Abnormal accumulation of elastase, a serine proteinase from human neutrophil, causes a number of acute and chronic inflammation diseases (Bernstein *et al.*, 1994, cited in (1)). There is a demand for specific and potent exogenous inhibitors of proteinases, such as HNE, associated with these inflammatory processes (Sternlicht and Werb, 1999, cited in (1)). The serine proteinase inhibitor from tamarind seeds needs to be studied to determine whether it could have such application. Anti-inflammatory properties of tamarind fruit pulp were reported (7).

Anti-microbial Activity

Tamarind fruits are reported to have anti-fungal and anti-bacterial properties (Ray and Majumdar, 1976, Guerin and Reveillere, 1984, Bibitha *et al.*, 2002, Metwali, 2003, and John *et al.*, 2004, all cited in (3)). According to Al-Fatimi and collaborators (11), in an agar diffusion assay, extracts from *T. indica* flowers showed antibacterial activity against four bacteria tested (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*). Antimicrobial activity of *T. indica* study has been attributed to lupeol (Ali *et al.*, 1998, cited in (11)).

Tamarind leaves possess a strong *in vitro* antibacterial activity against more than 13 (81%) common gram positive and gram negative bacteria that were tested (10). The latter also reports that tamarind leaf extract was very effective against *E. coli*. Not much is known, however, about the antibacterial compounds present in the tamarind leaves (10) nor the specific compounds responsible for such activity. Tamarind plant extracts have been used to purify drinking water in Burkina Faso and Vietnam (Bleach *et al.*, 1991, cited in (3)).

Anti-fungal Activity

Tamarind fruits are reported to have anti-fungal as well as anti-bacterial properties (Ray and Majumdar, 1976, Guerin and Reveillere, 1984, Bibitha *et al.*, 2002, Metwali, 2003, and John *et al.*, 2004, all cited in (3)). Extracts from the fruit appear promising as a potential fungicidal agent against cultures of *Aspergillus niger* and *Candida albicans* (3).

Moluscicidal Activity

Extracts from tamarind fruit pulp have shown molluscicidal activity against *Bulinus truncatus* snails. This is probably due to the presence of saponins in the fruit (Imbabi and Abu-Al-Futuh, 1992a, cited in (3)).

Anti-diabetic Activity

Frequent research on aqueous extracts of seeds has shown a strong anti-diabetic effect in rats (Maitin *et al.*, 2004, cited in (3)).

Cytotoxic Activity

Al-Fatimi *et al.* (11) reported that extracts of *T. indica* methanol showed remarkable cytotoxic activity against FL-cells with IC₅₀ values below 50 µg/ml.

Use in Traditional Medicine

Tamarind is used in herbal medicine in many parts of the world (12) (Table XII). Medicinal uses of tamarind can be found in many cultures and for a wide array of applications (8). The medicinal value of tamarind has been mentioned already in traditional Sanskrit literature (3).

Traditionally, tamarind products, leaves, fruits and seeds have been extensively used in traditional Indian and African medicine (Jayaweera, 1981 and Parrotta, 1990, cited in (3)). A number of recent surveys have listed local folk uses of tamarind as remedies for a number of ailments (Rimbau *et al.*, 1999, Kristensen and Lykke, 2003, Sen and Behera, 2000, Punjani and Kumar, 2002, Patil and Yadav, 2003 and Rajendran *et al.*, 2003, all cited in (3)).

There is medical interest in the use of purified xyloglucan from tamarind in eye surgery for conjunctival cell adhesion and corneal wound healing (Burgalassi *et al.*, 2000 and Ghelardi *et al.*, 2000, cited in (3)). Other medicinal interest relates to the use of tamarind fruit to manage fluoride toxicity (Khandare *et al.*, 2000, cited in (3)).

Fruit

The fruit pods are regarded as a digestive, carminative, laxative, expectorant and blood tonic (Komutarin *et al.*, 2004, cited in (16)). Other parts of the plant present antioxidant (Tsuda *et al.*, 1994, cited in (16)), anti-hepatotoxic (Joyeux *et al.*, 1995, cited in (16)), anti-inflammatory (Rimbau *et al.*, 1999, cited in (16)), anti-mutagenic (Ramos *et al.*, 2003, cited in (16)), and anti-diabetic activities (Maiti *et al.*, 2004, cited in (16)).

Tamarind preparations are universally recognized as refrigerants for fevers, and as laxatives and carminatives. Alone, or in combination with lime juice, honey, milk, dates, spices or camphor, the pulp is considered to be effective as a digestive, even for elephants, as a remedy for biliousness and bile disorders, and as an antiscorbutic (8). The laxative properties of the pulp and the diuretic properties of the leaf sap have been confirmed by modern medical science (Bueso, 1980, cited in (3)).

In traditional practice, the pulp is applied on inflammations, is used in a gargle for sore throat and, mixed with salt, as a cream for rheumatism. It is, further, administered to alleviate sunstroke, *Datura* poisoning, and alcoholic intoxication. In southeast Asia, the fruit is prescribed to counteract the ill effects of overdoses of false chaulmoogra, *Hydnocarpus anthelmintica*, given in leprosy. In Colombia, an ointment made of tamarind pulp, butter, and other ingredients is used to rid domestic animals of vermin (8).

Tamarind fruits were well-known in Europe for their medicinal properties, having been introduced by Arab traders from India (Rama Rao, 1975, cited in (3)). The pulp has been reported in several pharmacopoeias, such as the British and American. Some 90,000 kg of shelled fruits are annually imported into the United States for the drug trade, primarily from the Lesser Antilles and Mexico. The European supply largely come from Calcutta, Egypt and the Greater Antilles (8).

Leaves and Flowers

Tamarind leaves and flowers, dried or boiled, are used as poultices for swollen joints, sprains and boils. The latter are usually applied after grinding leaves and flowers into powder whereby they are used in lotions or infusions. Lotions and extracts made from them are used in treating conjunctivitis, as antiseptics, as vermifuges, treatments for dysentery, jaundice, erysipelas and haemorrhoids, and various other ailments. Fruit shells are burned and reduced to an alkaline ash which enters into medicinal formulas (8). The leaves, mixed with salt and water, are used to treat throat infections, coughs, fever, intestinal worms, urinary troubles and liver ailments. Leaves and pulp act as a cholagogue, laxative and are often used in treating liver 'congestion', constipation and haemorrhoids (3).

Seeds

The powdered seeds are made into a paste for drawing boils and, with or without cumin seeds and palm sugar, are prescribed for chronic diarrhoea and dysentery. The seed coat, too, is astringent, and is also specified for the latter disorders. An infusion of the roots is believed to have curative value in chest complaints and is an ingredient in prescriptions for leprosy (8).

Bark

The bark of the tamarind tree is regarded as an effective astringent, tonic and febrifuge (3, 19). It is used as a tonic and in lotions or poultices to relieve sores, ulcers, boils and rashes (3). Fried with salt and pulverized to an ash, it is given as a remedy for indigestion and colic. A decoction is used in cases of gingivitis, asthma and eye inflammations. Lotions and poultices made from the bark are applied on open sores and caterpillar rashes (8). The bark of the tree should be peeled off if needed for medicinal purposes during the time when the tree is not flowering or when the flowering season ends (3).

Table XII. Medicinal Uses of Tamarind

Continent/ Country ¹	Part(s) Used	Ethnomedical Uses	References
Africa, Asia, America	Pulp	laxative, digestive, carminative, remedy for biliousness and bile disorders, febrile conditions	(3)
Thailand		gentle laxative for reduction of excess weight	Bhumibhamon, 1999, cited in (3)
-		alleviation of sunstroke	(3)
-		treatment of <i>Datura</i> poisoning	Gunasena and Hughes, 2000, cited in (3)
-		treatment of intoxication effects of alcohol and <i>Cannabis sativa</i>	(3)
India, West Africa, Uganda		gargle for sore throats, dressing of wounds	Benthall, 1933, Dalziel, 1937, Eggeling and Dale, 1951 and Chaturvedi, 1985, cited in (3)
-		restoration of sensation in cases of paralysis	(3)
Haiti		cure of malarial fever	Timyan, 1996, cited in (3)
Southeast Asia		counteraction of ill effects of overdoses of chaulmoogra (<i>Hydnocarpus anthelmintica</i> Pierre), given to treat leprosy	(3)
Mauritius		liniment for rheumatism	(3)
Burkina Faso, Vietnam		purify drinking water	Bleach <i>et al.</i> , 1991, cited in (3)
-		enhance bioavailability of ibuprofen in humans	Garba <i>et al.</i> , 2003, cited in (3)

¹When '-' is mentioned, no information about country, nor continent is mentioned in the reference.

Table XII. Medicinal Uses of Tamarind (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
India	ashes of fruit shells	curing enlarged spleen	(3)
Cambodia, India	seed	treat boils and dysentery	Rama Rao, 1975, Jayaweera, 1981, cited in (3)
-		externally applied on eye diseases and ulcers	(3)
India		ulcers and bladder stones	Rama Rao, 1975, cited in (3)
India		anti-diabetic	Rama Rao, 1975, cited in (3)
-		chronic diarrhoea, jaundice	(3)
-		external use to prevent formation of pimples	(3)
Indonesia		hair dressing (oil treatment)	(3)
-	seed coat	astringent	(8)
-	leaves, pulp	cholagogue, laxative, treating 'congestion' of the liver, habitual constipation, haemorrhoids	(3)
Brazil		purgative, diaphoretic, emollient	(3)
-	leaves, flowers	conjunctivitis, antiseptic, vermifuge, dysentery, jaundice, erysipelas, haemorrhoids	(8)

Table XII. Medicinal Uses of Tamarind (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
-	leaves	throat infections, coughs, fever, intestinal worms, urinary troubles, liver ailments	(3)
-		in cardiac and blood sugar reducing medicines	(3)
-		applied to boils to prevent suppuration and inflammatory swellings	(3)
-		ulcers	(3)
Philippines, India, West Africa		rheumatism, external swelling	Jayaweera, 1981, Rama Rao, 1975, cited in (3)
Philippines		cough remedy	(3)
-		eye infections, sprains, wounds	(3)
-		conjunctivitis	(3)
Nigeria		against trypanosomiasis	(19)
Philippines	flowers	treatment of eye diseases and conjunctivitis	Brown, 1954, de Padua <i>et al.</i> , 1978, cited in (3)
-		remedy for jaundice and bleeding piles	(3)
Yemen		skin antiseptic, insecticide	(11)
Cambodia, India, Philippines	bark, flower, root	treatment for digestive tract ailments and indigestion	Jayaweera, 1981, Rama Rao, 1975, cited in (3)

Table XII. Medicinal Uses of Tamarind (Continued)

<i>Continent/ Country</i>	<i>Part(s) Used</i>	<i>Ethnomedical Uses</i>	<i>References</i>
-	roots	curative in chest complaints, ingredient in prescriptions for leprosy	(8)
India (Tamil Nadu)	root bark	abortion and prevention of pregnancies	Lakshmanan and Narayanan, 1994, cited in (3)
-	bark	astringent, tonic, febrifuge, used in cases of gingivitis, asthma and eye inflammations	(8)
-		applied on open sores and caterpillar rashes	(8)
-		indigestion, colics	(8)
-		recover loss of sensation due to paralysis	(3)
-		treatment of sore throats, to heal aphthous sores, heal urinary discharges and gonorrhoea	(3)
Philippines, Eastern Sudan		astringent, tonic	Dalziel, 1937, cited in (3)
Philippines, Eastern Sudan		relieve sores, ulcers, boils and rashes	Dalziel, 1937, cited in (3)

Conclusions

Virtually every part of *Tamarindus indica* L. (wood, root, leaves, bark and fruits) has either nutritional or medicinal value, with a number of industrial and commercial applications.

Tamarind is a versatile, nutritious fruit with a great variety of uses. Tamarind fruit pulp is used for seasoning, as a food component, to flavour confections, curries and sauces, and is a main component in juices and certain beverages. Tamarind fruit pulp has a sweet acidic taste due to a combination of high contents of tartaric acid and reducing sugars. The pulp is relatively poor in protein and oil, though rich in several amino acids. It is a good source of Ca, P, Cu, Mn and Zn, but low in Fe. Vitamin B content is quite high; carotene and vitamin C contents are low.

Tamarind seed is a by-product of the tamarind pulp industry. Presence of tannins and other dyeing matters in the seed testa make the whole seed unsuitable for consumption, but they become edible after soaking and boiling in water. Tamarind seed is also used as raw material in the manufacture of polysaccharide (jellose), adhesive and tannin. Seeds and kernels are high in protein content, while the seed coat is rich in fiber and tannins (anti-nutritional factors). Seeds are gaining importance as an alternative source of proteins, and are besides a good source of fatty acids and rich in some essential minerals, such as Ca, P, Mg and K.

Tamarind leaves are a fair source of vitamin C and β -carotene; mineral content is high, particularly P, K, Ca and Mg.

Anti-oxidant, anti-inflammatory, anti-microbial and anti-fungal activity has been documented from several plant parts. Tamarind is also extensively used in traditional medicine in many cultures and for a wide array of applications.

References

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Chapter 6

Popular Traditional Herbal Medicines from the Jóolas of Essyl in the Rural Community of Enampor (Ziguinchor, Sénégal): An Ethnographic Survey

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Joola people from Essyl inhabit an area located about twenty kilometers southwest of Ziguinchor, the capital city of the southern region of Senegal. The Joola constitute an ethnic group which despite the heavy external cultural influences, have managed to preserve their traditions. Their medical practices which are primarily based on the use of plants are very much dependent on traditional religious beliefs. An ethnographic survey was conducted among these Joola people in order to document their traditional medicinal practices and the wealth of their medical recipes. In the conduct of this study, a series of investigations were carried out with the traditional healers and people with a strong knowledge base of herbal medicine. The results show that in Essyl, medical treatment is often carried out by specialists from different fields that include traditional and modern medicine: traditional healers, obstetricians, physiotherapists, orthopedists, surgeons and herbal medicine men. These medical practitioners and experts work with a wide range of plants available in their natural environment. We collected their responses and organized their recommendations which focused on identifying the specific plants used in their healing tradition and how each of the plants are used. These results permit us to then examine the scientific knowledge that supports, justifies or explains the traditional uses of these plants for particular

health conditions and illness as well as identifies those gaps in scientific understanding that remains to be explored. Furthermore, the results show a disturbing concern in that the present way the plants are collected, prepared and extracted, there likely will be loss in the natural populations of medicinal plants. This threat in this local biodiversity needs to be addressed.

The Joola of Essyl are part of a linguistic and cultural homogenous community which occupy a former kingdom named Mof Avvi, which today counts approximately five thousand inhabitants distributed over ten villages. Administratively, Mof Avvi constitutes the main part of the Enampore rural community (Department of Ziguinchor). In Senegal, Joola people are among the ethnic groups which have preserved their cultural values, with traditional ways including their spiritual animist practice which is rare in Senegal today but still very much alive in this region. This work aims at identifying the determinants of illness and the medicinal plants used in its therapeutic treatment among the Joola people of Essyl.

In the floristic field, Mof Avvi is an area characterized as a Sudanean-Guinean dry forest, with a Guinean forest type. Mangrove swamps are found near the villages bordering the Casamance River and the savanna, an area where agricultural and pastoral activities are practiced. Due to the abundance of flora, use of medical plants is highly developed in these areas. While the use and acceptance of plants for medicine is not only accepted but popular, the specifics of the medical knowledge is often held by a minority of experts named 'ullah' or 'medicine men' who form a closed community. We tried through our investigations to provide a better understanding of this medical heritage, to benefit the general public, the involved community and to contribute toward the preservation of this unique knowledge.

Methodology

Using a participatory approach, about 100 consultants took part in this investigation to document and record this traditional knowledge. This included traditional healers and volunteers with a good knowledge of traditional medicine from the ten villages of Mof Avvi. Our investigations were also conducted in all ten villages of Mov Avvi. During those investigations, the questions asked aimed to document the: a) various specialties of Joola traditional medicine; b) the individual herbal medicinal plants and their main attributes and uses; c) the plant parts used in medical practices; and d) the name of the plants in the local language.

For each plant discussed, individual plants were collected and a herbarium voucher specimen was prepared. Botanical identification of the individual plants were authenticated by experts in the Laboratory of Pharmacognosy and Botany of the Faculty of Medicine, Pharmacy and Dentistry of the University Cheikh

Anta Diop, Dakar, Senegal. Voucher specimens were compared with the Herbarium of the Fundamental Institute of Black Africa, Cheikh Anta Diop (IFAN), Dakar, Senegal.

Results and Discussion

Medical Specialties

As found across Africa (*J*), the Joola of Essyl have two ways of accounting for an illness: a 'mystic-magic' and a rational one. In the 'mystic-magic' vision, an illness is explained as a consequence of the violation of an interdict or caused by a badly disposed person (e.g. witch). As the social organization is strongly regulated in Mof Avvi, an illness is conceived to be a punishment inflicted on the victim as a reprisal for the faults committed. The illness is thus an act of the spirits or shrines which are the minders of social cohesion and which punish any transgressions by a member of the community. The therapy consists of making a sacrifice to the shrine to beseech forgiveness. However, a symptomatic treatment consisting of plant preparations is often taken in conjunction with the above proceedings.

In addition to the mystic-magic origin of illnesses discussed above, an illness can also be caused by an individual endowed with supernatural malevolent capacities whose motivations are often resentment or pure spite. In this case, the witch (assay) seizes the soul of their victim or casts a magic spell, which resembles a common illness (e.g. malaria, hepatitis, madness etc.). Here, the therapy aims at restoring the soul or undoing the magic spell. This magic treatment is also accompanied with a symptomatic treatment with plants.

The medical practices of the Joola of Essyl also have a rational approach. Indeed an illness can be regarded as the result of environmental factors, the dietary habits of the person and of the functional balance of the body. This more realistic perception of pathology and the proposed cures account for the particularly wide botanical knowledge of traditional healers. In addition to this botanical knowledge, the Joola also have mastered the skill of blending plant based medicine. The Joola people of Essyl have a practical knowledge of the human body. The treatment of an illness takes into account two essential parameters: the diagnosis and the therapy itself. These two parameters lead to the classification of traditional healers into six groups.

The Spiritual Healers (Shrine Holders)

Three subcategories of healers are found among shrine holders:

1. The soothsayers who through their shrine can determine the origin of an illness.
2. The patient can consult a healer (*ásottena*) who receives his knowledge from a spirit or a shrine (*epañ*) of which he is the servant. The diagnosis and

the treatment which often includes the use of plants are communicated by the shrine of which the healer is the only possible interpreter.

3. A third subcategory of healers (ullah) are specialized in undoing the magic spell cast by malevolent spirits or witches. The diagnosis is made by divination by the healer and the patient receives a mystic treatment which involves biting the part of the body affected in order to extract the cause of the illness. The healers in this subcategory may operate with the help of the shrine.

Obstetricians:

This group of healers is exclusively composed of women who constitute a very tightly closed circle in which all the practitioners remain anonymous. In fact, tradition dictates that men do not get involved nor know anything about childbirth.

Surgeons:

Their intervention is limited to incision of abscesses, extraction of thorns, circumcision or ganglia extraction.

Physiotherapists:

They are specialized in back pain, pain of the rib cage and chest pain. The treatment is given by applying pressure with hands followed by massages with *Carapa procera* oil (commonly or locally known as Touloucouna or bitterfat from Wolof of Senegal, Tallicoonah oil tree, Kunda oil tree, or Monkey kola) or with *Butyrospermum parkii* butter (Shea butter) or even snake fat.

Orthopedists:

This area of expertise which comprises various fields of intervention can only be inherited. Orthopedists cure bone fractures, luxations and sprains. They often use plants to prevent infections or to aid muscle relaxation.

Herbal medicine men:

They constitute the largest population of traditional healers and generally acquire this art of healing from their parents. This group contains general practitioners and specialists. The diagnosis requires anatomic-physiological knowledge of the human body and knowledge of symptoms of specific illnesses.

Plants and Their Indications

Naming illnesses and translating the definitions of diseases is difficult because of the medical syncretism of the Joola people of Essyl. Even though the source of disorders can be characterized by physical manifestations such as wounds, pimples, coughs, and much more, their oral language lacks specific terms to designate certain illnesses and disorders. The most typical case is that of the word “bújjusa” whose meaning includes ‘cold’, ‘cough’, ‘malaria’, ‘aches’, ‘fever’ etc. This lack of specific terminology to name illnesses creates diagnostic problems and makes referral of patients to relevant healers problematic.

The classification of pathologies or disorders is primarily symptomatically based (Table I). However, a number of disorders and indications (e.g. plants used in ‘poison test’, ‘successful fishing’, and ‘protection against witchcraft’, ‘predictions’) are directly related to mystical beliefs of the people (Table I). For instance, the “poison test” requires the use of an extract of *Erythrophleum guineense* which is ingested in order to detect a culprit. The culprit is then identified when they are poisoned by the ingested substance, whereas the innocent person is the one that always vomits the substance as soon as it is ingested. The use of this plant is rather common among various people in Africa (2).

Due to the absence of a modern medical denomination, we adopted the local name of “corté” to refer to an illness affecting the feet, characterized by a burning sensation with small oozing wounds (Table I).

Our survey and interview’s resulted in a collection of 143 plant species used in 73 therapeutic indications (see Table II). The majority of the species belong to the local native flora. These results demonstrate the wealth and the medical applications of the local flora. Certain plants have a wide range of usage for a variety of treatments. These species, commonly found in the area include: *Combretum micranthum*, *Elaeis guineensis*, *Paulinia pinnata*, *Annona senegalensis*, *Calamus deerratus*, *Carica papaya*, *Cassytha filiformis*, *Citrus aurantifolia*, *Ficus exasperata*, *Guiera senegalensis*, *Landolphia heudelotii*, *Leptadenia hastata*, *Salacia senegalensis*, among other plants. However, some species are not native or they are very rare in the area. Medicinal plants of this latter category include *Butyrospermum paradoxum*, *Erythrophleum guineense*, *Euphorbia balsamifera*, *Mitragyna inermis*, *Nicotiana tabacum* and *Ximienta americana*.

Scientific research confirms the medical attributes of many of these plants used by the traditional healers. For instance, the medical attributes of *Guiera senegalensis* (3-4) justifies its widespread use in the treatment of coughs and bronchitis. Likewise, *Combretum micranthum* is used to treat liver disease (1, 5-6) and *Anacardium occidentale* to treat arterial hypertension (7). However, further studies are required to confirm the use of many other of the medicinal plants they use as well as establishing or validating their healing or curative properties. From our survey, we can report that it is the leaves that are the most commonly used plant tissue for medicinal purposes. As expected, however, other plant parts such as the roots and barks are also commonly used.

From our data, the plant roots were the 'tissue' used in 49 cases. In contrast, the bark of the plant was used in 27 and the entire plant was used in 16 cases. Since these plants are heavily used and extensively harvested and collected in rural communities in Senegal, the ways in which the plants have been harvested and collected, particularly those plants where the roots are the prime tissue used for medicinal purposes can lead to decreased populations of these genetic materials. Their overuse and over collection can constitute a continual threat to the regions biodiversity that ultimately could reduce the availability of these plants as medical resources (8). Thus, the collectors and harvesters as well as the traditional healers and users need to be trained and educated to ensure that the medicinal plants are harvested in a sustainable manner which will then protect and conserve these valuable genetic resources (9).

Whether any of these genetic resources could be practically brought into cultivation as a vehicle to ensure plant preservation of the wild populations remains to be evaluated. As the objective of this study was not to examine the sustainability and ecology of the regions medicinal plants but rather their health care system, we did not collect data on the potential threat to the overharvesting, the current system of destructively removing harvesting the entire plants for roots, or the manner in which the bark was collected, relative to maintaining the health of the tree and shrub. Rather, we note that there is not a tradition to replant, propagate and or grow any of these plants in gardens or fields. When coupled with the observation that the forest density is decreasing, the use of the plant materials increasing, and virtually all medicinal plants are collected in the wild, the threat of population loss for these medicinals is a concern that needs to be addressed.

Table I. The Medicinal Plants, Their Local Names, Plant Parts Used and Their Medicinal Uses in Essyl-Joola Traditional Medicine

<i>Genus and Species</i>	<i>Local Name in Essyl-Joola</i>	Diseases, Applications and Plant Part ²	Method(s) of preparation
<i>Acacia albid</i> Del.	Butëful	Convulsions, epilepsy (B or R); "Corte" (B)	Water maceration
<i>Acanthospermum hispidum</i> DC.	Sibay eteun	Conjunctivitis; Toothaches (P)	Water boiling
<i>Adansonia digitatta</i> L.	Bubax	Horns; Rickets (B)	Water boiling
<i>Afromosia laxiflora</i> (Benth.) Harms	Bukola kola	Physical asthenia; Rheumatoid arthritis aches (L)	Water boiling
<i>Afelia africana</i> Sm.	Bupau	Asthma (R)	Water maceration
<i>Alchornea cordifolia</i> (S. et T) Müll. Arg	Büjjoy	Pericardial pains, palpitations; Nausea and vomiting; (L)	Water boiling ; Fresh leaves juice; water boiling or juice
<i>Allophylus africanus</i> P. Beauv.	Busobol	Delay of walk (R or L)	Water maceration
<i>Anacardium occidentale</i> L.	Bükkaju	Arterial hypertension (B); Mycoses (N); 22Diarrhoea (R); Angina (B or L)	Water boiling, juice

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Annona senegalensis</i> Pers.	Bototox	Diarrhoea; Whooping-cough; Venom of scorpion; Bone break (R); lower back pain (L)	Water boiling; fresh roots paste; juice
<i>Anthocleista nobilis</i> G. Don.	Evvuł eñaru	Intestinal parasites ; anaemia (B)	Water boiling
<i>Anthocleista procera</i> Lepr.	Evvuł eñaru	Intestinal parasites; anaemia (B)	Water boiling
<i>Anthostema senegalense</i> A. Juss.	Búulax	Constipation (La)	Latex in water
<i>Arachis hypogaea</i> L.	Egerete	Purulent urethritis (S)	Seeds paste
<i>Avicennia africana</i> P. Beauv.	Bibej	Horns (B); (L)	Fresh bark paste; water boiling
<i>Azadirachta indica</i> A. Juss.	Bukkasia	Fever and malaria (L)	Leaves on head or water boiling
<i>Baïssa multiflora</i> A. Juss.	Bifem bëine	Thinning; Physical asthenia; Rheumatoid arthritis aches (St); Sexual asthenia (R); Repulsive of snakes (R or L)	Water boiling; water maceration; maceration
<i>Bombax costatum</i> Pell. et Vuill.	Bussana bëine	Galactagogue (B)	Powder
<i>Borassus flabellifer</i> L.	Ñuvuul	Mycoses (AsS), Angina (Fb), Asthma (R)	Water infusion; water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil'-Joala</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Borreria verticillata</i> (L) G. F. Mey.	Eyeyel	Venom of snake (L or R)	Parts mashed or powder
<i>Butyrospermum paradoxum</i>	Karité	Hemorroides (Bu)	Local use
<i>Calamus deerratus</i> Mann. Et Wendl.	Gacet	Pericardial pains, palpitations (L) ; Arterial hypertension; Oedema ; Pregnancy (maintenance and against spontaneous abortion) Obesity (L & St) Toothaches (La)	Water boiling
<i>Calotropis procera</i> Ait.	Bupom		Latex on the tooth aches
<i>Capparis polymorpha</i> G. et Perr.	Bubbun gaggaj	Purulent urethritis, Gastritis (R)	water maceration
<i>Capsicum frutescens</i>	Bébbèbè	Angina (Fr)	Fruit mashed
<i>Carapa procera</i> DC.	Buxunum	Pruriginous eruptions, scale (R) ; Mycoses (S) ; Hémorroides (B)	Maceration; water boiling
<i>Carica papaya</i> L.	Buppapa	Constipation (S) ; Sexual asthenia; Purulent urethritis (R) ; Gastritis; Icter, liver diseases, yellow fever (R &L or Fr)	Seeds powder; water maceration; water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Cassia occidentalis</i> L.	Buputa	Whitlow, Conjunctivitis, headache, migraine (L)	Leaves paste; juice of leaves; leaves on the head, juice of leaves or seeds
<i>Cassia podocarpa</i> G. et Perr.	Bua?	Conjunctivitis (L or S)	
<i>Cassia siamea</i> Lam.	Bukkasia	Fever and malaria; Rheumatoid arthritis aches (L)	Leaves on the head or water boiling; water boiling
<i>Cassia sieberiana</i> DC.	Busaet	Diarrhoea (R); Hematuria (schistosomiasis) (L&St)	Water boiling
<i>Cassytha filiformis</i> L.	Bubbun eddi?	Arterial hypertension; Oedema; 41 Mammalian pain; lower back pain; (P); Rheumatoid arthritis aches (L)	Water boiling
<i>Ceiba pentandra</i> (L.) Gaertn.	Bussana	Mycoses (As); Physical asthenia (L or B); Rheumatoid arthritis aches (L&St)	Ashes in local use; water maceration or boiling; water boiling
<i>Cephaelis peduncularis</i> Salibs.	Búbubuay	Abdominal pains; Diarrhoea; Toothaches (L)	Water boiling
<i>Cissampelos mucronata</i> A. Rich.	Búsugalax	Abdominal pains; Nauseas and vomiting (R); Predictions (L)	Water boiling or maceration; powder by oral route; mashed leaves in water
<i>Cissus aralioides</i> (Welw.) Planch.	Biringisen	Infected wounds (L); Otitis (ySt); Chews (yFr)	Juice in local application

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Cissus quadrangularis</i> L.	Bubbun gannu	42 Métrorragies, dysmenorrhoeas (P); Otitis (ySt)	Water boiling; juice
<i>Citrus aurantifolia</i>	Billemuña	secondary amenorrhoeas(L or R); Purulent urethritis (L); Delay of walk (Fr); Brochitidies, chest pain (L-); Gastritis (R)	Water boiling; water maceration
<i>Clerodendrum capitatum</i> (Willd.) Sc et Th	Búluoro	Abdominal pain (R)	Water boiling
<i>Cnestis ferruginea</i> DC.	Suffot emundumo	Dysmenorrhoea (R); headache, migraine (L&St)	Water boiling
<i>Cola cordifolia</i> (Cav.) R.Br.	Búbbam	Abscess; Infected wounds (B or R), Oeytotic (B)	Paste; powder in local use; water maceration
<i>Cola nitida</i> (Vent.) Sch. et Endl.	Búguru	Mammalian pain (FrH)	Powder
<i>Combretum micranthum</i> G Don.	Bititix	Abdominal pains; Diarrhoea; Fever and malaria; Bronchitides, chest pain; Icter, liver diseases, yellow fever (L)	Water infusion; mastication & water infusion; water boiling
<i>Combretum racemosum</i> P. Beauv.	Unknown name	Physical asthenia; Insomnia (L&St)	Water boiling
<i>Combretum tomentosum</i>	Bititix báine	Sexual asthenia (R)	Water maceration

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Crotalaria retusa</i> L.	Buputa bëine	Childbirth, abortive; Asthma (P)	Water boiling
<i>Daniella oliveri</i> (R.) <i>Hutch. et Dalz.</i>	Bitippi	Bone break (B)	Cast
<i>Desmonium velutinum</i> (Willd.) DC.	Buas bëine	Diarrhoea; Rheumatoid arthritis aches (L)	Water boiling
<i>Detarium senegalense</i> J.F.Gmel.	Búbukkut	Infected wounds (Fr); Diarrhoea (L or B); Chews (L)	Pulp in local application; water maceration
<i>Dialium guineense</i> Willd.	Buffalax	Abdominal pains; Cough (L); Protection against the wizards R&L	Water boiling
<i>Dioscorea bulbifera</i> L.	Egolen	Pericardial pains, palpitations (R)	Powder in oral route
<i>Elaeis guineensis</i> Jacq.	Ñiit	Mycoses (FrO); Abdominal pains; Purulent urethritis (yL); Childbirth, abortive (Fb); Sexual ashenia; Hematuria (schistosomiasis) (R)	Oil in local use; water boiling; paste dilution; water maceration
<i>Enneasteomon barbateri</i> (Baill.) Keay.	Buxaal bëine	Cold(L)	Water boiling
<i>Erythrina senegalensis</i> DC.	Bàsalum bëine	Infected wounds; Nauseas and vomiting; Secondary amenorrhoea (B)	Powder in local use; water maceration

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Erythrophleum guineense</i> G. Don	Birem	Whitlow; Infected wounds; cardiotoxic poison to kill noxious animals (B)	Powder; dilution of mashed bark
<i>Euphorbia balsamifera</i> Ait	Unknown name	Hémorroïdes (P); Galactagogue (L&St)	Water boiling
<i>Fagara leuprieurii</i> (G. et Perr.) Engl.	Birikkit	Intestinal parasites (L); Repeated infant mortality (R)	Water boiling; water maceration
<i>Fagara xanthoxyloides</i> Lam.	Birikkit baine	Hematuria (schistosomiasis) (B)	Water maceration
<i>Ficus asperifolia</i> Miq.	Unknown name	Trypanosomiasis (L)	Leaf for scraping
<i>Ficus capensis</i> Thunb.	Bupox bútuay	Pregnancy (maintenance and against spontaneous abortion) (R)	Water maceration
<i>Ficus exasperata</i> Vahl.	Buas baine	Burns; Mycoses (L), Infected wounds, Conjunctivitis, Wounds, cuts (La);	Juice); leaf for scraping; local use
<i>Ficus glumosa</i> Del.	Bifi baine	Repeated infant mortality (L)	Water maceration
<i>Flemingia faginea</i>	Buas	Pericardial pains, palpitations; Arterial hypertension; Pregnancy (maintenance and against spontaneous abortion); Obesity (P)	Water boiling
<i>Gardenia ternifolia</i> K. Schum	Biembaxa	Icter, liver diseases, yellow fever (R)	Dilution of mashed roots

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Gardenia triacantha</i> DC.	Biembaxa	Icter, liver diseases, yellow fever (R)	Dilution of mashed roots
<i>Gossypium barbadense</i> L.	Bibil	Icter, liver diseases, yellow fever; Cough (L), Otitis (L or Fr)	Juice; water boiling; juice
<i>Güera senegalensis</i> J.F.Gmel.	Búfunux	Pruriginous eruptions, scale; Bronchitides, chest pain; Whooping-cough; For profitable fishing (L) Cough (L or B)	Water boiling; dilution of mashed leaves & water maceration
<i>Harungana madagascariensis</i> Lam.	Bufep	Arterial hypertension (L); Pruriginous eruptions, scale (P)	Water infusion; water boiling
<i>Holarrhena floribunda</i> (G. Don) D. et Sch.	Birixanu	Childbirth, abortive; Dysmenorrhoea (R&L); 31	Water boiling; water maceration
<i>Hypophila auriculata</i> (Sch.) Heine	Elalañ	Galactagogue; "Corte" (R) Enuresis; Hematuria (schistosomiasis) (L&St)	Water boiling
<i>Hymenocardia acida</i> Tul.	Koronkonda	Arterial hypertension; Pregnancy (maintenance and against spontaneous abortion) (L)	Water infusion; water boiling
<i>Hyptis psicigera</i> Lam.	Búilen	Mycoses; Abdominal distension; headache, migraine; Cold (L)	Leaves mashed; water infusion; leaves for inhalation

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Icacinna senegalensis</i> A. Juss.	Burabaa?	Fever and malaria (L&St)	Water boiling
<i>Ipomoea asarifolia</i> (Dest.) R. et Sch.	Erarax	Abscess (L); Oedema; Purulent urethritis; Bone break (L or P)	Leaves paste; water infusion; water boiling; leaves for touching Leaves paste
<i>Ipomoea batatas</i> (L.) Lam.	Batata	Abscess; Whitlow (yL)	
<i>Jatropha curcas</i> L.	Bicirit	Oedema (B); Constipation (S); Wounds, cuts (La)	Bark paste; seeds powder; local application;
<i>Kaempferia</i> <i>aethiopica</i> Benth.	Buvvomman	Abdominal pains; Purulent urethritis (R); Hematuria (schistosomiasis) (L&St)	mashed roots in water; water maceration; water boiling
<i>Khaya senegalensis</i> (Dest.) A. Juss.	Búxol	Pruriginous eruptions, scale (B); Cough (Fr)	Wwater maceration ; water boiling
<i>Laguncularia</i> <i>racemosa</i> Gaertn.	Bujaxaen	Abdominal pains (L)	Water maceration
<i>Landolphia dulcis</i> (R. Br.) Pichon.	Bubot	Infected wounds, Antipoison, Burns (R); 39 Tooth aches (St)	Rroots powder; toothbrush
<i>Landolphia</i> <i>heudelotii</i> A. DC.	Bifem	Abdominal distension; Mammalian pain (R); Galactagogue (R& L); Conjunctivitis (L); Trypanosomiasis (La)	Water boiling; roots mashed; water boiling or maceration; direct use

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil¹ -Joola</i>	<i>Diseases, Applications and Plant Part²</i>	<i>Method(s) of preparation</i>
<i>Landolphia hirsuta</i> (Hua) Pichon	Bineñ	Anaemia; Thinning (St)	Water boiling
<i>Lannea nigritana</i> (Sc. Ell.) Keay.	Unknown name	Intestinal parasites (B)	Water boiling
<i>Lannea velutina</i> A. Rich.	Unknown name	Secondary amenorrhoea; Métrorragies; anaemia (Fr or R)	Water boiling
<i>Lepidagathis sericea</i> Ben.	Bupaapapab	Delay of walk (L &St)	Water maceration
<i>Lepisanthes senegalensis</i>	Biec	Arterial hypertension; Intestinal parasites (L)	Mashed leaves in water
<i>Leptadenia hastata</i> (Pers.) Decne.	Bubbun akkoña	Infected wounds (La); Galactagogue (P); headache, migraine (La); Cold; Wounds, cuts (L or La)	Latex in direct use; water boiling; juice of leaf in nares; leaves or latex in nares; latex or mashed leaves in direct use
<i>Macaranga heudelotii</i> Baill.	Bulanit	Abdominal distension, Pregnancy (maintenance and against spontaneous abortion), Female sterility (R)	Water boiling
<i>Macrosphyra longistyla</i> (DC) Hiem.	Bujúu sijjamen	Delay of growth, Thinning (R)	Water maceration

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Mangifera indica</i> L.	Bumangu	Conjunctivitis, Bronchitides, chest pain (L)	Juice in eyes ; water boiling
<i>Manihot esculenta</i> Crantz.	Eex	Abscess, Hemorroides (L)	Mashed leaves
<i>Mezoneurum benthamianum</i> Baill.	Fañumora	Antipoison (R)	Water boiling
<i>Mitragyna inermis</i> (Willd.) O. Kze.	Unknown name	Elephantiasis (R)	Water boiling
<i>Morinda geminata</i> DC.	Bulogoñ	Abdominal pains (L)	Water boiling
<i>Moringa oleifera</i> Lam.	Binebeday	Convulsions, epilepsy, Conjunctivitis, Rheumatoid arthritis aches (L)	Leaves for friction; leaves juice in eyes
<i>Mucuna cochinchinensis</i> (Lour.) A. Chev.	Gusaax kafe	Repulsive of snakes (S)	Seeds by oral route
<i>Mucuna pruriens</i> (L) DC.	? aña	Sexual asthenia (R)	Water maceration
<i>Musa sapientum</i>	Balullumay	Arterial hypertension, Obesity, Otitis (L), Lice (AsL)	Water infusion; juice; ash in local use
<i>Naucllea latifolia</i> Sm.	Birillo	Pregnancy (maintenance and against spontaneous abortion) (R)	Water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part</i>	<i>Method(s) of preparation</i>
<i>Nelsonia canescens</i> (Lam) Spreng.	Guil sijaamen	Pruriginous eruptions, scate (P)	Water maceration
<i>Newbouldia laevis</i> (P. Beauv.) Seem.	Bipeleen	Whitlow (B) Trypanosomiasis (L); Repulsive of snakes (R)	Mashed bark; water boiling; water maceration
<i>Nicotiana tabacum</i> L.	Esumbba	Cough (L)	Water boiling
<i>Opilia celtidifolia</i> (G. et Perr.) Endl.	Bakol effulum	Sexual asthenia (R)	Water maceration
<i>Oryza sativa</i> L.	Emmano	Abdominal distension (S)	Brun of seeds for friction
<i>Oxytenanthera</i> <i>abyssinica</i> Mumro.	Buttara	Pericardial pains, palpitations, Arterial hypertension (L)	Water boiling
<i>Parinari excelsa</i> Sabine.	Billi	Diarrhoea (L), Cough (B)	Water boiling; water boiling or fruit like food
<i>Parinari macrophylla</i> Sabine.	Biel	Toothaches (B)	Water boiling
<i>Parkia biglobosa</i> (Jacq.) Benth.	Buxompo?	Whooping-cough (R)	Water boiling
<i>Paullinia pinnata</i> L.	Bubbun ullax	Thinning, 8 Physical asthenia, 58 Rickets, 60 Delay of growth, 59 Delay of walk (P), 64 Cough (R)	Leaves for inhalation; water maceration; water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part²</i>	<i>Method(s) of preparation</i>
<i>Ptilostigma reticulatum</i> (DC.) Hochst.	Bielexaw	Hemorroides (L&St), Ocytoic(L)	Water boiling; mashed leaves in water
<i>Ptilostigma thonningii</i> (Sch) Miln.-Redh.	Bielexaw	Hemorroides (L&St), Ocytoic (L)	Water boiling; mashed leaves in water
<i>Prosopis africana</i> (G. er Perr.) Taub.	Biix	Mouth ulcer, gingivitis, stomatitis (L), Bronchitides, chest pain (L&St), Antipoison (B)	Water boiling; powder
<i>Psidium guajava</i> Radd.	Buyyaba	Diarrhoea (Fr or yL)	Water boiling
<i>Rauwolfia vomitoria</i> Afz.	Bubbun buccac	Headache, migraine (B), Mental disorders, Lice, Antipoison (L)	Latex in nasal route; leaves on the head; water boiling
<i>Reissantia indica</i> (Willd.) Hallé.	Bulenguen	Oedema (L), Giddiness (L&St),	Leaves in direct use; leaves for inhalation
<i>Rhizophora racemosa</i> G.F. Mey.	Bumaax	Fever and malaria, headache, migraine, Angina, Wounds, cuts (L)	Water boiling; mashed leaves
<i>Ricinus communis</i> L.	Bubbun fuxow	Mycoses, Convulsions, epilepsy, Mental disorders (L)	Mashed leaves; leaves for friction; leaves on the head
<i>Ritchiea capparoides</i> (Andr.) Britt.	Gabuuful	Venom of snake (L)	Mashed leaves

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part²</i>	<i>Method(s) of preparation</i>
<i>Saba senegalensis</i> (A. DC.) Pichon.	Búbur	Abdominal distension, Diarrhoea (R), Trypanosomiasis (La)	Water boiling or maceration; water boiling; latex in local use
<i>Salacia senegalensis</i> (Lam.) DC.	Bunga?	Enuresis, Cough, Burns, Predictions, Repeated infant mortality (R)	Water boiling or maceration; water maceration; powder; water maceration
<i>Scoparia dulcis</i> L.	Bubbun jabbut	Whitlow, Delay of growth, Thinning (P)	Mashed plant; water maceration
<i>Secamone afzelii</i> (Sch.) K. Schum.	Buxararar	Constipation (P)	Water boiling
<i>Sida rhombifolia</i> L.	Bitel	Abscess (L), Infected wounds (R), Venom of snake (L or R)	Mashed leaves; powder, mashed leaves or roots
<i>Smeathmannia laevigata</i> Sol.	Buffo	Secondary amenorrhoea (R)	Water boiling
<i>Smilax kraussiana</i> Meissn.	Gáparula	Rheumatoid arthritis aches (L)	Water maceration
<i>Solanum aethiopicum</i>	Buijxata	Sexual asthenia (R)	Water maceration
<i>Solanum incanum</i> L.	Bitega nifuxow	Whitlow (Fr)	Fruits
<i>Spondias mombin</i> L.	Biceccel	anaemia (B)	Water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil' -Joola</i>	<i>Diseases, Applications and Plant Part²</i>	<i>Method(s) of preparation</i>
<i>Sirophanthus sarmmentosus</i> DC.	Butaxaruba	Abdominal pains, Intestinal parasites, Antipoison (B)	Water boiling or maceration); water boiling; powder
<i>Sirophanthus hispidus</i> DC.	Butaxaruba	Abdominal pains, Gastritis (R), Lice (L&St)	Water boiling; water boiling or maceration; leaves & stem
<i>Syzygium guineense</i> (Willd.) DC.	Biec eñaru	Female sterility (L)	Water maceration or powder
<i>Tapinanthus bangwensis</i> (Engl. et Kr.) Danzer.	Eccorix	Asthma, Convulsion, Epilepsy, Diarrhoea, Intestinal parasites	Water boiling or powder; water boiling
<i>Terminalia albida</i> Sc. Ell.	Buanka baafit	Secondary amenorrhoea (P)	Water boiling
<i>Terminalia macroptera</i> G. et Perr.	Bubbun emandi?	Icter, liver diseases, yellow fever (R)	Mashed roots in water
<i>Tetracera alnifolia</i> Willd.	Furat fuñuget	Physical asthenia, 38 Insomnia, 10 Asthma (L&St)	Water boiling; water maceration
<i>Tetracera potatoria</i> Afz.	Fukkuxus	Galactagogue, Conjunctivitis, Cough (SaSt)	Sap in oral route; sap in eyes
<i>Uvaria chamae</i> P. Beauv.	Buxaal	Abdominal pains (B), lower back pain (L&St)	Water boiling

Table I. Continued.

<i>Genus and Species</i>	<i>Local Name in Essil¹-Joola</i>	<i>Diseases, Applications and Plant Part²</i>	<i>Method(s) of preparation</i>
<i>Vermonia colorata</i> (Willd.) Drake.	Búfyu	Nauseas and vomiting (L or R), Sexual asthenia (R), Obesity (L&St or R)	Water maceration
<i>Vitex doniana</i> Sw.	Butteñ	Whooping-cough, Cough (yL)	Water boiling
<i>Voacanga africana</i> Stapf.	Bulèn	Giddiness (St)	Water maceration
<i>Walteria indica</i> L.	Búpanda?	Intestinal parasites (B&R), Venom of scorpion (L)	Wwater boiling
<i>Ximenia americana</i> L.	Unknown name	Abdominal pains (Fr), Hematuria (schistosomiasis) (L&St), Conjunctivitis (Fb)	Water boiling
<i>Xylopia aethiopica</i> (Dunal) A. Rich.	Biyew	Antipoison (L)	Water maceration; powder
<i>Xylopia parviflora</i> (A. Rich.) Benth.	Biyew báine	Cold, Bronchitus, chest pain, Rheumatoid arthritis aches (L)	Juice on nasal route; water boiling

NOTE: ¹Orthographical notation in language Joola Essyl: á : pronounced like the first vowel in "above"; u : pronounced like the phonetic vowel in "good" or the French "ou"; e : pronounced like the phonetic vowel "read"; x : represents an aspirated h; g : pronounced like good; ñ : pronounced like the Spanish mañana 'morning'; ? : pronounced like the English final consonant "sing". The doubling of consonants is used to indicate consonant length i.e., pronunciation of those consonants is characterised by emphasis on them. ²Plant tissue used includes B= Bark; R= Root; P= Whole plant; N= Nuts; La= Latex; S= seeds; St= Stems; L= leaves; AsS= Ashes from stalks; Fb= Foliar bud; Bu= Butter; LS= leaf and stem; Fr= Fruit; As= Ash; ySt= young Stem; FrH= Fruit hull; FrO= Fruit oil; yL=young leaves; yFr= young Fruit; SaSt= Sap of stem; AsL= Ashes from leaves.

Conclusion

In this study, we have shown that even though the therapeutic approaches of the Joola people of Essyl are based on both rational and supernatural principles they are ultimately based on herbal medicine. This tendency towards herbal medicine is motivated by the wealth of flora in the natural environment which provides traditional healers with a variety of therapeutic tools. Our investigations have confirmed the effectiveness of these plant species that were identified in our study. However, a great majority of plant species still need to be examined. Therefore, there is for a real need for further research to both preserve traditional medical knowledge and to discover whether new applications from these plants can be of benefit to the larger Senegalese and international community. Despite the relevant abundance of flora, the biodiversity of Essyl is at this time in potential jeopardy due to overharvesting and the collection of medicinal plants in a nonsustainable manner. There is an urgent need to educate those involved in the collection and use of these plants to ensure these medicinals will be abundant in the future. The importance of conserving the regions rich biodiversity and their natural environment has not been a real issue among the Joola. Benefits of conservation and preservation must be presented to the Joola in a manner that can illustrate its link to the continued availability and supply of the critical medicinals that they now enjoy and depend upon for their health, welfare and spiritual needs.

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Chapter 7

The Diversity of Medicinal Plants in Nigeria: An Overview

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Plants constitute a major economic resource of most countries of the world including Nigeria. They have taxonomic classes which enable their classification with respect to their role in economic development. In the health sector, plant parts are employed in pharmaceutical research and in the development of new drugs. In Nigeria and some other countries of the world, plant parts or herbal preparations are used as herbal-drugs for the treatment of various ailments. In Nigeria herbal practices, the practitioners claim that plant parts possess various phytochemicals which exhibit diverse pharmacological and biological responses and diversities. Nigeria is a country steeped in the use of and belief in traditional medicines in which plants play a major role. This paper provides a brief snapshot or overview to several indigenous medicinal Nigeria plants.

The majority of the Nigerian populace depends on herbal preparations in the treatment of certain ailments and diseases. This can be attributed to the high cost of orthodox drugs as compared to herbal preparations, to the efficacies of some of the preparations to their health needs, and notably to the traditional and historical role that plants have played in Nigerian health care. Although some medicinal plants are found used for similar purposes in different places and countries, there are plants that exhibit different properties dependent upon the environment in which they grow.

Phytopharmacology

The significant use of medicinal plants in the Nigerian traditional health care system prompted an increase in the number of scientific studies on medicinal plants including their chemistry, biology, and pharmacological activities. The results were sufficiently promising to suggest that Nigerian indigenous medicinal plants used in traditional medicine would be excellent candidates for further study and for pharmaceutical development for the treatment and eradication of some infectious diseases.

Global research on the medicinal properties of plants is highly impressive and increasing and provides yet a further justification for an examination of Nigerian medicinal plants (1). A Medicinal plant has been defined as one which contains substances that can be used for therapeutic purposes or contains biological active substances that are precursors for development of new drugs (2). Research on medicinal plants has further proved that some plants have excellent ability to strengthen human resistance to diseases as well as the ability to support good health. The health potentials of medicinal plants have given rise to the development and production of food supplements from plants and other natural products for the improvement of health care delivery (3). The pandemic explosion of diseases such as malaria, diabetes, hepatitis and AIDS etc, in Nigeria has posed challenges even to traditional medical practitioners who do believe that cures to each and every of these diseases are within the herbal resources of their nation. These serious infectious diseases have prompted increased interest on the part of the Traditional herbal practitioners to work with those in science to configure ways to both study traditional medicines in a more integrated approach and in seeking avenues to screen and validate herbal formulas as was done with the Nigerian herbal formula NIPRISAN available now as a pharmaceutical drug Nicosan in Nigeria, shown to be effective in the treatment of sickle cell anemia (4). The long term goal of such programs is to ensure the best health care can reach the most people in the most affordable way and strongly suggests the need to integrate traditional herbal practices into the network of modern health care delivery.

As a result of the intense research on Nigerian medicinal plants, sources of new therapeutic agents have been identified. The National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria has developed a plant-based cure for sickle cell anaemia using extract from four plants (5, 6, 7). This and this product was later commercialized and is being manufactured as Nicosan in Nigeria (4). *Gongronema latifolium*, another

indigenous herb found in the Niger Delta of Nigeria which is now being used for the treatment of diabetis (8).

Studies on several Nigerian herbs have led to the identification of plant extracts with therapeutic efficacies against diabetes. Extracts from *Garcinia kola* commonly called bitter kola reduces gastrointestinal spasm in rats thereby showing promise in the treatment of diarrhea (4). Their findings also revealed that the plant extract has the potential for the treatment of respiratory infections. Phytochemical analysis shows that the seed has a high content of alkaloid and biflavonoids.

Nauclea latifolia, a plant indigenous to Nigeria possesses a high activity against cardiovascular disorders and a depleting effect on the cholesterol LDL and VLDL (9,10). Earlier, Udoh et al (unpublished) had shown that extract from the leaf of *N. latifolia* possesses potent myotensive activity in rat. Using hypertensive human subjects, he further showed this extract to exhibit antihypertensive activity. The plant contains a high level of alkaloids. Locally, *N. latifolia* is called Mfang mbube in the Nigerian region to which it is indigenous.

Other Nigerian medicinal plants have also been studied intensively. Ethanol extract of *Piper guineense* with high contents of alkaloidal amides was found efficacious against helminth parasites (Monogenean) of cultured fish (11,12). Petroleum ether extract of seeds of *Carica. papaya* and ethanol extract of leaves of *Mucuna pruriens* were effective against protozoan parasites (*Ichthyophthirius multifiliis*) of fish (13). The research on these plants led to the pioneering discovery on the use of extracts from plants for the treatment of diseases in fish. Ethanol extracts of *P. guineense* have also shown efficacies in the control of conception in mice (14). The antiobesity properties of *Aframomum melegutta* by pancreatic lipase inhibition has also been demonstrated (15).

There are so many edicinal plants indigenous to Nigeria, a selected few are highlighted by their medicinal uses and pharmacological active substances (Table I).

Table I. Selected Nigerian medicinal plants and their biological activities.

Scientific and common Name	Plant Family	Biological Activities	Responsible Bioactive Compounds. References
<i>Piper guineense</i> Schumacher et Thonn; West African black pepper, Ashanti pepper <i>Garcinia kola</i> Bitter kola	<i>Piperaceae</i>	Neuromuscular activity, contraceptive, antiparasitic, aphrodisiac Abdominal disorders, chest complaints, used as an enema, Bronchitis, Headache, Anthelmintic, Caries, diarrhea, hemorrhoids, antibiotic,	Alkaloidal amide, 12, 16, 17
<i>Nauclea latifolia</i> <i>Aframomum meleguitta</i> Grains of Paradise	<i>Rubiaceae</i> <i>Zingiberaceae</i>	Cough, nasal congestion, control of diarrhea, antidiabetic, Aphrodisiac, hypertension, stimulant, gastrointestinal problems, dysentery, headache, backpain, skin ailments, stimulates digestion, antiviral activity,	Alkaloids and biflavonoid. 4, 17, 18
		Antihypertensive, antimicrobial Fever, stomachache, diarrhea Antiobesity, dysentery, antibacterial, Abortifacient, hemorrhoids (used with <i>Garcinia kola</i>), antifungal, colds, migraine, stimulant	Alkaloids. 10, 17, 19 Gingerols, shogaols and paradol. 15, 17

Table I. Continued.

<i>Scientific and common Name</i>	<i>Plant Family</i>	<i>Biological Activities</i>	<i>Responsible Bioactive Compounds. References</i>
<i>Carica papaya</i> Melon tree	<i>Caricaceae</i>	Antifertility, antiparasitic, antihelminthic, abortifacient, amnesia, insomnia, headache, arthritis, asthma, coughs, dysentery, hepatitis, gall bladder problems, diuretic, stomachic, AntiparasiticSnakebite	13, 17
<i>Mucuna pruriens</i> <i>Gongronema latifolium</i>	<i>Fabaceae</i>	Diabetes, Digestive disorders, colic, worms and as a purgative	13, 17 17
<i>Cleome rutidesperma</i>	<i>Caparaceae</i>	Anti-inflammatory, antihelminthic, carminative, ear aches.	Alkaloids, tannin, saponin, flavonoids, cardiac glycosides. 17, 20, 21
<i>Emilia coccinea</i>	<i>Asteraceae</i>	Fever, convulsion, ringworm, ulcer, dizziness, epilepsy, cardiac problems, eye inflammation, gonorrhea, ovarian problems, respiratory problems, snakebite, scabies, wound healing, stomachic	Alkaloids, tannin, saponin, steroid, terpenes, flavonoids, cardiac glycosides. 17, 20, 22
<i>Euphorbia heterophylla</i>	<i>Euphorbiaceae</i>	Erysipelas, cough, bronchial paraxymal asthma, hayfever. Constipation, parasitosis, chest abscess.	Alkaloids, tannin, phlobatanin, flavonoid, trepenoid, cardiac glycosides. 17, 21, 23, 24

Table I. Continued.

<i>Scientific and common Name</i>	<i>Plant Family</i>	<i>Biological Activities</i>	<i>Responsible Bioactive Compounds. References</i>
<i>Richardia bransilensis</i>	<i>Rubiaceae</i>	Eczema, Treatment of boils, avine malaria, cataract	Alkaloids, tannin, saponin, steroid, terpenes. 17, 20
<i>Scoparia dulcis</i>	<i>Scrophulariaceae</i>	Antiviral, antitumor chest pains, sore throat, gonorrhoea, otitis, vomiting, cough, snake repellent, antelmintic, conjunctivitis, rickets, dizziness, constipation, diabetes, diarrhea, antibiotic, fever, respiratory problems, ease childbirth.	Alkaloids, tannin, saponin, phlobatanin, terpenes. 17, 21, 25
<i>Sida, acuta</i>	<i>Mahvaceae</i>	Stops bleeding, treatment of sores and wounds; antipyretic, catarrh, dysentery, gonorrhea, chest pains, fevers, aphrodisiac, dizziness, galactagogue	Alkaloid, flavonoid, cardiac glycosides. 17, 26
<i>Spigelia anthelmia</i>	<i>Lonaniaceae</i>	Worm expeller, antiparasitic, sore throat, chest complaints, .	Alkaloid, saponin, tannin, steroids, flavonoids, cardiac glycosides. 17, 21
<i>Stachytarpheta cayennensis</i>	<i>Verbanaceae</i>	dysentery, syphilis, gonorrhea, measles	Alkaloids, tannin, saponin, phlobatanin, flavonoid, cardiac glycosides. 17, 21
<i>Tridax procumbens</i>	<i>Asteraceae</i>	Stops bleeding. Treatment of diarrhea, malaria and stomachache. Hypertension, edema, jaundice, wound healing.	Alkaloids, tannin, saponin, flavonoid. 17, 20, 24

Table I. Continued.

<i>Scientific and common Name</i>	<i>Plant Family</i>	<i>Biological Activities</i>	<i>Responsible Bioactive Compounds. References</i>
<i>Terminalia avicennioides</i>	<i>Combretaceae</i>	Asthma, cough, hemoptysis, tuberculosis, sore throat, diarrhea, shigellocidal activity ascites, edema, conjunctivitis, ophthalmia, ulcers, wounds, bums, bruises, rheumatism, swollen joints, diarrhea, dysentery, toothache, epilepsy	Anthraquinones, saponin, steroids, Tmmin, terpenoids. 17, 27, 28
<i>Anogeissus leiocarpus</i>	<i>Combretaceae</i>	Asthma, cough, tuberculosis, anthelmintic, tapeworm, Gonorrhoea, tonic, enema, jaundice, body pain, chest pain, wounds, ulcers, skin rashes, itching, hemorrhoids, abscesses, hemia, diarrhea, ophthalmia, abdominal pain, rheumatism, fishing poison, stimulant, toothache.	Anthraquinone, flavonoid, saponin, steroids, tannin, terpenoids. 17, 28
<i>Ocimum gratissimum</i>	<i>Lamiaceae</i>	Antibacterial, gonorrhea, eye health, eye and ear diseases, emetic, fever, anthelmintic, constipation, abdominal pain, gastrointestinal disorders childrens colds, cough, bronchitis, headache, otitis, conjunctivitis, fever, rheumatism, colic, diarrhea, toothache, hemorrhoids, nosebleed, purgative, respiratory problems, nasal congestion, mosquito repellent, convulsions, epilepsy,	Alkaloids saponin and tannins. 17, 29, 30
<i>Phyllanthus discoides</i> (<i>P. diandrus</i>)	<i>Euphorbiaceae</i>	Antibacterial, palpitations.	Alkaloids saponin and tannin. 17, 30
<i>Acalypha wilkesiana</i>	<i>Euphorbiaceae</i>	Antibacterial	Alkaloids saponin and tannin. 30

Table I. Continued.

<i>Scientific and common Name</i>	<i>Plant Family</i>	<i>Biological Activities</i>	<i>Responsible Bioactive Compounds. References</i>
<i>Anogeissus leiocarpus</i>	<i>Combretaceae</i>	Antimicrobial, tonic, body pain, chest pain, coughs, wounds, ulcers, skin rashes, itching, worms and tapeworms, coughs, gonorrhea, jaundice, hemorrhoids, amenorrhea, abscesses, hernia, diarrhea, ophthalmia, rheumatism, fishing poison, stimulant, aphrodisiac, toothache, fevers, wounds, sores, ulcers.	Terpenes, triterpenoid saponins. 16, 17
<i>Capparis brassei</i>	<i>Capparidaceae</i>	Antimicrobial, tuberculosis, coughs	Terpenes, saponin. 17, 28
<i>Entada africana</i>	<i>Mimosaceae</i>	Bronchitis, cough, whooping cough, dysentery, fever, wound healing, abdominal pain, dysentery, ulcers, sore eyes, constipation, stomachic, abortifacient, throat problems, colds, emetic, rickets, stimulant, gonorrhoea, diuretic, diarrhea, headache, internal bleeding, chest abscesses, dermatosis, snakebite, jaundice.	Terpenes, triterpenoid saponins. 17, 21, 28
<i>Canarium (spp)</i>	<i>Burseraceae</i>	Antioxidant activity, colic, hemorrhoids, jaundice, enema, scabies, gland inflammation, liver disease, wound healing, skin dermatitis, coughs, stomachic, Several species reported to be used in premature birthing, to expel the placenta, leprosy	17, 31
<i>Cnestis ferruginea,</i>	<i>Conrtraceae</i>	Antioxidant activity, bronchitis, abortifacient, wound healing, purgative, coughs, dysmenorrhea, fever, body pain, dysentery, diarrhea, migraine, sinusitis, threatened abortion, ophthalmia, pneumonia, mouth and wound infections, coughs, tuberculosis, whooping cough, toothache, snakebite, hemorrhoids, abscesses, gum pain headache, appetite stimulant, stomachic.	17, 31

Table I. Continued.

<i>Scientific and common Name</i>	<i>Plant Family</i>	<i>Biological Activities</i>	<i>Responsible Bioactive Compounds. References</i>
<i>Funtumia elastica</i>	<i>Apocynaceae</i>	Antioxidant activity, gonorrhoea, burns, bronchitis, whooping cough, skin fungus and wound healing, diarrhoea, diuretic, hemorrhoids, purgative, anthelmintic, respiratory problems, stomachic.	17, 31
<i>Landolfia owariensis</i> ,	<i>Apocynaceae</i>	Antioxidant activity, dental care, caries, toothache, diarrhoea, anthelmintic, dizziness, epilepsy, rheumatism, edema, purgative, stomachic, stiffness.	17, 31
<i>Sphenocentrum jollyanum</i> ,	<i>Menispermaceae</i>	Antioxidant activity, stimulant, stomachic, constipation, poisoning, ulcers, coughs, wound healing. aphrodisiac, dysentery, epilepsy.	17, 31
<i>Voacanga africana</i>	<i>Liliaceae</i>	Antioxidant activity, cardiac spasms, caries, disinfectant, fatigue, amoebic dysentery, asthma, diarrhoea, edema, leprosy, convulsions in children, to calm the insane, epilepsy.	17, 31

NOTE: *The medical applications cited from Neuwinger (2000) (17) contain the original research and traditional reports.

Plant-Drug Discovery

The use of plants as sources of bioactive chemicals and as inspiration for new chemistry with medicinal applications has been a fruitful approach in search for new drug entities (32-34). The diversity of plant species is an invaluable repository of unusual chemical compounds, given that secondary metabolites are like chemical finger prints of individual's species (35). The pharmacological activity of these chemical substances can be identified with test procedures at various biological levels, ranging from sub-cellular elements to the normal or modified intact animal. Studies on the biological activity of some African medicinal plants are performed with *in vitro* and *in vivo* methods. Since such biological screens can reveal prototype drugs with unknown mechanisms of action, the inclusion of *in vivo* animal models to complement the *in vitro* approach can prove advantageous by "short-gun" or "target-directed". The short-gun approach screens for active substances by means of tests that are irrelevant to the subsequent application of the drug. In contrast, the target-direct or mechanistic approach is employed when a specific and ultimately molecular biological mode of action is desired for a given therapeutic area (10, 36).

One strategy towards reducing drug importations and making drugs available at a lower cost in Nigeria would be to cultivate and locally process native species that are known to contain therapeutically useful compounds. That would involve communities and growers in the collection and cultivation of medicinal plants (37), thus providing additional cash income opportunities to many. This would also involve the development of standardized botanical and herbal formulas, extracts and products, often now lacking in the manufacturing of herbals, again generating additional income opportunities and an improvement of available locally made herbal products now found in the Nigerian marketplace.

The need to authenticate the botanical materials used in the commercial herbal remedies and the need to develop standardized products could strengthen this sector and not only provide again more income generating opportunities but ensure safer and higher quality traditional herbal products. Lastly, this approach would be both culturally acceptable as these same products are used today and if done in concert with the public health care system help to alleviate the over pressured national health care system that some consider to be failing (2). This approach could help integrate our modern Nigerian health care and medical system with our traditional medicines which are largely based upon plants and for which the majority of our population already use and rely.

Drug discovery has been primarily an industrial effort and true innovation has generally been achieved by intensive screening of many compounds for selected activities (37). Plant products have been employed in the identification of biological receptors in mammalian systems and their uses in investigation of the physiological and pathological functions of potential drug targets. Classical examples include nicotine, physostigmine, curare, muscarine, pilocarpine, and tropane alkaloids in the study of nicotinic and muscarinic receptors (38). Innovative discovery of drugs leans heavily on the basic physiological and

biochemical knowledge with much help from serendipitous pharmacological and clinical observations. It is the availability of drugs that permits new biochemical, physiological and especially, pharmacological knowledge to accrue (9). Therefore the importance of careful evaluation of really novel pharmacological effects has to be seriously considered, rather than limiting drug discovery and development to previously determined therapeutic goals. This is of particular relevance when discussing Nigerian and African medicinal plants. Many of our indigenous medicinals have not been subjected to these modern biological screens. By either conducting more of these screens in-country and/or in partnership with others that would support and champion the processing and value-addition in Nigeria, then greater economic benefit will filter down to the country. The validation of traditional applications could also lead to a significant economy developed that is solely geared to providing, local, regional or national standardized traditional medicinal products.

Concluding Remarks

Nigeria is home to thousands of indigenous and naturalized plants, many that have been used for centuries as medicinal plants. Many of these plants have a diversity of biological activities and applications. Many of these plants serve a dual function both as a food and medicine. In Nigeria over 60% of the population depends on plants and plant products for their medicines. Common ailments traditionally treated with plants include: malaria fever, jaundice, diabetes, arthritis and typhoid fever. Each have been treated efficaciously with some herbs from plants indigenous to Nigeria. Recently, some Nigerian phytotherapists have claimed to successfully treat HIV infected patients with plant-extracts but this claim must be sufficiently investigated.

Because of the dual nature of the plants, being consumed both as a food and used as a medicine, it is important to recognize that besides the direct medicinal benefits, plants are often rich sources of the macro- and micronutrients, vitamins and other chemicals we need to maintain and improve our health and repair damaged tissues.

Improved quality, increased attention to the scientific study of these plants, and more favorable public policies are needed to strengthen this sector and provide economic opportunities to all those involved as well and provide more affordable medicines.

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Chapter 8

Bioactivity and Bioactive Compounds of African Amaryllidaceae

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Members of the family Amaryllidaceae are widely distributed in the tropics and the warm parts of the temperate regions of the world. They are known for their ornamental value and the production of structurally unique alkaloids, the Amaryllidaceae alkaloids. Some of these alkaloids possess a wide range of biological activities including: antitumor, antiviral, antibacterial, antimalarial, central nervous system diseases, immunomodulatory and anti-inflammatory. This chapter emphasizes the bioactivity and bioactive compounds of African Amaryllidaceae.

Plants of the family Amaryllidaceae are perennial or biennial herbs with subterranean bulbs containing thick, fleshy bulb scales or without a typical bulb but a rhizome as in *Scadoxus* and *Clivia*. The Amaryllidaceae are widely distributed. They are richly represented in the tropics and have pronounced centres in South Africa and to a lesser extent in Andean South America. Other groups have their centre in the Mediterranean. Groups of phylogenetically related genera often have a particular geographic concentration (1).

The family is classified into nine tribes. The tribe Lycorideae is Asiatic in distribution while Stenomesseae and Eucharideae are South American in distribution. The tribe Pancratieae consists of Old World genera ranging from South Africa to Macronesia and the Mediterranean region and further eastward

into tropical Asia. The genus *Narcissus* of the tribe Narcisseae has a typically west Mediterranean distribution while the genus *Sterenbergia* ranges from the Mediterranean to Iran. The tribe Galantheae has a Mediterranean-western Asiatic distribution. Southern Africa is the centre of the tribes Haemanthae and Amaryllideae. The latter being centred in the winter rainfall area of Southern Africa, although the genus *Crinum* has a pantropical distribution (1).

The mostly African tribe Amaryllideae consists of 11 currently recognized genera and approximately 155 species. It is classified into two monophyletic sub-tribes. The sub-tribe Crininae has four genera *Boophone*, *Crinum*, *Ammocharis* and *Cybistetes* while the sub-tribe Amaryllidinae includes the genera *Amaryllis*, *Nerine*, *Brunsvigia*, *Crossyne*, *Hessea*, *Strumaria* and *Carpolyza* (2). Within the sub-tribe Crininae, the genus *Cybistetes* is a Western Cape representative while *Ammocharis* is a widely distributed Sub-Saharan genus (3). The genus *Boophone* is also known to occur in the temperate winter-rainfall region of southern Africa, although the species *B. disticha* occurs widely in Central Africa (4).

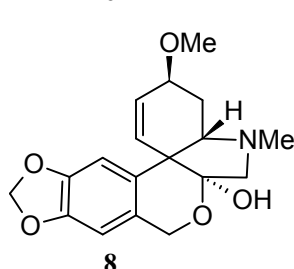
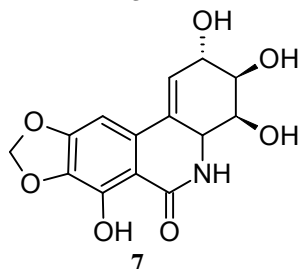
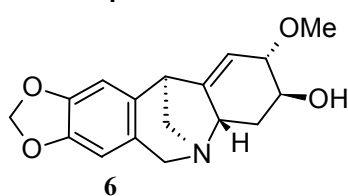
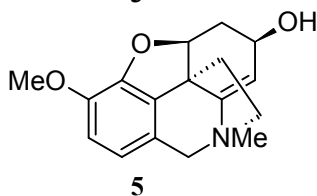
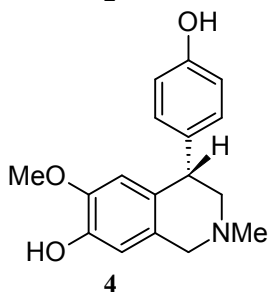
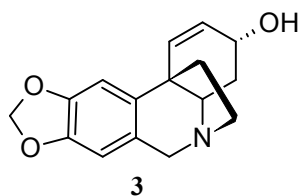
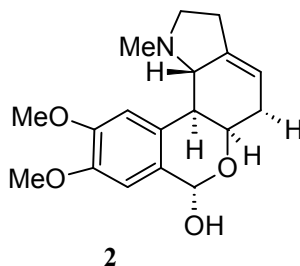
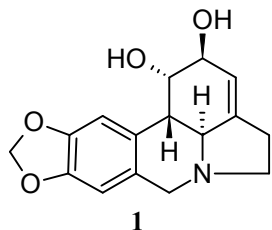
Phytochemistry

The poisonous and medicinal properties of certain species of the family Amaryllidaceae prompted many investigators in the nineteenth century to seek for active compounds from this family. The investigation of *Narcissus pseudonarcissus* in 1877 led to the isolation of lycorine **1** as the first alkaloid from this family (5). Since then, more than 300 such alkaloids have been reported. They are present in the species of almost all of the genera of the family Amaryllidaceae. More than 150 were isolated during the last two decades.

Most of the Amaryllidaceae alkaloids contain a ring system of fifteen carbon atoms which is divided in two parts. The first, containing an aromatic ring (ring A) and the benzylic carbon atom which is attached to either nitrogen or oxygen. The second contains an eight-carbon fragment and is composed of a six membered ring and a two carbon side chain which is attached to the nitrogen atom (6,7). The two fragments invariably attach to each other and to the basic nitrogen atom to give the different ring types of the Amaryllidaceae alkaloids listed below (8):

- Lycorine-type alkaloids derived from pyrrolo[3,2,1-*de*] phenanthridine/pyrrolophenanthridone;
- Lycorenine-type alkaloids derived from [2]benzopyrano[3,4g]indole) including both lycorenine and homolycorine;
- Galanthamine-type alkaloids derived from a dibenzofuran ring;
- Crinine-type alkaloids derived from 5,10b-ethanophenanthridine;
- Montanine-type alkaloids derived from 5, 11b-methanomorphanthidine;
- Cherylline-type alkaloids derived from tetrahydroisoquinoline;
- Narciclasine-type alkaloids; and
- Tazettine-type alkaloids derived from the [2]benzopyrano[3,4c]indole.

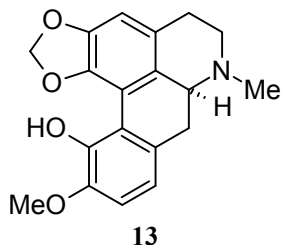
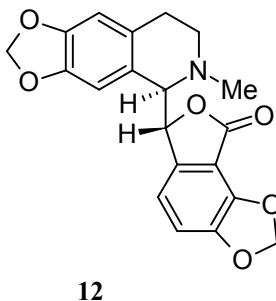
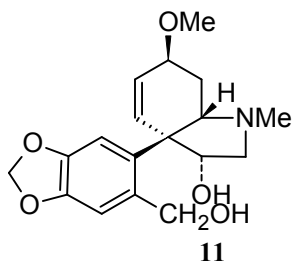
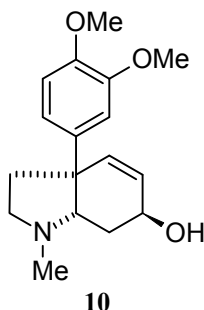
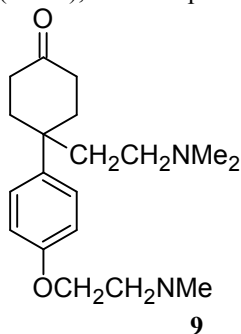
Representative examples of Amaryllidaceae alkaloids are lycorine **1**, lycorenine **2**, crinine **3**, cherylline **4**, galanthamine **5**, montanine **6**, narciclasine **7**, and tazettine **8**.



A characteristic feature of the Amaryllidaceae alkaloids is that they are produced exclusively by the members of this family (4,5,8). The only exceptions are amisine **9** from *Hymenocallis arenocola*, egonine **10** from *Hippeastrum equastre* and mesembrenol **11** from *Crinum oliganthum*. These three alkaloids belong to the mesembrine-type alkaloids which were found originally in the family Aizoaceae (9). The alkaloids (-)-capnoidine **12**, reported in Fumariaceae, and (+)-bulbocapnine **13**, from Papaveraceae, Fumariaceae and Lauraceae, were also isolated from *Galanthus nivalis subsp. cilicicus* (10).

Several reviews on the occurrence, distribution and synthesis of Amaryllidaceae alkaloids have been published (5, 6, 11-31). Special reviews have been made for the alkaloids of the genus *Crinum* (8, 32, 33) and alkaloids of the southern African Amaryllidaceae (4).

Non-nitrogenous constituents have also been isolated from the Amaryllidaceae. These includes lignans (10), flavonoids (34-36), chalcones (36-38), and triterpenes (39).



Ethnobotanical Use

Species belonging to the family Amaryllidaceae are used in different parts of the world for the treatment of various ailments and diseases. In Africa, the use of the amaryllidaceous plants in traditional medicine is largely centred in southern Africa for two reasons. Firstly, most African Amaryllidaceae tribes have their centre of variation in southern Africa. Secondly, traditional medicine forms an integral part of southern African culture (40, 41). The uses of different members of the African Amaryllidaceae are summarized below:

Bulb decoctions of *Apodolirion buchananii* are taken by Zulu people as purgative or administered as an enema for stomach ailments. Zulu also use unspecified parts of *Ammocharis coranica* medicinally for cattle and bulbs for witchcraft (42, 43).

Bulb decoctions of *Boophone disticha* are used by the Zulu to treat headaches, chest and bladder pains and hysteria. The Sotho and Xhosa use bulbs as a dressing for circumcision and narcotics while the leaves are used for the treatment of skin diseases. Khai and San people use bulbs as arrow poison (43, 44, 45). In Zimbabwe, the bulb is used for constipation, burns, oedema, wounds, rash, dizziness and lucky charms (46).

In Zulu traditional medicine, bulb decoctions of *Brunsvigia* species are used for coughs, colds, renal and liver complaints (44, 45). The southern Sotho take the bulb of *B. minor* for the relief of backache (44). The Xhosa use the outer skin of the bulb of *B. grandiflora* as a circumcision wound dressing to promote rapid healing. Roots of *Clivia miniata* are taken for snake bites, wounds, fever and to facilitate child birth. The leaves are used to induce labour while bulbs are used for infertility and urinary tract complaints (43, 44).

There is widespread use of *Crinum* species throughout the African Continent for the treatment of a variety of ailments. In southern Africa, *Crinum bulbispermum* is used by the Zulu and Tswana for the treatment of aching joints, rheumatism, varicose veins, backache, septic sores and abscesses. Tswana also use the bulb to treat kidney or bladder infections while in Sotho cultures the bulb is used for stimulation of milk production (44, 43). Bulb decoctions of *Crinum macowanii* are taken for the treatment of swelling of the body and treatment of urinary tract problems by the Zulu and for itchy rashes by the Xhosa (43, 44, 45). In Zimbabwe, bulbs are used as emetics and to stimulate milk production in both women and cows. The Zulu also use bulb decoctions of *Crinum moorei* and *C. delagoense* for swelling of the body and for urinary tract problems (43, 46).

In west Africa, the bulb of *C. jagus* is used in Nigeria by the Oju and Fulani tribes for the treatment of various cases of snakebite. In Seirra Leone, a cold infusion of the fresh leaves is used to bathe young children suffering from general body debility and rickets (47). Bulbs of *Crinum jagus* and *C. glaucum* are used in southern Nigeria to treat memory loss (48). Decoctions of bulbs of *C. giganteum* is also used in Nigeria as a vermifuge, purgative and in urinary ailments. Roasted bulbs, however, are used as rubefacient in rheumatism. The bulbs, eaten raw, are used for snake bites, for chronic cough and the treatment of asthma (49).

In east Africa, *C. kirkii* is used in Kenya for the treatment of sores. In Tanzania, the fruit and inner parts of the bulb are used as a purgative and the outer scales are used as a rat poison (50). In Madagascar, the bulb of *C. firmifolium* is used for the treatment of various parasitic skin diseases (38).

Members of the predominantly southern African genus *Cyrtanthus* (51) such as *C. breviflorus*, *C. contractus*, *C. mackenii*, *C. stenanthus* and *C. tuckii*, together with *Scadoxus multiflorus*, are famous as charms either as love charms or against storms and evil. Bulbs of *C. breviflorus* are also used by the Zulu to treat round- and tapeworms. Bulb infusions of *C. sanguineus* are taken regularly to ensure easy labour (43), while bulbs of *C. obliquus* are taken for scrofula and chronic coughs (44).

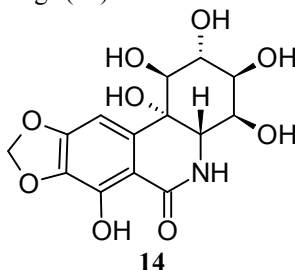
Alcoholic brandy or tincture of the fruit of different *Gethyllis* species such as *G. afra*, *G. ciliaris* and *G. linearis* have been used as a remedy for colic,

flatulence and indigestion (44, 45). The leaves of *Zephyranthus candida* is used by the Sotho as a remedy for diabetes mellitus (44).

The southern Sotho and Zulu tribes of South Africa have made use of decoctions of bulbs of the genus *Nerine* to treat coughs and colds, in renal and hepatic conditions, for the relief of backache and as a remedy for infertility (44).

Biological Activities

Extracts of some amaryllids, as well as alkaloids isolated therefrom, have exhibited various biological activities. These activities cover central nervous system, antitumor, antiviral, antibacterial, anti-inflammatory and antiparasitic conditions (8, 18, 52, 53, 54). However, the more recently demonstrated potent anticancer activity of pancratistatin **14** and the selective, reversible acetylcholinesterase (ACHE) inhibitory activity of galanthamine **5** has fuelled the search for bioactive Amaryllidaceae alkaloids toward the development of anti-tumor and anti-Alzheimer's drugs (55).



Central Nervous System Diseases

Alzheimer's disease

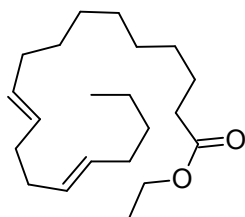
Alzheimer's disease is characterized by a progressive impairment of cognitive functions including the loss of memory and the inability to perform basic daily life (56). Based on the cholinergic hypothesis, these symptoms are the results of the reduction in brain acetylcholine activity due to the catabolism of acetylcholine by ACHE (57).

Recently, the Amaryllidaceae alkaloid galanthamine **5**, commonly found in the family Amaryllidaceae, was approved in many European countries for the treatment of Alzheimer's disease (58). Galanthamine **5** and other ACHE inhibitors act by inhibiting the activity of ACHE hence maintaining the levels of acetylcholine in the brain (59). Although galanthamine was originally isolated from European amaryllids it is also found in several African Amaryllidaceae (4). The long acting, selective, reversible and competitive ACHE inhibitory effect of galanthamine led to the search for other ACHE inhibitors from the family Amaryllidaceae (60-62).

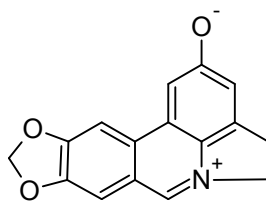
A number of African Amaryllidaceae species have been screened for ACHE inhibitory activity. Extracts from *Crinum jagus* and *C. glaucum*, collected from Nigeria, inhibited ACHE activity. The alkaloids hamayne **15** ($IC_{50}=250\ \mu\text{M}$) and lycorine **1** ($IC_{50}=450\ \mu\text{M}$) were isolated from the bulbs (48). *In vitro* activity against ACHE was also demonstrated for extracts of *C. moorei* (63,64), *C. macowanii*, *C. camanulatum*, and *C. graminicola*. All the species contained a band corresponding to galanthamine (64). Extracts of *C. variable* and *Nerine bowdenii* have also demonstrated marked inhibition of ACHE (64, 65). Linoleic acid ethyl ester **16** (66) and ungeremine **17** ($IC_{50}=0.35\ \mu\text{M}$) (67) have been identified as the compounds responsible for the inhibitory activity of these two species, respectively.

Twenty-two Amaryllidaceae alkaloids isolated from the South African amaryllids *Crinum bulbispermum* (68), *C. macowanii* (69), *C. moorei* (70) and *Cyrtanthus falcatus* (71) have been screened in our laboratory for ACHE inhibition activity (72). The alkaloid 1-*O*-acetyllycorine **53** ($IC_{50}=0.96\ \mu\text{M}$) exhibited inhibitory activity two-fold more potent than galanthamine ($IC_{50}=1.9\ \mu\text{M}$). However, the alkaloids crinine **3** ($IC_{50}=461\ \mu\text{M}$), crinamine **18** ($IC_{50}=300\ \mu\text{M}$), epivittatine **19** ($IC_{50}=239\ \mu\text{M}$), 6-hydroxycrinamine **20** ($IC_{50}=490\ \mu\text{M}$), *N*-desmethyl-8 α -ethoxypretazettine **21** ($IC_{50}=234\ \mu\text{M}$), *N*-desmethyl-8 β -ethoxypretazettine **22** ($IC_{50}=419\ \mu\text{M}$) and lycorine **1** ($IC_{50}=213\ \mu\text{M}$) only had weak activity. The studies have also revealed differences in activity related to different ring types of Amaryllidaceae alkaloids. Lycorine-type and galanthamine-type alkaloids were the most active against ACHE. Crinine-, tazettine- and cherylline-type alkaloids had only weak activity against ACHE.

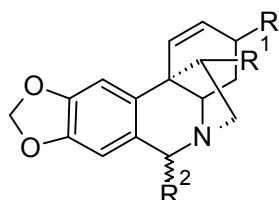
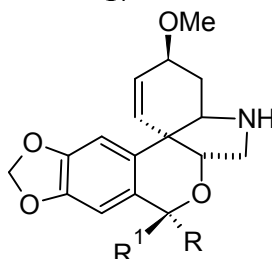
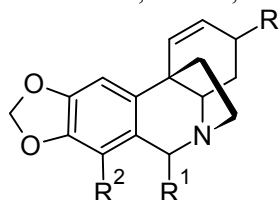
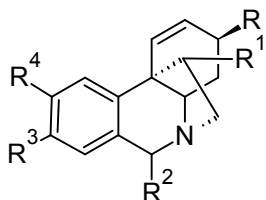
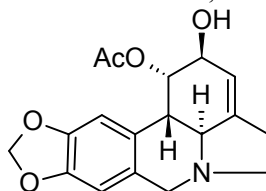
The mechanism of binding of galanthamine **5** to ACHE has been investigated. This has revealed that the double bond of the cyclohexene ring of galanthamine **5** stacks against the indole-ring binding site of the enzyme while the *O*-methyl group of galanthamine **5** occupies the acetyl-binding pocket of acetylcholine (73). Research in our laboratory revealed that the mechanism of binding of 1-*O*-acetyllycorine **53** to ACHE enzyme might not be the same as that of galanthamine (74). From the analysis and superpositioning of 1-*O*-acetyllycorine **53** and other related lycorine-type alkaloids on galanthamine, it appears that the methoxy group of galanthamine partially aligns with the methylene dioxy group of the lycorine-type alkaloids, while the double bond of the cyclohexene does not align with any part of lycorine-type alkaloids (74).



16



17

15 R=OH α , R¹=OH, R²=H18 R=OM α , R¹=OH, R²=H19 R=OH α , R¹=H, R²=H20 R=OM α , R¹=OH, R²=OH $\alpha\beta$ 21 R=OEt, R¹=H22 R=H, R¹=OEt25 R=OH α , R¹=H, R²=OMe27 R=OMe β , R¹=R²=H35 R=OMe α , R¹=OH α , R²=H26 R=OH, R¹=R²=H, R³=R⁴=OMe28 R=OMe, R¹=R²=H, R³=R⁴=OMe29 R=OMe, R¹=OH, R²=OH, R³+R⁴=OCH₂O30 R=OMe, R¹=OH, R²=H, R³+R⁴=OCH₂O

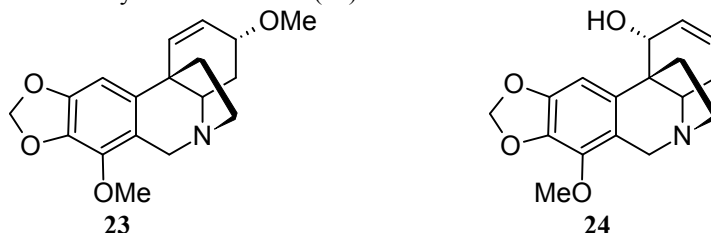
53

Depression

Depression is a common psychopathology that causes significant impairment and incurs large personal and social costs in the form of lost productivity and health care expenses (75). Reduction of serotonergic neurotransmission is strongly implicated in the neuropathology of depression (76). There are several antidepressants in the market exerting their effect by

selective inhibition of serotonin re-uptake and are known selective serotonin re-uptake inhibitors (SSRI) (77). The mechanism of SSRI is to bind to a specific site on the neuronal serotonin transporter with resulting inhibition of the transportation of serotonin from the synaptic gap back to the neuron (78).

A few South African amaryllids have been investigated for their affinity to the SSRI site on the serotonin transporter *in vitro*. Extracts of the leaves and bulbs of *Boophone disticha* have exhibited affinity to the SSRI site. The alkaloids buphanadrine **23** ($IC_{50}=274 \mu M$) and buphanamine **24** ($IC_{50}=1799 \mu M$) were found to be the active compounds (79). Leaf extracts of *Brunsvigia grandiflora* have moderate affinity while root extracts of *Gethyllis ciliaris* had low affinity to the SSRI site (77).



Research conducted on twenty-one Amaryllidaceae alkaloids isolated from different members of the South African Amaryllidaceae, for their affinity to the SSRI site, showed that cherylline **4** had the highest affinity ($IC_{50}=3.4 \mu M$) followed by epivittatine **19** ($IC_{50}=12.1 \mu M$), powelline **25** ($IC_{50}=20 \mu M$) and maritidine **26** ($IC_{50}=20 \mu M$). The alkaloids epibuphanisine **27** ($IC_{50}=78.2 \mu M$) and *O*-methylmaritidine **28** ($IC_{50}=40.1 \mu M$) had moderate affinity while crinine **3** ($IC_{50}=267.2 \mu M$), crinamine **18** ($IC_{50}=608.7 \mu M$) and 1-*O*-acetyllycorine **53** ($IC_{50}=452 \mu M$) showed weak affinity to the protein (80).

The majority of the alkaloids that had affinity to the serotonin transporter (SERT) were crinine-type alkaloids. However, cherylline **4**, tazettine **8** and 1-*O*-acetyllycorine **53** are the only alkaloids that showed affinity to SERT among the cherylline-, tazettine- and lycorine-type alkaloids, respectively (80). The activity of the crinine-type alkaloids was attributed to the presence of a 1,3-dioxole moiety in common with the clinically used SSRI paroxetine (79).

Others

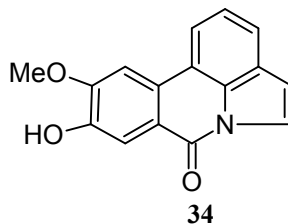
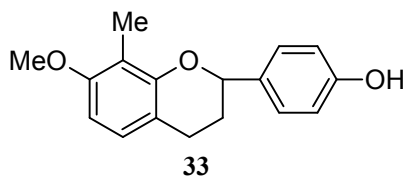
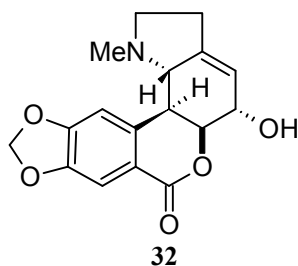
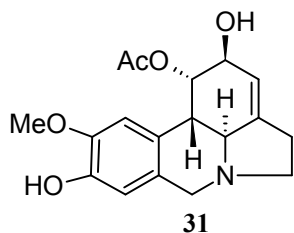
Members of the Amaryllidaceae have also shown activity against central nervous system diseases other than depression and memory loss. For instance, the aqueous extracts of *C. giganteum* bulbs prolonged the duration of penobarbital sleeping time in rats. It also reduced spontaneous motor activity, decreased the exploratory activity and attenuated amphetamine-induced stereotype behavior in mice. These results seem to be predictive of the central sedative properties of the extract and the possible application in anxiety conditions (49).

Antitumor Activity

The use of extracts from the family Amaryllidaceae in the treatment of tumors can be dated back to the times of Hippocrates and Pliny. The application of these extracts in the treatment of tumors continued by practitioners of the middle ages throughout the old world (81). Lycorine **1**, the most widely distributed alkaloid in this family, including species endemic to Africa, showed various biological effects on tumor cells. Lycorine **1** inhibited protein synthesis in eukaryotic cells by preventing peptide bond formation (8, 54). It also showed significant cytotoxic effects against human breast cancer, human fibro sarcoma, human lung cancer, human melanoma, human colon cancer, murine lymphoid neoplasm, human epidermal carcinoma, hormone dependent human prostatic cancer, hormone dependent breast cancer and human glioblastoma cell lines (82, 83). It has recently been established that lycorine **1** acts as an anti-cancer agent by arresting the cell cycle at G2/M phase and induction of apoptosis in HL-60 cells (84). After treatment of human multiple myeloma cell line KM3 with **1**, typical apoptotic events could be observed. Lycorine was also able to block the KM3 cell cycle at G0/G1 phase through the down-regulation of both cyclin D1 and CDK4 (85). Lycorine, when tested in the human leukemia xenograft model, appeared to exhibit anti-tumor activity *in vivo*, thus is a useful therapy against acute promyelocytic leukemia (86). However, other studies concluded that lycorine together with haemanthidine **29** and haemanthamine **30** are not substrates of the glycoprotein responsible for the efflux-pump activity of the tumor cells (87).

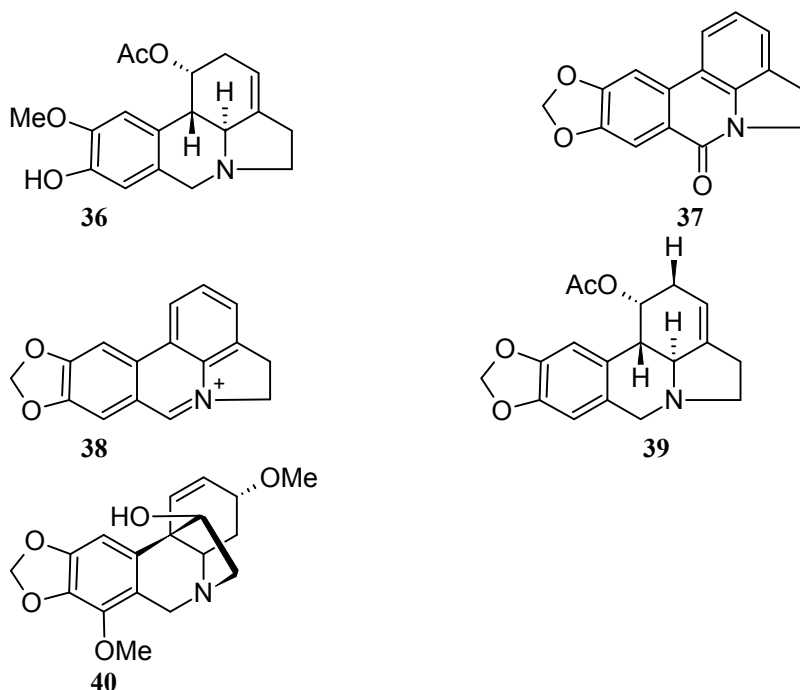
The alkaloids crinamine **18**, from *Brunsvigia radulosa*, (88) and haemanthamine **30**, from *Cyrtanthus elatus*, (89) showed a selective apoptosis-inducing activity to animal cancer cells over non-cancerous human cells (55). Structure-activity relationships demonstrated the requirement of the alpha-5, 10b-ethano bridge and a free hydroxyl at C-11 as pharmacophoric requirements for the activity. In another study, crinamine **18**, from *Brunsvigia josephinae*, (90) proved to be cytotoxic against human tumor cell lines (Molt4 and HepG2) and a murine non-tumor cell line (91). Sternbergine **31** (from *Crinum firmifolium*) and hipeastrine **32** exhibited similar activities in the same assay.

Investigation of the cytotoxic effects of the chemical constituents of the two *Crinum* species growing in Egypt namely *C. bulbispermum* and *C. augustum* revealed that the flavan 4'-hydroxy-7-methoxyflavan **33** together with the alkaloids pratorinine **34** and 6 α -hydroxybuphanisine **35** showed cytotoxic activity against human leukemic Molt4 cells (34).



A hot water bulb extracts of *Crinum delagoense*, taken orally in South Africa to cure human cancer, have shown activity against BL6 mouse melanoma cells. Subsequently, an investigation of the ethanolic extracts of the bulb of this species led to the isolation of six alkaloids of which only the water soluble alkaloids lycorine **1** and 6-hydroxycrinamine **20** were active against BL6 mouse melanoma cells (92).

Inhibitory activity of extracts of *Haemanthus natalensis* against KB cell cultures, *Amaryllis belladonna* and *Brunsvigia radulosa* against P 388 lymphocytic leukaemia in mice were reported (93). Further investigation of *B. radulosa* resulted in the isolation of eight alkaloids. Three of these alkaloids namely, 1-*O*-acetylnorpluviine **36**, anhydrolycorin-6-one **37** and sternbergine **31** gave strong to moderate toxicity against BL6 mouse melanoma cells (88). *Amaryllis belladonna* however, yielded anhydrolycorinium chloride **38** as the principal antineoplastic component. The alkaloids acetylcaranine **39** and ambelline **40**, from this plant, have also shown activity against these cell cultures (94).

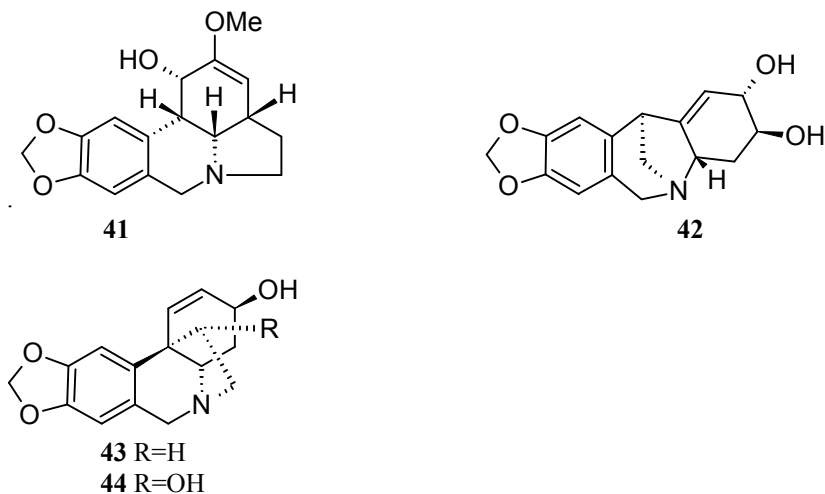


Antibacterial Activity

Dichloromethane extracts of *Cyrtanthus falcatus*, *C. mackenii* and *C. suaveolens* had low antibacterial activity against both Gram-positive and Gram-negative bacteria (95).

Lectin-like proteins from *Crinum moorei* aggregated *Staphylococcus aureus* at 19 $\mu\text{g/ml}$ and inhibited bacterial growth at a concentration of 0.8 mg/ml. No such effects were observed against *Bacillus subtilis* (96).

Crinamine **18** was the principal antibacterial constituent of the bulbs of *Crinum jagus* (97). The alkaloids amarbellisine **41** (MIC=22 $\mu\text{g/ml}$), pancracine **42** (MIC=22 $\mu\text{g/ml}$), vittatine **43** (MIC=19 $\mu\text{g/ml}$), and 11-hydroxyvittatine **44** (MIC=17 $\mu\text{g/ml}$), isolated from the Egyptian *Amaryllis belladonna*, had activity against the Gram-positive *Staphylococcus aureus*. Both amarbellisine **41** (MIC=22 $\mu\text{g/ml}$) and vittatine **43** (MIC=22 $\mu\text{g/ml}$) exhibited activity against the Gram-negative *Escherichia coli* whereas, pancracine **42** (MIC=16 $\mu\text{g/ml}$) showed activity against *Pseudomonas aeruginosa* (98).



Antifungal Activity

Extracts from Zimbabwean *Crinum macwanii*, *C. moorei* and *Amaryllis belladonna* have exhibited activity against *Candida albicans* (4, 99). Further investigation of *Amaryllis belladonna* led to the isolation of lycorine **1** (MIC=39 $\mu\text{g/ml}$), hippastrine **32** (MIC=125 $\mu\text{g/ml}$), amarellisine **41** (MIC=63 $\mu\text{g/ml}$), pancracine **42** (MIC=188 $\mu\text{g/ml}$), vittatine **43** (MIC=31 $\mu\text{g/ml}$), and 11-hydroxyvittatine **44** (MIC=156 $\mu\text{g/ml}$), as the active compounds against *Candida albicans* (98).

Antiviral Activity

The hot water extracts of bulbs and leaves of *Haemanthus albiflos* had strong antiviral activity against Poliovirus 1, Herpes simplex 1 virus, Vesicular stomatitis virus and simian Rotavirus SA II (100, 101). The bulbs of this species also showed strong antiviral activity against Moloney murine leukemia virus and HIV (102).

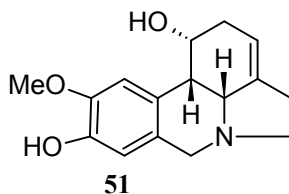
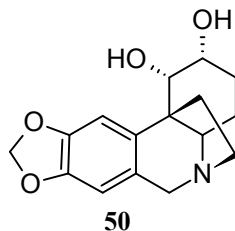
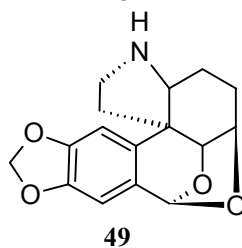
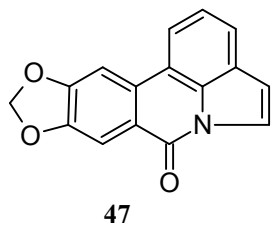
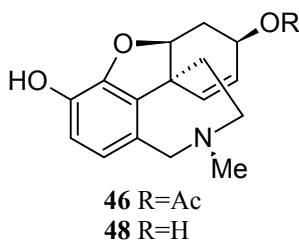
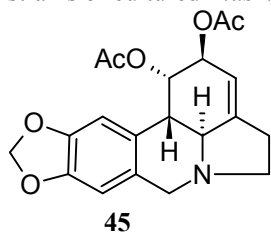
Crude extracts from the roots and leaves of *Clivia miniata* showed strong activity against Poliomyelitis, Coxsackie, Semliki forest, measles and herpes simplex viruses (103). This has led to the isolation of lycorine with inhibition occurring as low as 1 $\mu\text{g/ml}$ against Poliomyelitis virus (104). The methanolic extracts of Zimbabwean *Crinum macowanii* also exhibited activity against exotic RNA viruses *in vitro* (105).

Anti-parasitic Activity

Alkaloids isolated from *Crinum kirkii*, from Kenya, have been investigated *in vitro* against *Trypanosoma brucei rhodesiense* and *T. cruci*. The alkaloids

1,2-di-*O*-acetyllycorine **45** and 3-*O*-acetylsanguinine **46** showed activity against *Trypanosoma brucei rhodesiense* with an IC₅₀ of 1.0 and 1.1 µg/ml, respectively. However, hippadine **47**, sanguinine **48**, noraugustamine **49**, amabiline **50** and kirkine **51** showed very low activity with an IC₅₀ of 8.4, 18.7, 22.5, 31.9 and 90 µg/ml, respectively. 3-*O*-acetylsanguinine **46** also showed some activity against *T. cruzi* with an IC₅₀ of 2.3 µg/ml (106).

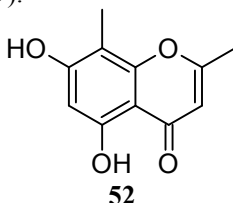
Alkaloids from the South African *Brunsvigia radulosa* have been evaluated for activity against two strains of cultured *Plasmodium falciparum*. The alkaloids 1-*O*-acetylnorpluviine **36**, anhydrolycorin-6-one **37**, crinamine **18**, hamayne **15** and sternbergine **31** demonstrated moderate antiplasmodial activity (88). Lycorine **1** and 1,2-di-*O*-acetyllycorine **45**, from bulbs of *Brunsvigia littoralis*, have also been reported to show antimalarial activity against two strains of cultured *Plasmodium falciparum* (107).



Anti-inflammatory Activity

The anti-inflammatory activity of extracts of different parts of *Cyrtanthus falcatus*, *C. mackenii*, *C. suaveolens*, *Gethyllis ciliaris*, *G. multifolia* and *G. villosa* have been investigated using Cyclooxygenase-1 and -2. All dichloromethane extracts tested showed strong inhibitory activity against both COX enzymes compared to aqueous methanolic extracts. The high inhibitory activity of the apolar extracts suggests that the non-alkaloidal constituents are responsible for this activity (95). This was supported further by the fact that

work in our laboratory on the activity of some Amaryllidaceae alkaloids had no or low anti-inflammatory activity (108). Further investigation of the underground parts of *G. ciliaris* has resulted in the isolation of the COX-1 inhibitor isoeugenitol **52** (109).



The ethanolic extracts of the inner scales of the bulb of *Boophone disticha* exhibited a significant decrease in ATP production while superoxide production was significantly inhibited by aqueous extracts of the inner and outer scales of the bulb when tested in isolated human neutrophils (110).

The anti-inflammatory, antilymphocytic and analgesic properties of the aqueous extracts of *C. giganteum*, widely spread in the northern states of Nigeria, have been investigated. The extracts produced significant effects in formalin-induced pain and on the cotton-pellet-induced granulomatous tissue formation in rats, and on abdominal constriction induced with acetic acid in mice (111). Aqueous extracts of *C. glaucum* have also shown inhibition of the oedema in the carrageenan-induced paw swelling model (112).

Micellaneous

Aqueous extracts of *Clivia miniata* and *Crinum glaucum* have caused contractions in both the uterus and ileum of guinea pigs (113, 114).

The aqueous extracts of *Crinum glaucum* caused an increase in tidal volume, increase in ventilatory rate and depth (115). The extract also inhibited the quantity of mediators antigenically released from the lungs, inhibited mast cell degranulation and reduced the mepyramine resistant activity from the lungs. These effects substantiate the efficacy of the extract in the treatment of asthma (116, 117). Aqueous and ethanolic extracts of *Crinum zeylanicum* have shown high mortality rate of the molluscides *Biomphalaria pfeifferi* and *Lymnaea natalensis* (118).

Conclusions and Future Directions

Most of the research conducted on the Amaryllidaceae plants during the last century focused on their chemistry. Few of these plants or their chemical constituents have been explored for their pharmacological potential. However, the discovery that galanthamine and pancratistatin have notable acetylcholinesterase inhibitory and antineoplastic effects has fuelled research on the therapeutic effects of Amaryllidaceae alkaloids throughout the world and Africa is no exception. While some of these alkaloids have demonstrated

various biological activities when investigated in *in vitro* assays only few of them, however, have been investigated *in vivo*.

Despite these important discoveries, African Amaryllidaceae remains untapped specially in the southern African region. Few species have been investigated for their biological activity mostly in South Africa and Egypt and to a lesser extend in Kenya and Nigeria. Information from other parts of the continent are sparse or lacking.

The majority of the active constituents from the family Amaryllidaceae are alkaloids. These alkaloids have been isolated first and thereafter evaluated for their biological activities. A bioassay guided isolation of active extracts may lead to the isolation of interesting non-nitrogenous compounds that could be used as leads for the synthesis of useful therapeutic drugs.

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Chapter 9

Biology and Chemistry of the Genus *Aloe* from Africa

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Aloe is a medicinal plant that has been used since ancient times by cultures in many countries and continents for many of the same applications. Africa is blessed with a wide variety of *Aloe* spp. each used by the indigenous cultures. Fleshy leaves are the source of two of the main products, gel and latex, with both products showing distinct differences in their chemical composition. Chemical composition of gel consists mainly of water with carbohydrate polymers and a range of other organic and inorganic components. In contrast, the chemical composition of the latex includes many phenolic compounds such as anthraquinones and chromones. The objective of this paper is to review the the botany, chemistry and pharmacological properties of Aloes from Africa.

Introduction, Botany and Ecology

Aloe's has been used in folk medicine for over 2000 years, and remains today, an important component in the traditional medicine of many cultures (1, 2). In the past decades, the popular use and interest of aloe gel products for the food, cosmetic and pharmaceutical industries has dramatically increased (2, 3).

Aloes belong to the Aloaceae family and comprise a large genus of over 300 sp (4, 5). The genus is native to Africa from the southern Cape in South Africa to about 15°N north to the southern parts of the Arabian Peninsula (4).

Aloes vary in size from 30 mm to over 15 m high and they are more or less succulent (Figure 1) (6, 7).

Aloe species are found naturally growing primarily in mainland Africa, with the majority of species in southern Africa and on the eastern side of the African continent. The largest numbers of *Aloe* species are found along the moister central north-south mountain ranges of mainland Africa, and in the arid south and south-west of the Madagascar island. Some species are very widespread in distribution, such as *A. buettneri* (6), while others have a very restricted distribution. Newton (8) prepared a geographical distribution list of *Aloe* species including the total number of species and endemic species in different countries of Africa. Madagascar and the isolated Indian Ocean Islands are the ones with highest rate of endemism (100%).

Aloes can grow in a wide range of habitats from forests to exposed rock surfaces, however, they are absent in moist lowland forest of mainland of Africa. The genus occupies a considerable altitudinal range from sea level up to about 3,500 meters above sea level. For example, *A. dichotoma* has been proposed as a candidate for studying climate change in the arid Desert and Nama Karoo biome in Namibia (9). In the wild, Aloes occur on a wide range of soil types and substrates. They can grow on dolomite, granite, gypsum and limestone (8). Garcia-Hernandez et al. (10) identified significant nutrient interactions of the Aloe crop growing in an irrigated calcareous desert soil.

The phenotypic diversity within and between *Aloe* spp. is significant. Some Aloe plants have short stems that are completely hidden by the leaves. They are succulent plants with perennial, strong and fibrous roots and numerous leaves, carrying spines in their margin. The leaves can be arranged in rosettes and some species have underground bulb. Yet, other species have long stems being arborescent, shrubby, sprawling, climbing or pendulous. Arborescent species can reach 15 meters height and may be branched or unbranched. Flowers are produced on racemose inflorescences. Usually the racemes are erect, but in some cases they are oblique or more or less horizontal. In most cases the flowers are brightly colored and very conspicuous (6-8).

Aloe juice has been used since ancient times in the treatment of several diseases as well as for cosmetic use by different cultures over history (11). It is likely that these diverse uses have been at least partially responsible for the spread of some of the Aloe onto different continents. Today, *Aloe vera* is considered to be the most popular and most widely internationally traded and the one most extensively researched (3).

As observed with many other species including medicinal plants, the destructive harvest of some of the Aloe, its over-collection and the inadvertent destruction of plants from harvesting the leaf exudates as well as the loss of the plants habitat has led in some regions to the Aloe being threatened. Some Aloe species have been reported as endangered species (8). In vitro culture is a powerful tool that offers the possibility to produce thousands of genetically identical plants within a short time frame, thus providing one avenue of preserving germplasm (12). A few *Aloe* species have been successfully propagated through tissue culture techniques. For instance, a rapid micropropagation protocol of endangered medicinal *Aloe vera* L var. *chinensis* (13) and *A. polyphylla* (14) have been reported. Also, in vitro regeneration of *A.*

arborescens via somatic embryogenesis from young inflorescences as source of starting material has been achieved (15).

Chemistry

The chemistry of aloe has been studied for many years (2, 4, 16, 17). *Aloe vera* is extensively recognized for containing a number of unique organic phytochemicals that showed medicinal properties. Although there is a large number of *Aloe* species, only few of them are of economic importance (16, 18). Though this paper focuses on the Aloes of Africa, we will be reported selected findings of *Aloe vera*, as the information is more extensive and lessons can be gleaned from the biological understanding of that species.

Most of the research has been focused on the commercial aloes including *A. vera* and *A. ferox*, the southern African aloe. Leaves of *A. vera* plants produce two major medicinal products: (i) a yellow latex, or exudate, mainly consisting of bioactive phenolic compounds such as anthraquinones and chromone (19-21) and (ii) a mucilaginous jelly from the parenchyma cells of the plants. The last one is known as “*Aloe vera* gel” (3, 17). The gel is mainly composed of water and mucilage containing a high content of polysaccharides. Enzymes such as oxidase, a catalase, and amylase have also been reported (3, 17, 22). Many of the beneficial effects of this plant have been attributed to the presence of polysaccharides. Acetylated mannan is the primary polysaccharide in the pulp (2, 22). The predominant monosaccharides in the pulp are manose and glucose, xylose, rahamnose, galactose, arabinose fructose and uronic acid (22).

Besides leaves, roots are also a storage site for the accumulation of many interesting secondary metabolites such as anthraquinones, pre-anthraquinones, anthrones, chromones, alkaloids, flavonoids, coumarins.

One of the main groups of phenolic constituents are anthraquinones, and these have been reported in roots of aloe (16, 24, 25), in leaf exudates of *A. elgonica* (26) and *A. ferox* (27). However, in one study, anthraquinone glycosides were not been detected in the root (16).

Other group of phenolic constituents are the chromones. Using HPLC analysis, aloesin, aloin and aloeresin A have been identified in many *Aloe* species (4, 16, 24, 28, 29) (Figure 2). 7-*O*-methylaloeresin A has been reported in *A. marlothii* and *A. ruperstris* in leaf exudates (30). In addition, a number of chromones with different structures present in the gel of *A. vera* have been identified and reported (19, 20, 31). Also, 5-methylchromone derivates have been described from *A. broomii*, *A. africana*, and *A. speciosa* (32) (Figure 1). Aloeresin H (8-*C*- β -D-glucopyranosyl-7-hydroxy-5methylchromone) has been elucidated by degradation experiments combined with 1D and 2D NMR data from *A. ferox* (33) (Figure 1).

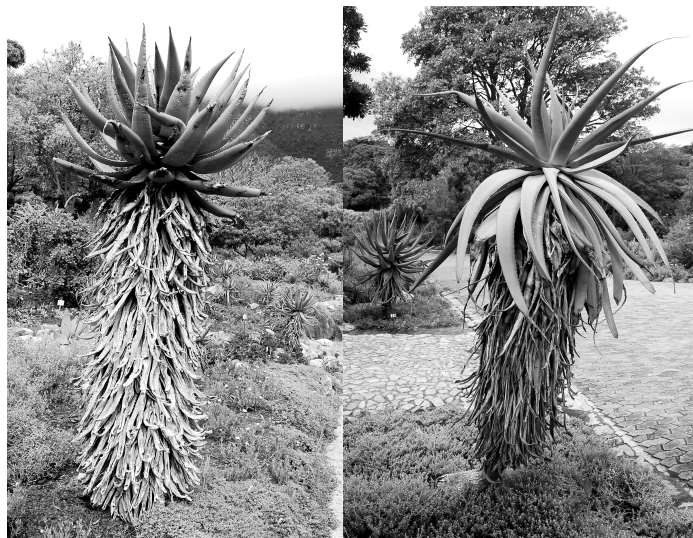


Figure 1. *A. ferox* (left) and *A. speciosa* (right) from South Africa.

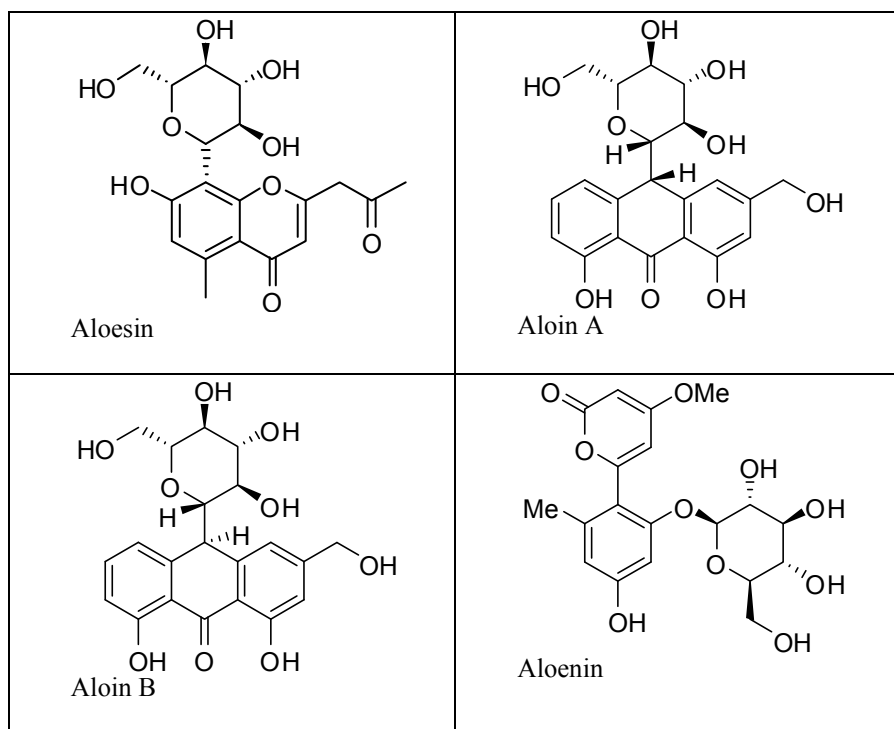


Figure 2. Chemical structures of some components isolated from *Aloe* species.

Recently, Duri et al. (34) isolated from Kenyan commercial aloes two new compounds, 10-*O*- β -D-glucopyranosyl aloenin and 8-C- β -D-glucopyranosyl-7-*O*-methyl-(*R*)-aloesol together with aloenin (phenylpyrone derivative), aloenin 29-*p*-coumaroyl ester, aloenin aglycone, orcinol and acetylorscinol.

Anthrones are also an important group of components present in *Aloe* species, Aloin A and B collectively known as barbaloin are the most important bioactive phenolic component (16, 35, 36). Also, a number of oxantrones have been reported occurring in different *Aloe* sp. 10-hydroxylaloin B 6'-*O*-acetate was isolated from *A. claviflora* (37), C-*O*-diglucosylated oxantrone (also known as littoraliside) from leaf exudates of *A. littoralis* (38), and 5 Hydroxylaloin was isolated from *A. microstigma* (39). Aloenin was isolated from *A. arborescens* and later reported in other *Aloe* sp (36, 40, 41).

Dagne et al. (42) revealed the presence of 10-hydroxylaloin B and deacetylittoraloin from leaf exudates of *A. littoralis*.

Several methods are currently used to identify the secondary metabolites of aloe, such as Thin Layer Chromatography (TLC) (30, 43), High Performance Liquid Chromatography (HPLC) (44), High Performance Liquid Chromatography in combination with nuclear magnetic resonance spectroscopy (HPLC-NMR) (45), High-Speed Countercurrent Chromatography (HSCCC) combined with traditional pretreatment (46), Gas Chromatography/ Mass Spectrometry (CG/MS) (47, 48).

Later, Karagianis et al. (45) showed the potential of using HPLC-NMR (High Performance Liquid Chromatography in combination with nuclear magnetic resonance spectroscopy,) in the structure elucidation of aloe metabolites without prior isolation.

Flavonoids are widely distributed in many groups of plants. Viljoen et al., (49) showed that flavonoids occur as major compounds in 31 *Aloe* spp. out of the 380 studied. Four major flavonoids were detected in aloe: naringenin (flavanone), dihydroisorhamnetin (dihydroflavonol) and aspienin and isovitexin (flavones). Moreover, the distribution of the different flavonoids provides valuable chemotaxonomic evidence (49).

Although aloe plants are known as medicinal plants, there are actually a few studies showing aloes to be poisonous (50). A few local species such as *A. gillilandii*, *A. ballyi*, *A. ruspoliana*, *A. ibitiensis* and *A. deltoideodonta*, among others, has been reported to contain toxic hemlock alkaloids especially γ -coniceine (51, 52). These compounds may be easily recognized because of their mousy smell. Further phytochemical investigations confirmed the presence of hemlock alkaloids in *A. sabaia* and also the presence of a new chlorinated amide, N-4'-chlorobutylbutyramide (53). In view of the potential implications of the presence of alkaloids, screening for alkaloids before recommending an aloe species as medicinal has been suggested (16).

Although *A. vera* and *A. ferox* species are the most important commercial in the international markets, it is largely the presence of anthrones and polysaccharides content that determine the plants effectiveness for therapeutic activity. There are other *Aloe* species that could be promoted for similar purposes when those species contain one or both groups of bioactive compounds. The biological activities of the *Aloe* sp could be attributed to synergic actions of the components rather than from a single component (16).

Moreover, *Aloe* spp. endemic from Madagascar such as *A. suzannae*, *A. helenae* and *A. vaotsanda* contain unique flavonoids (16, 49).

Biological Activity

Aloe plants have always been used for its medicinal and therapeutic properties (54), although there was not any clear understanding of the basis of such properties.

Antioxidant Activity

Oxidation represents a crucial part of aerobic life and our metabolism. It consists in the transfer of electrons from one atom to another. Sometimes, the electron flow becomes uncoupled, generating thus free radicals. These molecules are unstable and very reactive with all biological molecules, producing in some cases irreversible damages or destruction in a variety of tissues. Oxidative damage plays an important role in many degenerative processes and diseases. Therefore, there is a strong interest in the search of natural antioxidant products from plants with low cytotoxicity. There are many mechanisms of antioxidant activity including scavenging free radicals, inhibiting the enzymes that produce free radical, or protecting the antioxidant defenses (55).

Several authors have investigated the antioxidant components in *A. vera* products of the exudate. Yagi et al., (56) reported the DPPH radical and superoxide anion scavenging activity in seven aloesin derivatives. Later, Beppu et al., (57) reported the oxygen scavenging activity of the two phenolic compounds (2'-*O-p*-coumaroylaloetin and 2'-*O*-feruloylaloetin), preventing thus the destruction of the pancreatic islets. The antioxidant activity of aloemodin depends also on scavenging hydroxyl radicals (58, 59). Moreover, it appears that the antioxidant or pro-oxidant activity of aloemodin is related to its concentration (60).

In vitro studies have demonstrated that *A. vera* latex derived anthraquinones in presence to ultraviolet light A (UVA) exhibited significant photo-oxidative damage to both cellular RNA and DNA (61). Several studies on photostability and phototoxicity of *A. vera* extracts indicate that in presence of UV light it can generate the formation of free radicals (62, 63).

Polysaccharides that are mainly found in the gel are also a group of compounds that exhibit antioxidant activities. It was demonstrated that APS-1 (mainly composed by mannose glucose in ratio 18:5) was effective in scavenging superoxide anion radical (dose-dependent fashion), hydroxyl radical, suppressed conjugated diene formation from LDL oxidation induced by Cu^{+2} , and exhibited a protective effect on hydrogen peroxide-induced injury in PC12 cells (64). Also Kardosova et al. (65) showed that *in vitro* experiments with acidic and neutral polysaccharides were able to prevent lipid peroxidation by scavenging hydroxyl radicals.

Finally, it has been reported that the growth stage of the aloe plants play an important role in the composition of the active constituents and thus in the antioxidant activity (66). Strong antioxidant activity was observed from the components of the inner gel. For example, Wu et al. (64) were able to isolate the main polysaccharide of leaf (APS-1) which is basically composed by mannose and glucose (18:5). APS-1 exhibit significant free radical scavenging and antioxidant activity, and protective effects on hydrogen peroxide-induced injury in PC12 cells.

Traditional uses of Malagasy *Aloe* sp (*A. vahombe* and *A. divaricata*) include as purgative (decoction of leaves, very bitter), the leaf juice is used to consolidate limb fractures and the plant is also known for its ocytotoxic properties (67).

Anti-inflammatory Activity

Inflammation is a normal and complex reaction by the body to an injury. Aloe has been used in traditional medicine for its anti-inflammatory use. The aqueous and chloroform extracts of aloe inhibit the effect on carrageen induced edema. This effect was associated with an inhibitory action on the arachidonic acid pathway via cyclooxygenase (68). Aloe gel components were able to suppress bacterial induced pro-inflammatory production of cytokines (systematically elevated after a bacterial invasion), namely TNF- α and IL- β (69).

Aloe vera was evaluated as topical anti-inflammatory agent, and cinnamoyl-C-glucosylchromone (isolated from the exudates) was able to reduce croton oil-ear inflammation at an activity level comparable to hydrocortisone (70). Speranza et al. (71) demonstrated that 5-methylchromone (aloesin I) from dried exudates from *A. ferox* was able to reduce the oedema response induced by croton oil in the mouse ear. Moreover, Yagi et al. (56) pointed out the activity of p-coumaroyl and feruloyl groups on the inhibition of the cyclooxygenase 2 and thrombosane A₂ synthase by aloin derivates. Species with higher concentrations of flavonoids (e.g. *A. pratensis*, *A. humilis* and *A. pretoriensis*) showed high anti-inflammatory activity with values similar to those plants that accumulate anthrones and chromones (*A. wickensii*) (72).

Anti-diabetic Activity

Diabetes mellitus is one of the world's major diseases, and since ancient times many cultures have been using medicinal plants to control diabetes (73).

Several reports have pointed out the beneficial effects of *A. vera* in controlling blood glucose. As such, *A. vera* has been cited as one of the seven most promising herbs or supplements to control blood glucose in blood (74). Aloe gel, rich in glucomannan has been reported to have hypoglycemic effects; however the mechanisms have not been elucidated (75). In addition, Aknimoludam et al. (76) postulated that aloe preparations not only reduce the plasma glucose levels, but also can be used as a prophylactic agent to prevent

the hyperglycemia. Okyar et al. (77) observed hyperglycemic activity in *A. vera* leaf extract and suggested the potential use on the treatment on non-insulin dependant diabetes mellitus.

Anti-cancer Activity

Aloe-emodin (hydroxyanthraquinone) has been reported with a unique *in vitro* and *in vivo* antineural tumor activity with selective toxicity (78). Aloe-emodin not only can behave as an anti-tumor agent, but also as anti-angiogenic compound. Angiogenesis is a complex process, and it is required both for cancer progression and metastasis. Under certain pro-angiogenic signal cells are activated and are involved formation and differentiation of blood vessels. It was also suggested, that it could be a candidate drug for photodynamic therapy (79).

Other compounds, such as di (2-ethylhexylphthate) have shown antileukaemic and antimutagenic effect and were extensively investigated by Lee and collaborators (43, 80).

The effect of aloe polysaccharides on chemopreventive agent was examined on inhibition of formation of B[a]P-adducts DNA *in vitro* and *in vivo* (81, 82). Also, a mannose rich polysaccharide, PAC-I isolated from aloe has shown to have potent stimulatory activity on murine peritoneal macrophage. When PAC-I was administrated *in vivo* is capable of prolonging the survival of tumor bearing mice (83).

Wound Healing Activity

Of all the uses of aloe, one of the most popular is for the treatment of wounds and burns (2, 3). However, the complete composition of aloe components and the component responsible of wound healing is still being unraveled. Restoration of the tissue integrity is one of the fundamental processes during wound healing. The early stages of wound healing are characterized by laying down provisional matrix, follow by the formation of granulation tissue and synthesis of collagen and elastin. Collagen is the major protein of extracellular matrix and it is the predominant constituent of the final scar. Wounds treated with *A. Vera* not only increased the provisional matrix (84) but also the collagen content (85).

Choi et al. (86) were able to isolate a glycoprotein fraction (5.5 kDa) that is involved in the wound healing effect of *A. vera* via cell proliferation and migration. This glycoprotein fraction was able to accelerate the wound healing on a monolayer of human keratinocytes and also to enhance wound healing in hairless mice with significant cell proliferation.

There is cumulative evidence supporting the effectiveness of *A. vera* products in burn healings, radiation skin reactions, but further well designed trials with sufficient details of contents should be carry out (87, 88).

In the wound healing process, infection also plays an important role. Aloe gel also exhibits antimicrobial, antifungal and anti-viral properties (89). The inner gel of aloe exhibited antimicrobial activity against *Shigella flexneri* and

Streptococcus pyogenes (90). The activity of three compounds (aloe-emodin, chrysophanol and aloin A) isolated from *A. ferox* have been shown to be effective against both Gram negative and Gram positive bacteria, demonstrating a broad spectrum potential of the plant as antimicrobial agent (91).

Other Activities

Aloe-emodin and Aloin A has been studied as hypotensive agents. Aloe-emodin aloe-emodin has emerged as a potent blood pressure reducing agent and caused 79% decline in blood pressure at a dose of 3 mg/kg in rats (92).

Conclusions

The genus Aloe from Africa is highly diverse with hundreds of species, however, only a minor number of these diversity has been explored in search for new bioactive components. Many of them are being used extensively by traditional healers, suggesting that the genus can be the source of potential new compounds that are waiting to be discovered or commercialized. *A. vera* and *A. ferox* are two popular and well species that contain many unique constituents with biological activity and with application to the pharmaceutical, cosmetic, personal care products sectors as well as to the continued uses in traditional medicines.

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Chapter 10

Chemistry and Biological Evaluation of Nigerian Plants with Anti-Diabetic Properties

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The use of plant drugs by the traditional herbalists for the treatment of ailments remains the mainstay and cornerstone of healthcare delivery in developing countries. The reliance on the use of plant drugs to manage diseases that could not be effectively treated with allopathic or synthetic drugs continues unabatedly in developing countries and to gain grounds in developed countries. Diabetes mellitus being one of such diseases that plant drugs are being exploited for its management. Diabetes is a heterogeneous disease and different plant drugs may act through one or more mechanisms to bring about their anti diabetic activities. The various mechanisms by which plant drugs bring about antidiabetic activities include: glycosidase (glucosidase) inhibitor mechanism, α -amylase inhibitor mechanism, inhibition of hepatic glucose metabolizing enzyme mechanism, antioxidant mechanism, inhibition of glycosylation of haemoglobin mechanism and modulation of glucose absorption from the gut. In this review a survey of Nigerian plants used by the three major ethnic groups was carried out. Their organic constituents and some identified active constituents including their methods of analyses were provided through a data base search.

Curative potentials of plants have been recognized since prehistoric era and plants with such properties are today known as medicinal plants, many with long history of use for the treatment of various human and animal ailments (1). The extracts from these plants have been used to heal and/or to kill (2). Research on plants for the discovery of new prototype drugs that could be of great use in the treatment of diseases is of increasing interest especially when there are limited synthetic drugs available.

Phytopharmaceuticals both as drugs and dietary supplements are of great interest in the treatment of some disease conditions that could not be effectively managed with allopathic drugs or diseases that are refractory to allopathic or synthetic drugs. Diabetes mellitus is one of such diseases that plant drugs are being exploited for treatment and there appears to be a high possibility for the discovery of new plant-based antidiabetic drugs (3,4) Diabetes is recognized as a genetic disease, yet significantly influenced by diet, lifestyle and other factors, caused by inherited and/or acquired deficiency in the production of insulin by the pancreas or by the ineffectiveness of the insulin produced (5). It is described as a metabolic disorder (6), and is characterized by hyperglycemia, altered metabolism of fats and lipids, carbohydrates and proteins (7,8). Chronic hyperglycemia during diabetes causes glycation of body protein, which in turn leads to secondary complications affecting the eyes, kidney, nerve and arteries (9). Along with abnormalities in the serum lipids and hyperglycemia, diabetes is accompanied with microvascular and macrovascular complications which are the major causes of morbidity and death in diabetic subjects (10). Diabetes is associated with increased risk of complicated cardiovascular diseases. The major defining characteristic feature of diabetes is chronic and sustained elevation of circulatory glucose level.

The extreme clinical manifestations have given rise to the basis for subdividing diabetes mellitus into insulin dependent diabetes mellitus (IDDM), known as type 1 and non-insulin dependent diabetes mellitus (NIDDM), called the type II. The latter is a disorder of dysregulated energy metabolism and has a higher incidence of occurrence (11). Insulin resistance has been recognized as one of the earliest defects in Type II diabetes and may be attributed to a primary defect in insulin stimulated glucose transport and disposal in muscles and fat, and impaired suppression of hepatic glucose output. Hyperglycemia in type II diabetes has been treated with sulfonylureas such as tolbutamide, chlorpropamide, glibenclamide and biguanide drug such as metformin, which are synthetic drugs, but they are unable to permanently lower glucose concentration to normal range and reinstate a normal pattern of glucose hemostats. The use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side-effects (1,12). There is need to search for effective additional remedies from natural sources that would be devoid of the undesirable side-effects of the currently used synthetic drugs, hence, the research into plants and plant products for the treatment of diabetes mellitus.

Plants and plant products have long provided many modern drugs and continue to provide the major source of medicines globally. The use of herbs has been long recognized as being the core first line treatment in the primary health care of 70-80% of the worlds population (13-15) and while the total percentages

likely vary based upon the estimate use, it is clear from all such estimates that herbs and their extracts are widely employed and relied upon globally. As such, it is in this context that in many developing countries, plants have been used in the treatment of diabetes (especially type II diabetes). Herbal medicine is also used in traditional health care systems to treat other ailments such as epilepsy and asthma that allopathic or synthetic drugs are only used as palliatives. Some plants have been employed only for curative purposes in the herbal medicine for the treatment of diabetes while others, e.g. *Vernonia amygdalina* (16) and *Ocimum gratissimum*, have served as medicines as well as food and condiments for several years in many countries and are associated with little or no side effects. (1,17). The physiologically active organic compounds in herbal drugs that exhibit antidiabetic activity can be found across a wide class of natural product compounds including alkaloids (17), terpenoids (18), peptides (19), amines (20), steroids (18), flavonoids (21), coumarins (22), sulphur compounds (23,24) and sometimes complex carbohydrates (20).

These plants containing various organic compounds have been employed singly such as *Parinari curatellifolia* Planch (25) or in combination e.g. *Treculia Africana* Decne and *Bryophyllum pinnatum* Lam.(26) for their anti-diabetic effects but only a small number of them have been scientifically evaluated, with the hope of providing useful sources for treatment of diabetes mellitus especially type II (1). In this review, we summarize and discuss the plants widely used in Nigerian traditional herbal medicine. We present a compiled list of species including the plant part used, the traditional method of preparation, the chemical constituents responsible for the antidiabetic properties and the method of assay. The list of plants used in Nigeria for their antidiabetic properties also includes information as to the major natural products that have been reported in the plants species but in most cases no scientific evidence has yet linked the natural products to the treatment of type II diabetes. From the wide range of chemical classes presented, we also review several possible mechanisms of action through which the antidiabetic activities may be achieved.

Methodology

The plants with antidiabetic activities were obtained from twelve Traditional Herbal Medical Practitioners who have practiced for at least twenty years and were attending a Diploma in Herbal Medicine Programme of the Department of Pharmacognosy, University of Lagos, 2006/2007 Session coordinated by Dr. S.O. Ogbonnia (a Senior lecturer and researcher in the Department). Twelve students of Herbal Medicine Programme were drawn from different tribes as follows: six Yorubas (Western Nigeria), three Igbos (Eastern Nigeria) and three Hausas (Northern Nigeria). For their research projects, the students were assigned to contact other herbalists in their various localities in addition to their knowledge and list herbs and plants that are used in their respective regions in the treatment of diabetes. Additional specific information were obtained from personal discussions with Agu Titus Iwuala, Odumosun Abimbola, Alade Raymond, Alao Michael, Osademey Magnus Jerome (All Traditional Herbal Medical Practitioners).

The interview instrument included a survey that asked for each to provide the plant name, the plant part used, other folk medicinal uses and traditional method of preparation while a database search shows the chemical constituents of these plants and the method of assay that could be used to analyze the chemical constituents.

Results and Discussion

Twelve traditional herbalists from the three Nigerian major ethnic groups and from three regions (north, west and east) were used in the study of some Nigerian plants with antidiabetic activity. The results of the survey are summarized as listed in Table I. Table II is a database search showing the chemical constituents and their assay methods. From the interviewer, it was observed that the knowledge of herbs used as antidiabetics is not generalized among the tribes except few plants eg *Ocimum gratissimum*, *Vernonia amygdalina* and *Vernonia colorata* that have wider applications. Each tribe has some common herbs generally used as antidiabetics and in rare occasions there are herbs used only by a particular practitioner.

Generally, plant materials are used either fresh or dried. Where fresh leaves or plant materials such as flowers, fruits and seeds are used, they may be ground and expressed for the juice. Fresh roots, barks and fruits are usually cut into small pieces and may in addition be ground and expressed for the juice. Methods of decoction with water or maceration with local gin may be employed but maceration is the commonest method of extraction of the dried plant parts whether leaves, flowers, fruits, seeds, barks and roots.

Mechanisms of Antidiabetic Activity

Diabetes is a heterogeneous disease state. There are various mechanisms through which different plant drugs can bring about their antidiabetic activities. Antidiabetic activity is not limited to or dwelled in a particular group or a class of natural products. The mechanism of action in general can be related to the ability of the plant in question or its active principle to lower plasma glucose level by interfering with one or more processes involved in glucose homeostasis (27). There are, however, a few reports on the medicinal plants that are beneficial in the treatment of diabetes mellitus but do not act by the direct lowering of the blood sugar level eg *Trigonella foenumgraecum* (fenugreek) (28). These agents may act by reducing hypercholesterolaemia and other cardiovascular risk factors that contribute to the death of diabetic subjects. The mechanisms of hypoglycemic activity are numerous and can be summarized as follows:

- (a) By altering the activity of some enzymes that are involved in the glucose metabolism - glycosidase (glucosidase) inhibitor and α -amylase inhibitor (29).
- (b) Stimulation of insulin secretion or enhancing glucose utilization by acting like insulin (28, 30).
- (c) Stimulation of glycogenesis and hepatic glycolysis (31).

- (d) By acting as a pancreatic beta cell potassium channel blocker (31).
- (e) Stimulation of cyclic adenosine monophosphate (cAMP) 2nd messenger. cAMP 2nd messenger is formed from ATP by adenylyl cyclase at the inner surface of cell membrane. It acts as an intercellular second messenger in response to hormones such as glucagons, epinephrine and norepinephrine. cAMP is hydrolyzed by phosphodiesterase thereby terminating the hormone action. In the liver insulin increases the activity of phosphodiesterase (32)
- (f) Diminishing the release of some hormones such as glucagon that counteract insulin action (27)
- (g) Modulation of glucose and carbohydrates absorption from the gut (28)
- (h) By antioxidant activity as found in some phenolics and phenolic compounds (33)
- (i) Inhibition of glycosylation of haemoglobin mechanism (31)

The hypoglycaemic activity of a plant drug may be brought about through one or combinations of two or more of these mechanisms. Some of these mechanisms are briefly described below,

Glycosidase (Glucosidase) Inhibitor Mechanism

One of the earliest features of type II diabetes (observed in pre-diabetic phase) is the loss of early phase secretion of insulin (34). Early phase insulin secretion is seen after a meal or after oral or intravenous ingestion of glucose and it is responsible for inhibition of hepatic glucose output and its absence results in postprandial hyperglycemia. The α -glucosidase inhibitors category of drugs has been found to decrease postprandial glucose level by interfering with carbohydrate digestion and delaying gastrointestinal absorption of glucose (35). Slowing down digestion and breaking down of starches may have beneficial effects on insulin resistance and glycaemic index control on people suffering from diabetes.

In this group, some alkaloids especially polyhydroxyalkaloids that are water soluble have been identified as potent glucosidase inhibitors (29). These natural products as a whole comprise of relatively simple monocyclic pyrrolidine and piperidine alkaloids, necines, amino alcohols which are derivatives of bicyclic pyrrolizidine, and are mostly esters of amino alcohols and of aliphatic carboxylic acids. The various types of necines can be distinguished as a function of degree of hydroxylation of the molecule e.g. crotanecine (Figure 1). Indolizidine ring system bearing alkaloids with a varying number of hydroxyl groups have also been found to have glycosidase inhibition activities (29,36).

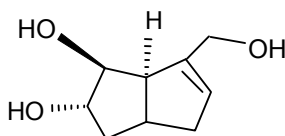


Figure 1: The molecular structure of crotanecine.

Polyhydroxynortropanes have been isolated from Solanaceae (e.g. *Datura metel*), Convolvulaceae (e.g. *Pharbitis-Ipomoea* and *Argyrea* species) and Moraceae (*Morus nigra*) and like the polyhydroxyindolizidines, these compounds appear to inhibit various glucosidases (α - and β -glucosidase, α - and β -glycosidase). Calystegines (Figure 2) characterized by a hydroxyl functional group at C-1 (can be tri-, tetra-, or pentahydroxy), have been isolated from the genera *Atropa*, *Datura*, among others. They are also highly water soluble alkaloids and can be extracted with water. The selectivity and intensity of the inhibition of glycosidases depend on the structure of the alkaloid. Herbal drugs containing polyhydroxyalkaloids may be useful for postprandial hyperglycaemia but may be associated with possible side effects such as neurological toxicity and cerebellar degeneracy in animals (36).

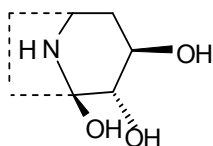


Figure 2: The molecular structure of Calystegine A₃.

Inhibition of Hepatic Glucose Metabolizing Enzymes Mechanism

Synthesis of glucose by the liver and kidney from noncarbohydrate precursors such as lactate, glycerol and amino acids constitutes a process known as gluconeogenesis. The liver hydrolytic enzymes glucose-6-phosphatase (G-6 – Pase) and fructose-1, 6- diphosphatase have been shown to play a crucial role in gluconeogenesis contributing to hyperglycemic condition found in diabetes. Herbal drug products may act by binding with the enzymes. Treatment with a herbal drug has been observed to decrease the activities of these liver enzymes significantly with a concomitant decrease in blood sugar level (37). The effect of a plant extract with antidiabetic activity on G-6 –Pase can thus be evaluated by isolating microsomes from alloxan or streptozotocin–induced diabetic rats using Vogel’s methods (38). The pallet obtained is re-suspended at a protein concentration of 40mg/ml in a homogenization buffer and G-6-Pase activity is measured by monitoring the release of phosphate from G-6-P. Microsomes (1.0 μ l of the preparation above) is incubated at room temperature in 200 μ l of a buffer at pH 7.2 containing 50 mmol/l HEPES, 100mmol/KCl. 2.5 mmol/l EGTA, 2.5 mmol/l MgCl₂ and 1.0 μ l G-6-P. The phosphate released is measured by adding 300 μ l of 1M HCl containing 10mg/ml ammonium molybdate and 0.38mg/ml malachite green. After 15 min incubation at room temperature the absorbance is measured at 620nm. 50, 100 and 150 μ g of water extract of the drug is added before the addition of enzyme and the same amount of water used as control.

Alpha-Amylase Inhibitor Mechanism

Inhibitory activity against α -amylase by flavonoids and anthocyanins has been reported (39). Herbs used in Nigerian traditional medicine for the treatment of diabetes mellitus are known to contain phenol compounds especially the flavonoid quercetin and the mechanism of their antidiabetic activity may be brought about by their amylase inhibiting activity and the antioxidant activity of the phenolic compounds.

Antioxidant Mechanism:

Antioxidants are chemical substances that donate an electron to the free radical and convert it to a harmless molecule. Antioxidants intercept free radicals and protect cells from the oxidative damage that leads to aging and some disease conditions (40). Numerous studies have shown that antioxidants have both preventive and therapeutic activities on diseases ranging from diabetes to cancer and to general deterioration of the body (40,41). Antioxidants keep the free radicals in check before they can impair the immune system and damage the cell (42).

Oxidative stress has been reported to increase in diabetic patients and is regarded as common pathway by which many classical cardiovascular disease (CVD) risk factors and postprandial dysmetabolism may initiate and promote atherosclerosis (43). There are evidences to suggest that oxidative cellular injury caused by free radicals contribute to the development of diabetes mellitus (44, 45). Studies have shown that treatment with antioxidants reduces diabetic complications (12). Flavonoids have been shown to scavenge reactive oxygen species that are produced under severe stress conditions and protect plant cell and animal cell metabolism from oxidative stress and may have important role in human health (46). Almost all flavonoids possess several common biological and chemical properties. They are phenolics and have antioxidants properties; and are endowed with the capacity to modulate certain cellular enzyme activities. They have also the ability to scavenge electrophiles and active oxygen species. The reactive oxygen species (ROS) removal rate is mostly controlled by a variety of low molecular weight antioxidants (47). Polyphenols have been shown to act as antioxidants and are associated with combating oxidative stress and reducing some cardiovascular disease.

The phenols and polyphenols act as antioxidants because of resonance, the oxygen atom of the OH group acquires a positive charge and so the proton release is facilitated. The carbon atom of the C-OH group is sp^2 hybridized; it is more electron- attracting than the sp^3 carbon and subsequently there is an increased electron withdrawing inductive (-I) effects that facilitates proton release (48). Hydrogen of the hydroxyl group of phenol is easily removed by 'one- electron transfer' oxidizing agents e.g. ferric ions, alkaline potassium ferricyanide etc.

Inhibition of Glycosylation of Haemoglobin Mechanism:

Both fasting and postprandial hyperglycaemia contributes to overall glycaemic burden and therefore total glycosylation of haemoglobin, HbA1C (34). Many studies have shown that there is substantial evidence and a very strong correlation between hyperglycaemia and the risk of developing cardiovascular disease and mortality (49-51). Postprandial hyperglycemia has been found to occur together with postprandial hyperlipidaemia which is also associated with increased oxidative stress and endothelia dysfunction. However, one could therefore postulate that herbal drug products that are effective in the reduction of postprandial hyperglycaemia may not only play a role in managing type II diabetes but could also offer a high possibility of reducing cardiovascular risk

Modulation of Glucose Absorption from the Gut.

Plants with blood glucose lowering activity in this category contain hypoglycemic polysaccharides. Natural products belonging to this chemical class of compounds has been found to lower glucose absorption from the gastrointestinal tract thereby reducing postprandial hyperglycemia (20). Guar and other gums belong to this class and bring about their hypoglycemic activity through this mechanism (52). Acidic polysaccharides and their degradation products have been investigated for their antidiabetic effects (53). Protein and polypeptides, in spite of their ability to act like insulin, would not add a great advantage to current antidiabetic therapies but some protein bound polysaccharides obtained from the water soluble fractions of pumpkin fruits (*Telfaria occidentalis*, *Cucurbita pepo* and *Cucurbita maxima*) have been investigated for their effect on insulin in diabetic rats (54).

Table 1: Selected Nigerian Medicinal Plants Traditionally used in the Treatment of Diabetes.

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Dicliptera alternans</i> (Acanthaceae)	Herbs ¹	Diabetes	Aqueous decoction of fresh or dried herbs is used taken as directed by the traditional herbalist often with food in the morning, afternoon and night
<i>Allium sativum</i> (Aliaceae)	Bulb	As condiment, in hypertension, Antimalaria, antidiabetic	The fresh bulb is cut into pieces and may be ground and extracted with water by maceration
<i>Allium cepa</i> (Aliaceae)	Bulb	In hypertension, Antimalaria, antidiabetic	The fresh bulb is cut into pieces and may be ground and extracted with water by maceration
<i>Alisma platago</i> (Aliaceae)	Herbs	Diabetes, bone setting	Aqueous decoction or alcoholic beverage of the herbs is used.
<i>Anacardium occidentale</i> (Cashew nut) (Anacardiaceae)	Stem bark	Diabetes	The bark is cut into small pieces and boiled with water and filtered
<i>Mangifera indica</i> (Anacardiaceae)	Leaves, stem bark	Diabetes, malaria	Fresh or dried bark is cut into small pieces and boiled alone or with the leaves using water

NOTE: ¹Herbs consist of stems but limited in girth by 'official' requirements and leaves often associated with flowers and young fruits

Table I. Continued

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Spondias mombin</i> (Anacardiaceae)	Leaves, fruits and seeds	Inflammation, diarrhea, venereal diseases and diabetes	Fresh or dried leaves are cut into small pieces and extract with water.
<i>Xylocopa aethiopica</i> (Annonaceae)	Fruits	Diabetes, good health, antiseptic	The crushed fruits are either extracted with palm kernel oil or with alcoholic beverages
<i>Alstonia boonei</i> (Apocynaceae)	Bark	Malaria, diabetes	Coarse powdered bark is extracted with palm kernel oil or with alcoholic beverages or by decoction with water
<i>Alstonia congensis</i> (Apocynaceae)	Bark	Malaria, diabetes	Coarse powdered bark is extracted with palm kernel oil or with alcoholic beverages or by decoction with water
<i>Rauwolfia vomitoria</i> (Apocynaceae)	Bark and roots	Diabetes, hypertension, tranquilizer and treatment of madness (antipsychotic)	Coarse powdered bark or root is extracted with alcoholic beverages or by decoction with water
<i>Picralima nitida</i> (Apocynaceae)	Bark of the stem and seeds	Diabetes, malaria, pyrexia, pneumonia, anti-inflammatory agent and analgesics, protozoan diseases and insect repellent	Powdered fresh or dried stem bark or seeds is extracted with water by decoction or by maceration
<i>Aristolochia albida</i> (Aristolochiaceae)	Leaves and climbing stem and roots	Good health tonics, stomach troubles, skin problems and diabetes	Herbal Medical Practitioners The fresh leaves are ground and extracted with palm kernel oil for treatment of skin infections. The infusion of dried roots is used for stomach troubles and aqueous decoction of the fresh or dried leaves alone or in combination with the roots is used in the treatment of diabetes (no incidence of poisoning has been reported)

Table I. Continued

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Aristolochia bacteolata</i> (Aristolochiaceae)	Leaves and roots	Diabetes, antihelmintics, applied topically for pimple and the roots used with lime for snake bites.	Dried leaves or dried roots are used singly or in combination in form of infusions.
<i>Aristolochia brasilienses</i> (Aristolochiaceae)	Leaves and climbing stem	Antibacterial property and diabetes	Aqueous decoction of the fresh or dried leaves alone used in the treatment of diabetes and as an antiseptic after birth.
<i>Gymnema sylvestris</i> (Asclepiadiaceae)	Leaves and roots	Used as condiment by Igbos, as diuretics and laxatives, and diabetes	The aqueous extract of the leaves is used in the treatment of diabetes. The aqueous or alcoholic extract of the roots are applied topically.
<i>Brassica napiformis</i> (Brassicaceae)	herbs	Aperitif, emetic, arthritis, diabetes, and lumbago	The fresh or dried herb is powdered and extracted with water or mixtures of water and alcohol.
<i>Carica papaya</i> (Caricaceae)	Leaves	Malaria, diabetes	The fresh leaves are cut into pieces and aqueous decoction obtained. Used alone or in combination with other herbs by Herbal Medical Practitioners
<i>Parinari</i> species (Chrysobalanaceae)	Bark, leaves and Seeds,	Pains and diabetes	The fresh or dried seeds are ground to coarse powder and extracted with alcoholic beverages by maceration
<i>Polygonatum humile</i> (Convallariaceae)	Roots and leaves	Antirheumatic, sedative, tonic and diabetes	The fresh or dried roots and/or leaves are ground and extracted with water or alcoholic beverages by maceration or decoction

Table I. Continued

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Vernonia amygdalina</i> (Asteraceae)	Leaves and roots	Diabetes, pile	The fresh leaves are mashed with water and the foamy juice with bitter principle used. Also the roots fresh or dried are cut into pieces and aqueous decoction obtained is used or can be extracted with alcoholic beverages and used
<i>Vernonia colorata</i> (Asteraceae)	Leaves and roots	Diabetes, pile	The fresh leaves are mashed with water and the foamy juice with bitter principle used. Also the roots fresh or dried are cut into pieces and aqueous decoction obtained is used or can be extracted with alcoholic beverages and used
<i>Calystegia spp</i> (Convolvulaceae)	Climbing plant	Diabetes and good health	The aqueous decoction of fresh or dried plant and sometime with the flower and fruits is used.
<i>Ipomoea aquatica</i> (Convolvulaceae)	herb	Gastric ,intestinal disorders and diabetes	Aqueous decoction of the herbs is used.
<i>Mormordica charantia</i> (Cucurbitaceae)	Unripe fruit	Diabetes	The unripe fruit is cut into pieces and its aqueous decoction is used.
<i>Citrullus lanatus</i> (Cucurbitaceae)	Leaves and seeds	Vermifuge, Tonic, diuretic, febrifuge and diabetes	The aqueous decoction of fresh or dried leaves and seeds are used
<i>Mormordica foetida</i> (Cucurbitaceae)	Unripe fruits, leaves, stem and flowers	Diabetes and variety of ailments such as malaria	The unripe fruit alone or in combination with other plant parts is extracted by maceration or decoction and used.

Table I. Continued.

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Cymbopogon citratus</i> (Poaceae) Lemon grass	Grass	Antimalaria, diuretic, abortifacient, and insecticides	The aqueous decoction of fresh or dried leaves are used traditionally by Herbal Medical Practitioners
<i>Garcinia cola</i> (Guttiferae)	Roots and stem bark	Cough, pains, diabetes	Powdered root or stem bark or both can be extracted by decoction
<i>Ocimum gratissimum</i> (Lamiaceae)	Leaves	anti-infective, spice, diabetes and diarrhea	The leaves are either extracted with alcoholic beverages or decoction with water
<i>Acacia nilotica</i> (Fabaceae)	leaves	Diarrhea, diabetes	Fresh or dried leaves are ground and extracted with water by maceration.
<i>Vigna unguiculata</i> (Fabaceae)	Seed pods	Nutritional value, diabetes	Fresh or dried pods are ground and extracted with water by maceration and used.
<i>Lagstroemia</i> <i>speciosa</i> (Lythraceae)	leaves	Weight loss, kidney and urinary problems and diabetes	Aqueous decoction of fresh or dried leaves is used.
<i>Azadirachta indica</i> (Meliaceae)	Leaves, stem bark and roots	Antimalaria and diabetes	Fresh leaves are pounded and extracted with water, while aqueous decoction of fresh or dried bark/ roots is used
<i>Leucaena</i> <i>leucocephala</i> (Fabaceae)	Leaves	Diabetes, livestock feed	Fresh leaves containing flowers and unripe seeds are mostly used. The leaves are with water by decoction or maceration.
<i>Morus nigra</i> (Moraceae)	Leaves	Food value, diabetes	Aqueous decoction or maceration of the leaves is used.

Table I. Continued

<i>Plant material</i>	<i>Part used</i>	<i>Folk medicinal uses</i>	<i>Traditional Preparation</i>
<i>Treculia Africana</i> (Moraceae)	Leaves	Seeds for food, diabetes and the bark as exhibits antibacterial	Aqueous decoction or maceration of the leaves or barks
<i>Psidium sp. P. acutangulum, P. striatulum, P. guaiava P. guineense</i> (Myrtaceae)	Leaves and stem	Fever, malaria and diabetes	Aqueous decoction or maceration of fresh plant or dried plant materials is used by Herbal Medical Practitioners
<i>Biophytum sensitivum</i> (Oxalidaceae)	leaves	Insulinotropic activity, diabetes	Aqueous decoction/maceration of fresh or dried plant leaves. Dried plant leaves can be extracted with alcoholic beverages and used.
<i>Morinda citrifolia</i> (Rubiaceae)	Roots	Malaria and diabetes	Aqueous decoction or maceration of fresh plant or dried plant materials is used. Also dried plant materials are extracted with alcoholic beverages and used.
<i>Morinda lucida</i> (Rubiaceae)	Leaves, bark	Malaria and diabetes	Aqueous decoction or maceration of fresh plant or dried plant materials is used. Also dried plant materials are extracted with alcoholic beverages and used.
<i>Capsicum frutescens</i> (Solanaceae)	Leaves, stem, bark	Rheumatism and diabetes	The aqueous decoction of leaves or /and stem bark are used by Herbal Medical Practitioners
<i>Stachyurus praecox</i> (Stachyuraceae)	leaves	Weight loss and diabetes	Fresh or dried leaves are boiled with water or can be extracted with alcoholic beverages and used
<i>Stachytapheta angustifolia</i> (Verbanaceae)	leaves	Diabetes, antibiotics and as a sedative	Fresh or dried lived are decocted with water or by maceration with alcohol

Table 2. Selected Nigerian Plants, with some of their known bioactive natural plant products linked with antidiabetes activities

<i>Plants</i>	<i>Class of Compounds Identified</i>	<i>Compounds Responsible, methodology of analysis, and references</i>
<i>Allium cepa</i> (Aliaceae)	Essential amino acid composing of arginine, histidine, lysine, tryptophan, phenylalanine and phenolic acids viz protocatechuic acid, ap-hydroxybenzoic, vanillin, o- and p-coumaric acid, cirtic abietic acid and oxalic acid	phenolic acids viz protocatechuic acid, ap-hydroxybenzoic, vanillin, o- and p-coumaric acid which are all antioxidants (GC) (59)
<i>Allium sativum</i> (Aliaceae)	Disulfide containing compounds, terpenoids	Terpenoids (GC) (60)
<i>Alisma platago</i> (Alismataceae)	Terpenoids	Triterpenes (GC and HPLC) (61)
<i>Anacardium occidentale</i> (Anacardiaceae)	Anacardic acid, alpha-linolenic acid, anacardol, antimony, cardol, eupopium, folacin, ginkgol, leucine, glucuronic acid, glutamic acid, hafnium, serine, phytosterol, praline, vitamin c, kaempferol, terpenes, quercetin-glycoside	Terpenes HPLC (59)
<i>Mangifera indica</i> (Anacardiaceae)	Polyphenols	Polyphenols, Cyanogenetic, glycs. (HPLC) (62)
<i>Xylopiya aethiopica</i> (Anacardiaceae)	β-Pinene, γ-terpinene, transpinocarveol, p-cymene	Terpenoids, β-pinene, γ-terpinene, trans-pinocarveol, p-cymene (GC) (63)
<i>Catharanthus roseus</i> (Apocynaceae)	Alkaloids: vincristine, ajmalicine, lochnerine, serpentine and tetrahydroalstonine, vinblastine.	Vincristine, vinblastine (GC) (59)

Table 2. Continued.

<i>Plants</i>	<i>Class of Compounds Identified</i>	<i>Compounds Responsible, methodology of analysis, and references</i>
<i>Alstonia boonei</i> (Apocynaceae)	Alstonine, alstoniline, cillastonine and echitamine	Not specified (HPLC) (59)
<i>Rauwolfia vomitoria</i> (Apocynaceae)	Alkaloid, reserpine, Rescinamine, phytosterols, fatty acids, unsaturated alcohols	Reserpine, rescinamine and ajmaline (HPLC) (59)
<i>Aristolochia bracteolata</i> (Aristolochiaceae)	Aristolochic acid, aristo red and yellow acidic compounds, alkaloids including magnoflorine (thalactrine) and aristolactam, nor-N-acetyl (-) nuciferine*	Alkaloids, magnoflorine and aristolochic acid (HPLC) (64)
<i>Gymnema sylvestre</i> (Asclepidaceae)	Gymnemic acid, quercitol, anthraquinone and glycosides	Gymnemic acid Gymnemosides, gmemasinoycones aglycones (HPLC) (65)
<i>Carica papaya</i> (Caricaceae)	Saponins, glucosinate, polyketide, alkaloids, proteolytic enzyme: papain, chymopapain, flavonols.	Papain, kaempferol (GC, HPLC) (60)
<i>Momodica charantia</i> (Cucurbitaceae)	Two acidic resin, a bitter substance called mormordicine. 0.3% alkaloid charantoin, a steroidal glycoside consisting of a mixture of β -D-glucose of β -sistosterol and of D-5,25-stigmastadiene-3- β -o1	Alkaloid charantoin (HPLC) (59, 64)
<i>Momordica charantia</i> (Cucurbitaceae)	Triterpene glycosides, called momordicosides A – L and the gogaglycoside A- H as well as momordicin, momordicin and cucurbitanes I, II, and III and gogasaponins I, II, and III. Proteins and Lectins present include α , β and γ momorcharins and momordins a and b.	Sterol glycoside mixture, charantins, insulin-like polypeptides and alkaloids (HPLC) (65)

Table 2. Continued.

Plants	Class of Compounds Identified	Compounds Responsible, methodology of analysis, and references
<i>Bridelia ferruginea</i> (Euphorbiaceae)	Flavonoids and biflavonoids based on apigenin and kaempferol moieties were isolated together with O and C glycoside from methanolic extract of this plant	Flavonoids, biflavonoids and glycosides (HPLC) (66)
<i>Euphorbia hirta</i> (Euphorbiaceae)	Gum-resin, sugar, mucilage, melisylpalmitic, oleic, linoleic, traces of ceryk alcohol, essential oil, succinic acids, stigmasterol, sitosterol, L-inositol, gallic acid, myricetin	(66)
<i>Tetrapleura tetraptera</i> (Fabaceae)	Triterpenoid glycosides, Aridanin, Aridan	Triterpenoid glycosides (GC, HPLC) (67)
<i>Viscum album</i> (Florathaceae)	Sugar, alkaloids, lectins, flavanoids, phenyl allyl alcohols, lignans, triterpenes, polypeptides	Lectins, Flavanoids, triterpenes (GC, HPLC) (60)
<i>Cymbopogon citratus</i> , lemongrass (Poaceae)	Volatile oils, aldehydes	Terpenes: citral and citronellal (GC) (68, 69)
<i>Garcinia cola</i> (Clusiaceae)	Biflavones, guttiferins, resins, volatile oils and alkaloids	Kolaviron (a biflavonoid) HPLC (70)
<i>Ocimum gratissimum</i> (Lamiaceae)	Essential oils, diterpenoids. polyphenols, tannins and sugars, phenylpropanoids.	Essential oils and terpenoids (GC) (59, 64)
<i>Mucuna pruriens</i> (Fabaceae)	L-DOPA, alkaloids, mucunine, mucuesdimine, mucunadine, pruriendine, β -sitosterol, lecithin	L-DOPA, alkaloids, mucunine, mucuesdimine, mucunadine, pruriendine, β -sitosterol (62)

Table 2. Continued.

<i>Plants</i>	<i>Class of Compounds Identified</i>	<i>Compounds Responsible, methodology of analysis, and references</i>
<i>Abrus precatorius</i> (Fabaceae)	Abrin, nicotine, esters, alkaloids, Abralin, choline	Abrin, hyaphorine, precatorine, abricin abrectorin (HPLC) (59)
<i>Azadirachta indica</i> (Meliaceae)	Azadirachtin, meliacin, azadironic acid limonoids (Nortriterpenoids). salanin. Sesquiterpenes. nortriterpenoids.	Desacetylnimbin, Nimbasterol, volatile oil (HPLC) (71, 72)
<i>Treulia africana</i> (Moraceae)	Polyterpenoids, glycosides Bergapten, polyketides	The polyketides of 3-phenyl-2,4,4- trihydroxychalcone (HPLC) (73)
<i>Musa paradisiacal</i> (Musaceae)	Fructosans, phenolic acids, anthocyanins, and steroids	Terpenoids and Steroids (HPLC) (60)
<i>Cryptolepis</i> <i>sanguinolenta</i> (Periplocaceae)	Cryptolepine, cryptosipirolepine quindoline	Cryptolepine (HPLC) (74)
<i>Borreria verticillata</i> (Rubiaceae)	Indole alkaloids, borrerine and a dimmer borreverine, dihydroborrecapine and borrecoxine, asperuloside/ diacetyl-asperulosideic acid, feretoside, campesterol, stigmasterol, β -sitosterol*	Volatile oil containing sesquiterpenes, sesquiterpene lactones and phenolic compounds (GC) (64)
<i>Blighia sapida</i> (Sapindaceae)	Hypoglycin A and hypoglycin B, cyclopropanoid amino acid	Hypoglycin A (HPLC) (59)
<i>Stachytarpheta</i> <i>angustifolia</i> (Verbanaceae)	Triterpenoid saponins	Triterpenoid saponins (HPLC) (75)

NOTE: * There are no studies linking the plant with diabetes

Herbal Antidiabetic Drug Interaction

Drug interaction is defined as any modification caused by another exogenous chemical (drug, herb or food) in the diagnostic, therapeutic or other action of a drug in or on the body (55). Combination of herbal medicines with prescriptive drugs may increase risks of interactions and many herbal drugs may have the potential to cause adverse drug interaction when combined with prescriptive medicines. In Nigeria, and many other developing countries, herbal medicines unlike conventional drugs are not regulated for purity and potency but are only screened for toxicity. Thus, some of the adverse effects and drug interactions reported for herbal products could be caused by impurities (e.g., allergens, pollen and spores) and even microbial contaminations (56).

Toxicity evaluations of herbal drugs in general and antidiabetic plant drugs (either prepared from a single plant material or polyherbal products) in particular are based on the effects of the drugs on haematological and biochemical parameters as well as metabolic enzymes and were measured as indices of organ toxicities. A mixture of *Alstonia boonei* and *Xylopia aethiopica* (57), and polyherbal mixtures *Maytenus senegalensis*, *Annona senegalensis*, *Kigella africana* and *Lannea welwitschii* (58) employed in the treatment of diabetes, were investigated in Wistar albino rats. The plasma aspartate aminotransferase, (AST), alanine aminotransferase, protein and creatinine levels were not affected at moderate and lower doses after 30 days exposure to the drugs. Further more the polyherbal preparation was found not to significantly affect zoxazolamine induced paralysis and phenobarbital induced sleeping time as well as certain CYP isoenzyme activities in rats. These findings suggest that the antidiabetic herbal preparation studied had no overt organ specific toxicity and did not demonstrate a potential for drug interactions through CYP- mediated metabolism in rats on subchronic administration (58).

Conclusion

The abundant tropical biodiversity hotspots and frontiers found in the rain forest belt and coastal area have endowed Nigeria as well as other tropical countries with a wide floristic diversity. Many of these plants found in our tropical rain forest areas in Nigeria are associated with some potential medicinal properties and are being exploited for their medicinal values by traditional herbal practitioners in the preparation of herbal medicines in the treatment of various ailments and diseases. Realization that many plant and plant-based remedies may either led to a new phytopharmaceutical drug or provide that model chemical template upon which a drug may be designed has led to a global awareness and growing interest in herbal drugs.

Diabetes mellitus, especially type II diabetes, is one of such diseases that herbal drugs should be examined and screened for validation and possible use in regions outside where they may be now only locally used, should safety and efficacy studies show success. The selected Nigerian medicinal plants as listed in Table 1 were obtained from the traditional herbalists, with their permission, who claimed to have employed them either singly or in combination in the

management of diabetes even though most of the claims are yet to be scientifically evaluated. The respect of traditional knowledge to provide leads and hints as to the selection of plants to screen coupled with their validation using modern scientific methodologies surely presents a sound strategy and would allow Nigerian medicinal plants and African natural plant products to contribute in a more substantive manner to the improvement of global health and nutrition needs.

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Chapter 11

Antiplasmodial Activity of Twenty Essential Oils from Malagasy Aromatic Plants.

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In search for new plant-derived biologically active compounds against malaria parasites resistant to commercially available drugs, twenty essential oils extracted from Malagasy aromatic plants were assessed for their anti-plasmodial activity against the multi-drug-resistant strain of *Plasmodium falciparum* FCM29. The plants were subjected to steam distillation and the aromatic volatile oils captured using a Clevenger trap. Fourteen essential oils were active against *Plasmodium falciparum* in culture with IC50s ranging from 27-225 µg/mL. While the essential oils (*Cymbopogon citratus* and *Lantana camara*) showed activities similar to that of chloroquine, none exhibited the high activity as achieved by quinine.

Malaria is a life-threatening parasitic disease transmitted by mosquitoes. Across 107 malaria-endemic countries, estimated incidence in 2004 totalled 402 million (range 350-500 million) cases, of which around 57% occur in Sub-Saharan Africa. Malaria is reported to cause in Sub-Saharan Africa alone almost one million deaths annually, of which the majority are children under five (1).

Disease control is hampered by the occurrence and increase of multi-drug-resistant strains of the malaria parasite *Plasmodium* sp. Many species affect humans though *P. falciparum*, is responsible for the most severe illnesses and deaths attributable to malaria in sub-Saharan Africa and in certain areas of South-East Asia and the Western Pacific (1).

The identification of new bioactive compounds with antimalarial activity, are needed to provide new leads or new compounds for drug development, particularly those with different underlying mechanisms of action to the current cadre of antimalarial drugs.

The most competent and efficient malaria vector, *Anopheles gambiae*, occurs exclusively in Africa and is also one of the most difficult to control. Many insecticides are no longer useful against mosquitoes transmitting the disease. Present antimalarials are derived largely from natural products from plants, quinine and artemisinin each isolated from, respectively, *Cinchona* sp. (Rubiaceae), a Peruvian plant, and *Artemisia annua* (Asteraceae), a Chinese plant. Both the alkaloid quinine and the non-volatile sesquiterpenes lactone artemisinin, are active on erythrocyte stage parasite life cycle. While tazopsine, a morphine-like alkaloid, isolated from the Madagascar rain forest plant *Strychnopsis thouarsii* (Menispermaceae) (2), was found to target the liver stage form of the parasite. Thus, plant derived bioactive compounds have been effective in developing modern antimalarial pharmaceuticals. A brief review of traditional African plants used to fight malaria highlights a wide range of promising species (3).

Aromatic plants and their essential oils have been used by societies for their medicinal value since ancient times. Used as sources of flavours, fragrances, cosmetics, personal health care products, industrial products, aromatherapy, healing, and for religious and spiritual ceremonies, essential oils have long established histories of use in the treatment of respiratory illness and as antimicrobial agents. Increased interest in the antimicrobial activities of essential oils came in part from the appearance of bacteria and fungi strains resistant to available antibiotics (4,5). In Madagascar, many aromatic plants and essential oils showed insect repellent activities (6), thus having an indirect impact in the occurrence of malaria. In Malagasy traditional healthcare, if aromatics plants were used for the treatment of fever, the specific use of essential oils for this purpose was not mentioned in the literature (7,8).

Anyway, several reports have studied the antimalarial properties of essential oils extracted from different species around the world including but not limited to *Tetradenia riparia* (9), *Cochlospermum tinctorium* and *C. planchonii* (10), *Virola surinamensis* (11), *Xylopiya phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, *Xylopiya aethiopica*, and *Hexalobus crispiflorus* (12), *Cymbopogon citratus* and *Ocimum gratissimum* (13), *Salvia* sp. (14) and oleanene constituents of *Lantana cujabensis* (15). *Artemisia annua* is also a rich source of well characterized volatile essential oils (16) though it is the non-

volatile terpene that exhibit the antimalarial activity (17,18) and from which modern artemisinin derived drugs originate. These reports and the discovery of artemisinin from *Artemisia annua*, a sesquiterpene sharing the same metabolic pathway as the majority of essential oil components (19), prompted us to study the *in vitro* antiplasmodial activity of twenty essential oils from Madagascar.

Material and Methods

Antiplasmodial test: *In vitro* antiplasmodial assays can be performed because of the adapted continuous culture of *Plasmodium falciparum* set by Trager and Jansen (20).

Previously, the traditional means of detecting parasite growth was microscopic examination of blood smears using Giemsa stain or the standard gold method [³H]hypoxanthine incorporation assay (21). The latter method requires radioactive materials that pose safety and disposal problems and has multiple steps that are technically demanding.

Recently, instead of incorporating radiolabelled compound into DNA, new nonradioactive screens have emerged, using DNA stains as a reporter to measure parasite growth (22,23,24,25). The use of DNA stains to detect parasite DNA has improved the ease of drug susceptibility testing. Developed first in Panama, PicoGreen® DNA stain method (22) was used for assessment of essential oils presented. The technique was transferred and used in Madagascar to assess antiplasmodial plant extracts (26). The method is based upon the intercalation of the fluorochrome PicoGreen® into *Plasmodium* DNA. PicoGreen® is an ultrasensitive fluorescent nucleic acid stain for measuring double-stranded DNA (dsDNA) in solution, and it enables the detection of quantities as low as 25 pg/mL of dsDNA with a spectrofluorometer using fluorescein excitation and emission wavelengths: respectively 485 nm and 518 nm (22).

Essential oils from selected Madagascar plant tested. Twenty essential oils were screened for determination of the concentration inhibiting 50% of culture growth (IC₅₀). The aromatic volatile oils were obtained by hydrodistillation using a Clevenger trap. The twenty essential oils were composed of thirteen essential oils extracted from different aromatic plants: *Schinus terebenthifolius* (Anacardiaceae); *Pelargonium* sp. (Geraniaceae); *Ocimum canum*, *Rosmarinus officinalis* (Lamiaceae); *Cinnamomum camphora* (Lauraceae); *Myristica fragrans* (Myristicaceae); *Eucalyptus globulus* (Myrtaceae); *Piper nigrum* (black pepper) (Piperaceae); *Elionurus tristis*, *Cymbopogon citratus* (Poaceae); *Vepris eliotii*, *Citrus hystrix* (Rutaceae); and *Lantana camara* (Verbenaceae); and with three oils derived from chemotypes of: *Ravensara aromatica* (Lauraceae), two from chemotypes of *Melaleuca viridiflora* (Myrtaceae) and two from fresh and dry *Pimenta dioica* (Piperaceae).

Results and Discussion

The results showed that the essential oil of *Cymbopogon citratus* (lemongrass) and *Lantata camara* were the most active against the plasmodium. *Cymbopogon citratus* showed the highest antiplasmodial activity with an IC₅₀ equal to 27 ± 1.69 $\mu\text{g/mL}$. The essential oil of *L. camara* showed an activity of 31.6 $\mu\text{g/mL}$. Both oils showed a similar activity to that found in chloroquine (25 $\mu\text{g/mL}$), one of the two controls used in this study. However, the both of these oils showed far lower activity when compared with quinine (3.3 $\mu\text{g/mL}$). The remaining essential oils screened exhibited lower activities ranging from no activity to 73.5 $\mu\text{g/mL}$.

While recognizing and expecting that significant differences would be expected between plant species and the chemistry of the essential oil on antiplasmodial activity, this study also shows the extension of that hypothesis, that chemotype within species as well as the final chemistry of the essential oil would also impact the antimalarial activity. The essential oil of *Ravensara aromatica*, dominated by sabinene and limonene, showed no activity, while the oils containing methyl eugenol and methyl chavicol showed antiplasmodial activity of 75 - 73 $\mu\text{g/mL}$, respectively (Table I). The essential oil of *Schinus terebenthifolius* dominated by monoterpenes hydrocarbons (24) showed also no activity. These results suggest the need of oxygen in the molecule for the antiplasmodial activity. The essential oil *Melaleuca viridiflora* dominated by $1,8$ cineole (a cyclic ether) showed no activity, while the oil dominated by the sesquiterpene alcohol (viridiflorol) exhibited antiplasmodial activity (Table I). These results are supported by the observation that essential oils (*E. globulus*, *C. camphora*) dominated by $1,8$ cineole usually exhibited lower activities (143 - 177 $\mu\text{g/mL}$). We also note that processing of the essential oils can also affect the chemistry of the antiplasmodial activity from screened oils since the oil of *Pimenta dioica* freshly distilled showed no activity while the oil coming from dried plant material showed parasitic activity (Table I). These results suggest that certain activities may be unintentionally overlooked if the oil is not properly prepared for the antimalarial screen.

Table I. The Main Chemical Constituents and Antiplasmodial Activity of Twenty Essential oils Against *Plasmodium falciparum* FCM29 strain

<i>Essential oil</i>	<i>Main constituent(s)</i>	<i>IC50 µg/ml</i>
<i>Cinnamomum camphora</i>	1,8-Cineole, sabinene (27)	177.3 ± 22.3
<i>Ravensara aromatica</i>	Methyl eugenol (28)	74.7 ± 3.3
<i>Ravensara aromatica</i>	Methyl chavicol	73.1 ± 3.4
<i>Ravensara aromatica</i>	Sabinene, limonene	NA
<i>Schinus terebenthifolius</i>	α-pinene (29), α-phellandrene	NA
<i>Myristica fragrans</i>	Sabinene (30)	NA
<i>Vepris elliotii</i>	Linalool (31)	117.6 ± 9.0
<i>Melaleuca viridiflora</i>	1,8-Cineole	NA
<i>Melaleuca viridiflora</i>	Viridiflorol	135.5 ± 17.2
<i>Piper nigrum</i>	(E)-caryophyllene (28,32)	120.7 ± 15.3
<i>Citrus hystrix</i>	Limonene, β-pinene (33)	119.5 ± 11.5
<i>Elionurus tristis</i>	Not identified	65.7 ± 8.1
<i>Pelargonium sp</i>	Geraniol, citronellol (34)	225.7 ± 13.0
<i>Eucalyptus globulus</i>	1,8-Cineole (35)	142.8 ± 25.9
<i>Cymbopogon citratus</i>	Neral, geranial	27.3 ± 1.6
<i>Pimenta dioica (Fresh)</i>	Eugenol (36)	NA
<i>Pimenta dioica (Dry)</i>	Eugenol(36)	165.8 ± 18.6
<i>Lantana camara</i>	(E)-caryophyllene, α-humulene (28,37)	31.6 ± 1.9
<i>Ocimum canum</i>	Camphor (38)	156.7 ± 9.4
<i>Rosmarinus officinalis</i>	α-pinene, camphor (39)	NA
Quinine		3.3 ± 0.5
Chloroquine		25.2 ± 1.4

NOTE: IC50: mean (triplicate); St.D.: Standard deviation error; NA: Not active

Conclusion

The majority of the essential oils exhibited some degree of activity against *P. falciparum*. Two of the essential oils (*C. citratus* and *L. camara*) showed activities similar to that of chloroquine, while none of the 20 Malagasy essential oils exhibited as high activity as from quinine.

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Chapter 12

Traditional Medicinal Plants and Malaria in Africa

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Malaria is considered one of the most prevalent diseases in Africa. Global infections are annually affecting 300-500 million people, with 90 percent of the cases in Sub-Saharan Africa. The incidence of infection has increased in recent times in many African countries despite ongoing programs seeking to reduce and alleviate the disease. Mortality associated with malaria is estimated as 1.5-2.7 million annually, and is rising as a result of increasing drug resistance. With global warming predicted to increase the emergence of malaria in many African countries and extend the regions where infections occur by expanding the 'habitat of the insect vector' additional national and international efforts are needed to address control strategies. In view of the problems that malaria infection is causing in Africa, this study sought to review the antimalarial properties of various medicinal plants, extracts and their components.

In regions where malaria is endemic, adults experience 1-2 attacks annually, whereas children may suffer from 1-7 infections. Furthermore, about three million people (mostly in Africa) suffer from long-term neurological damage of malaria. Recent estimates of the global malaria burden have shown increasing levels of illness and death caused by malaria, reflecting the deterioration of the malaria situation in Africa rather than the improvement and reduction in cases as had been hoped. About 90% of all deaths from malaria occur in Africa, and the great majority of these are in children under the age of five (1-2). About 40% of the world's population is at risk of contracting malaria in 109 countries and territories around the world (2). While new programs seeking to develop effective immunizations and engineering microbes to produce antimalarials capture the world's attention and funding for it represents exciting new science, the continued screening of natural plant products used today against malaria is being sourly overlooked.

Traditional medicinal plants have been used for treating malaria and/or the symptoms of malaria for thousands of years, and remain a major source of the two main groups (artemisinin and quinine derivatives) of modern antimalarial pharmaceutical drugs. In developing countries, plants remain the main sources of medicine, and when the origin of modern drugs, it is the antimalarials that have largely come from plants or the chemistry of the synthetic antimalarials have been nature inspired. According to the World Health Organization, as many as 80% of the world's people rely for their primary health care on traditional medicine, most types of which use remedies made from plants (3). The use of traditional medicine in developing countries is increasing, in part because populations are increasing, because modern pharmaceutical drugs are often beyond the economic reach of a large part of the population, insufficient supply of some pharmaceutical drugs, the realization by some governments that want to encourage indigenous forms of medicine rather than rely on imported drugs, and strong moves to revive traditional cultures which include and rely on the traditional medicines (4-5).

Traditional medicine or ethnomedicine is a set of empirical practices embedded in the knowledge of a social group often transmitted orally from generation to generation with the intent to solve health problems. It is an alternative to Western medicine and is strongly linked to religious beliefs and practices of indigenous cultures. Medicinal plants lore or herbal medicine is a major component of traditional medicine. The evidence for effectiveness of traditional medicinal plants in the treatment of infections is quite different from that, for example, of modern antibiotics. Traditional healers have for decades used many plants throughout African countries, to treat malaria, the symptoms of malaria as well as a wide range of diseases (5-6).

Key among the factors contributing to increasing malaria mortality and morbidity is the widespread resistance of *Plasmodium falciparum* to conventional antimalarial drugs, such as chloroquine, sulfadoxine-pyrimethamine and amodiaquine (7).

***Artemisia annua* and Artemisinin (antimalarial)**

Artemisinin, the principal bioactive antimalarial compound and its derivatives from *Artemisia annua*, a Traditional Chinese Medicinal plant used against fevers and malaria, have yielded a potent new class of antimalarials. The anti-malarials derived from *A. annua* are considered an integral part of the solution where malaria has become resistant to other medicines and even in areas where resistance is not yet a problem (8). Artemisinin-based combination therapies (ACTs) have been recommended in the countries where falciparum malaria - the most resistant form of the disease- is endemic (9). While “ACTs for all” would be the ideal strategy, it is most impractical for poor and remote communities, politically unstable areas, and people who dislike the use of modern medicine (10).

African scientists should be contributing to the improvement of *A. annua* L quality and to further develop artemisinin- based medicines, to help ensure a sustainable supply to meet market demand. While the world market for products containing artemisinin derivatives has grown rapidly, the available supply has been limited and not all artemisinin meets the required standards to produce the raw ingredients for pharmaceutical processing, making it all the more urgent to promote best practices in the cultivation, collection and processing of the raw material used to make the combination therapy.

African scientists must advocate in the use and implementation of good agricultural and collection practices and growers and producers provide traceability and records for the African production of *A. annua*. Such records provide a detailed description of the cultivation and collection techniques and measures required for a harvest to meet quality requirements (11).

However, the production of this crop requires a high degree of skills, excellent genetic materials, and the processing capabilities that also meet pharmaceutical requirements for the initial extraction of artemisinin from the harvested plant materials. Cultivation of *A. annua* requires a minimum of 6 months and extraction, processing and manufacturing of the final product require at least 2-5 months depending on the product formulation (12). High temperatures and moisture levels during post-harvest handling can damage the quality of the plant leading to a significant loss of artemisinin during the post-harvest handling and storage period (13). It is in the processing and the next set of industrial steps in the purification and preparation of the artemisinin derivatives that are lacking in general in Africa.

The objective of this study is to discuss the uses of African traditional plants to treat malaria in Africa, and to review the modern literature research on the bioactivities of these plants against the vector and the parasite (*Plasmodium falciparum*).

Traditional Medicinal plants and Malaria

There are numerous medicinal plants used in folkloric remedy of malaria, and the healers of African continent described these plants and their uses. *Swartzia madagascariensis*, *Combretum glutinosum* and *Tinospora bakis* are three plants of the folk medicine used by healers in Burkina Faso for the treatment of malaria (6). Cameroon healers used the boiling water extraction of stem bark and seeds of four medicinal plants, *Entandrophragma angolens*, *Picaralima nitida*, *Schumanniphyton magnificum* and *Thomandersia hensii* against malaria and report good results (14). Stem and leaves of *Pothomorphe umbellata* (Piperaceae), stem of *Enantia polycarpa* (Annonaceae) and the root of *Trichilia emetica* (Meliaceae) were used successfully in treating malaria in Cote D' Lvoire (15). Meanwhile, traditional healers often use water decoctions and macerations of *Emilia discifolia*, *Senecio stuhlmannii*, *Indigofera emarginella* and *Aspilia africana*, traditional medicinal plants of East and Central Africa for malaria remedy (16).

Ethiopian medicinal plants, Dingetegna (*Taverniera abyssinica*) and Endod (*Phytolacca dodecaandra*) are used for malaria disease (17). In Ghana, dried root decoctions of *Cryptolepis sanguinolenta*, prepared by boiling the powdered roots in water, are used in traditional medicine to treat various forms of fevers, including malaria (18). Some of the anti-malarial species for example, *Warburgia ugandensis* are already known to be over-exploited and in some parts of Kenya now rare (19). In Madagascar, a decoction of *Cinnamosma fragrans* (Canellaceae) leaf and bark is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms, while a decoction of the leaf and bark of *Desmodium mauritanium* (Leguminosae), is mixed with a selection of five plants from (*Ficus megapoda*, *Nymphaea lotus*, *Noronhia linocerioides*, *Vepris ampody*, *Zanthoxylum madagascariense*, *Gambeya boiviniana*, *Peddia involucrata*). This decoction is drunk (1 bowl, 3–4 times daily) to relieve malarial symptoms (20) (Table I).

While specific plants have been used against malaria, herbalists and healers have long recognized that not all plant tissues are equally effective, and thus they recommend and use different plant tissues dependent upon the species. *Opilia celtidifolia* (Opiliaceae) and *Trichilia emetica* (Meliaceae) are well known to the traditional healers of Mali for their use against malaria. The leaves are the most frequently used plant part (56.3%), the root and fruits are used about 30% and 8.5% respectively, and the less used plant part is the bark (5.3%) (21). Malaria was reported to be the most common condition treated by traditional healers in Uganda. These healers used plants as their most important source of natural products for malaria treatment. Plant extracts of *Maesa lanceolata*, *Conyza sp.*, *Rhus natalensis*, *Toddalia asiatica*, *Bothriocline longipes* and *Trimeria bakeri*, showed high activity against the blood stage of *P. falciparum* (22).

Table I. Traditional medicinal plants (leaf and bark) preparations and doses used against malaria in Madagascar.

Scientific name	Family	Doses
<i>Cinnamosma fragrans</i>	Canellaceae	1 bowl, 3-4 times daily
<i>Desmoium mauritianum</i>	Fabaceae	1 bowl, 3-4 times daily
<i>Dracaena reflexa</i> L.	Agavaceae	1 bowl, 3-4 times daily
<i>Ficus megapoda</i>	Moraceae	1 bowl, 3-4 times daily
<i>Nymphaea lotus</i> L.	Nymphaeaceae	1 bowl, 3-4 times daily
<i>Peddiea involucreata</i>	Thymelaeaceae	1 bowl, 3-4 times daily
<i>Tristellateia madagascariensis</i>	Malpighiaceae	2-3 glasses daily
<i>Vepris ampody</i>	Rutaceae	1 bowl, 3-4 times daily
<i>Zanthoxylum tsihanimposa</i>	Rutaceae	1 bowl, 3-4 times daily

NOTE: All preparations are decoctions from leaf and bark.

SOURCE: Modified from Randrianariveolojosi et al. 2003 (20).

In the French Guiana, many medicinal plants are used as traditional antimalarial remedies. Of the different 23 species that were tested, *Irlbachia alata*, *Picrolema pseudocoffea*, *Quassia amara*, *Tinospora crispa* and the multi components recipe showed showed high in vitro activity against *P. falciparum* (23) (Table II).

Bioactive Compounds of Medicinal Plants and Malaria

The antiplasmodial activity of five alkaloids (γ - fagarine, N-benzoyltryamine, skimmianine, dictamnine and 4-methoxy-1 methyl-2(1H)-quinoline), extracted by decoction from *Zanthoxylum tsihanimposa* stem bark were tested upon *P. falciparum*. The quinoline alkaloid γ -fagarine was the most potent of five alkaloids, being found to be 3.4 times more active than dictamnine and 1.4 times more active than skimmianine. The presence of the methoxy group in positions C4 and C8 appears to be important for bioactivity (20).

Argemone subfusiformis dried leaves, flowers and seeds were extracted with ethanol-water (70-30%) for 48 h, the aqueous-solution obtained was evaporated under vacuum and the residue was directly assayed. The antiplasmodial activity was good (24), and extracts were rich in isoquinollic alkaloids. In above ground parts, protopine, berberine and allocryptopine were identified (27). According to Sriwilaijaron et al. (28) berberine prevents the development of *Plasmodium falciparum* by inhibition of its telomerase activity.

Aspidospermine one indole alkaloid was isolated from bark of *Aspidosperma quebracho-blanco*, displaying antimalarial activity on *Plasmodium falciparum* (29).

Table II. Mode of preparation of selected antimalarial remedies.

<i>Species</i>	<i>Family</i>	<i>Part</i>	<i>Preparation of recipe</i>
<i>Geissospermum laevis</i>	Apocynaceae	Bark	40 g in 1l water, boiled for 15 min.
<i>Irlbachia alata</i>	Gentianeaceae	Leaves and roots	150 g fresh leaves are boiled in 300 ml water for 10 min. 150 g roots are boiled in 200 ml water for 10 min.
<i>Picrolemma pseudocoffea</i>	Simaroubaeae	Leaves	20 g are boiled in 1l water for 10 min.
<i>Pseudoxandra cuspidate</i>	Annonaceae	Bark	200 g of inner bark, boil for 15 min in 500 ml water.
<i>Pterocarpus rohrii</i>	fabaceae	Leaves and bark	15 g fresh leaves are boiled in 1500 ml water for 10 min., 15x 10 cm bark piece, boil for 15 min in 800 ml water.
<i>Quassia amara</i> L.	Simaroubaeae	Leaves and stem	20 g entire fresh leaves, boil for 10 min in 1l water, 100 g stem (cut pieces) are boiled in water for 15 min.
<i>Tinospora crispa</i> L.	Menispermaceae	Stem	15 cm is cut in small pieces, boil for 15 min in 500 ml water
<i>Zanthoxylum rhoifolium</i>	Rutaceae	Bark	400 g of inner bark are boiled in 1500 ml water until liquid reduces by half.
<i>Multi ingredient recipe</i>			<i>P. pseudocoffea</i> , 20 g fresh leaves, <i>Q. amara</i> , 8 g fresh leaves, <i>G. laevis</i> bark, 40 g bark boiled in 1500 ml water/15 min

SOURCE: Partial data from Bertani et al., 2005 (23).

Ethanollic leaves extract of *Castela texana* was evaluated, and antimalarial activity was reported for five quassinoids, with level of activity ranking from 0.01 µg/ml to 0.92 µg/ml (28). If the activity observed in the ferriprotoporphyrin biomineralization inhibition test (FBIT) of this extract is due to quassinoids-like molecules, the same mechanism of action could be suggested, as structure/activity relationship studies of quassinoids have shown that the oxymethylene bridge is necessary for antimalarial activity (29). The bark of *Vallesia glabra* stem has been shown to contain indole alkaloids such as apparicine, aspidospermatine, condylocarpine, haplocidine, tubotawine, vincadifformine (30). As these types of alkaloids are known to possess antimalarial activity, it is not surprising to have found such promising *in vitro* activity very much alike the one of *Aspidosperma quebracho-blanco* bark.

Methanolic and methylene chloride extracts of the leaves of *Hymenocardia acida* were found active against *P. falciparum* (31). Tannins and the alkaloid hymenocardine have been identified in the leaf extracts of *H. acida* (32). From

investigations of *in vitro* antimalarial screening of medicinal herbal extracts, the *n*-butanolic extract from the root of *Wikstroemia indica* showed a potent inhibitory effect. Fractionation of the active extract led to the isolation of two biflavonoids, sikokianin B and sikokianin C with IC_{50} values 0.54 $\mu\text{g/mL}$ and 0.56 $\mu\text{g/mL}$, respectively, against the chloroquine-resistant strain of *Plasmodium falciparum* (33). Phytochemical investigation of the 80% ethanolic extract of stem bark of *Vismia orientalis* Engl. (Clusiaceae), a plant used in traditional medicine in Tanzania, resulted in the isolation and spectroscopic characterization of 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone, emodin, vismione D and bianthrone A_1 . Vismione D exhibited a broad range of antiprotozoal activities against *Trypanosoma brucei rhodesiense* and *T. cruzi* ($IC_{50} < 10 \mu\text{g/mL}$), *Leishmania donovani* ($IC_{50} 0.37 \mu\text{g/mL}$) and *Plasmodium falciparum* strain K1 ($IC_{50} 1.0 \mu\text{g/mL}$). However, it was also slightly cytotoxic against human L6 cells ($IC_{50} 4.1 \mu\text{g/mL}$). Emodin showed antileishmanial activity ($IC_{50} 2.0 \mu\text{g/mL}$), while its IC_{50} against L6 cells was 20.3 $\mu\text{g/mL}$. Other antiprotozoal activities observed for emodin against both *Trypanosoma* species and *P. falciparum*, for bianthrone A_1 against *T. b. rhodesiense* and *P. falciparum*, and for 3-geranyloxy-6-methyl-1,8-dihydroxyanthraquinone against *T. b. rhodesiense*, *L. donovani* and *P. falciparum* were in the range of 10 to 50 $\mu\text{g/mL}$. (34).

In the search for new plant-derived biologically active compounds against malarial parasites, five essential oils extracted from the Cameroonian plants *Xylopia aethiopica*, *Xylopia phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, and *Hexalobus crispiflorus* were evaluated in regard to their antiplasmodial activity against the W2 strain of *Plasmodium falciparum*. The oils were obtained from the plants with 0.12, 0.13, 0.18, 0.6 and 0.1% yields (relatively to dried material weight) respectively. Analysis by gas chromatography and mass spectrometry identified mainly terpenoids, among which α -copaene, γ -cadinene, δ -cadinene, α -cadinol, spathulenol and caryophyllene oxide were most commonly found. The five oils were active against *Plasmodium falciparum* in culture. The most effective was the oil of *Hexalobus crispiflorus*, with an IC_{50} of 2 $\mu\text{g/mL}$. (35).

The Amazon Indians Waiãpi living in the West of Amapá State of Brazil, treat malaria with an inhalation of vapor obtained from leaves of *Viola surinamensis*. The antimalarial activity of the aromatic volatile plant extracts from leaves, showed that nerolidol (an acyclic oxygenated sesquiterpene) was identified as one of the active principles (36). Another recent study suggested the presence of an active isoprenoid pathway for biosynthesis of isoprenic chains of coenzyme Q in *P. falciparum* (37), parasites treated with nerolidol showed decreased ability to synthesize coenzyme Q in all intraerythrocytic stages. A challenge would be to now identify which African medicinal plants contain similar chemistry.

Traditional Plants that Repel Mosquitoes

Mosquitoes are important vectors of many diseases, particularly malaria, as well as being nuisance pests. Repellents minimize human contact with

mosquitoes. Repellents and insecticides have long been recognized as an important strategy in the control of malaria. Repellents based on essential oils are being developed as an alternative to synthetic components such as DEET (N,N-diethyl-m-ethylbenzamide). The effect of essential oils is varied depending on geographic origin of the plants and the oil chemistry. Many essential oils have been studied for their mosquito repellent activity.

The essential oils from *Pogostemon cablin*, *Syzygium aromaticum* and *Zanthoxylum limonella* plants performed as mosquito repellents about as equally well as citronella oil. (38). In addition, essential oils extracted from multiple species of *Tagetes*, such as *T. patula*, *T. erecta*, and *T. minuta*, have shown nematocidal, fungicidal and insecticidal activity (39), extracts of *T. minuta*, whose main component was β -ocimene (62.8%), were toxic against mosquitoes (40). The main components of *Minthostachy mollis* essential oil repellent against mosquitoes were pulegone (52.6%), menthone (35.8%) and limonene (10.1%) (40). The essential oil from *Rosmarinus officinalis* was effective in terms of repellence time against mosquitoes (41). The main components of essential oil for *R. officinalis* include camphor (34%), verbenone (25%) and (E)-caryophyllene (15%). Camphor also found in *Baccharis spartioides* (50.5%), may be responsible for repellence of these plants (42).

Some *Eucalyptus* species have been evaluated for their potential as mosquito repellents, principally *Corymbia citriodora* Hook (also known as *Eucalyptus maculate citriodora*) (43), the repellent properties of *E. saligna* may be attributed to 1,8-cineole, as this compound accounted for 93.2% of the total extracted essential oil. This essential oil was also the only one to significantly decrease in terms of repellence time at concentrations below 90%. A study using 1,8-cineole showed moderate effects as a feeding and oviposition deterrent of mosquitoes (44). *cis*-Carveol is one of constituents in the essential oils of six plants growing in Kenya which were screened for repellent activities against *Anopheles gambiae* sensu stricto. The oils of *Conyza newii* (*Asteraceae*) and *Plectranthus marrubioides* (*Lamiaceae*) were the most repellent (45).

Repellence tests with essential oils at 90% concentration indicated that five essential oils were effective repelling mosquitoes for 90 min (Table III). The essential oil of *Acantholippia seriphioides* was repellent even at the lowest concentration tested (12.5%). Repellence by this essential oil was expected because its main components are p-cymene (53%) and thymol (47%), both components showed repellent activity for approximately 1h against mosquito species (46). At concentrations of 12.5%, *Aloysia citriodora*, *Baccharis spartioides* and *Rosmarinus officinalis* showed the longest repellency times. Comparisons of the principal components of each essential oil suggest that limonene and camphor were the main components responsible for the repellent effects (46).

Table III. Essential oils that repel mosquitoes listed in order of repellency time (46).

Essential oil (concentration at 90%)	Repellence time
<i>Achyrocline satureioides</i>	3.3 ± 3
<i>Hyptis mutabilis</i>	20.0 ± 10
<i>Anemia tomentosa</i>	60.0 ± 30
<i>Acantholippia seriphioides</i>	60.0 ± 30
<i>Baccharis spartioides</i>	90.0 ± 0
<i>Eucalyptus saligna</i>	90.0 ± 0
<i>Minthostachy mollis</i>	90.0 ± 0
<i>Rosmarinus officinalis</i>	90.0 ± 0
<i>Tagetes minuta</i>	90.0 ± 0

SOURCE: Partial data presented (46).

The components of the essential oil extracts from *Callicarpa americana* and *Callicarpa japonica*, containing callicarpenal, intermedeol, and spathulenol proved to be highly effective biting deterrents against *Anopheles stephensi* and *Aedes aegypti*. These compounds and other terpenoids may represent useful alternatives to conventional, so-called synthetic insect repellents currently on the market. (47).

In laboratory tests, ethyl acetate extracts of *Hyptis suaveolens* from Guinea-Bissau and *Rhododendron tomentosum*, *H. Harmaja*, and *Myrica gale* significantly reduced probing activity of *Aedes aegypti*. The essential oils of these species were dominated by terpenes hydrocarbons, (*H. suaveoles*, β -caryphyllene, *R. tomentosum*, p-cymene, *M. gale*, myrcene, phellandrene and α -pinene) (48).

While, nepetalactone, the essential oil of catnip (*Nepeta cataria*) that gives the plant its characteristic aroma, has been reported to be about ten times more effective at repelling mosquitoes than DEET (49).

Traditional Medicinal Plants and Clinical Trials

There are and have been clinical trials conducted in Africa with medicinal plants for the treatment of malaria. For example, the aqueous root extract of *Cryptolepis sanguinolenta* shows promise in the treatment of falciparum malaria. Parasite clearance was only one day longer with this remedy than with chloroquine, and the clearance of fever was faster by 12 hours (50). In another trial, comparing the treatment of falciparum malaria with quinine or with infusions of *Artemisia annua*, the infusions resulted in good parasite clearance at day 7 (51). Promising trypanocidal activity with IC₅₀ values below 10 μ g/ml was found in 32 extracts of 13 plant species. The most active extracts with IC₅₀ values below 1 μ g/ml were derived from *Annona senegalensis*, *Bussea occidentalis* and *Physalis angulata* (52).

Extracts from the plants *Emilia discifolia*, *Senecio stuhlmannii*, *Indigofera emarginella* and *Aspilia africana* were reported to possess antiplasmodial

activity. *Aspilia africana* exhibited the highest antiplasmodial activity against both the chloroquine-sensitive, and chloroquine-resistant strains of *P. falciparum* (16).

Aqueous, methanol, hydromethanol extracts from the roots bark of *Swartzia madagascariensis*, methanol and hydromethanol extracts from the leaves of *Combretum glutinosum* and aqueous and alkaloidal extracts from the roots of *Tinospora bakis* were also screened against *Plasmodium falciparum* chloroquine-resistant strain W2 *in vitro*. Results of these screens showed that the methanol and hydromethanol extracts of *Swartzia madagascariensis*, hydromethanol extracts of *Combretum glutinosum* and alkaloidal extracts of *Tinospora bakis* were active ($5 \mu\text{g/ml} < \text{IC}_{50} < 50 \mu\text{g/ml}$) (6). Other compounds with antiplasmodial activity derived from *Senecio selloi* and *Eupatorium rufescens* plants were also found to be active (53).

Fifteen crude extracts from the stem bark and seeds of four medicinal plants, *Entandrophragma angolense*, *Picralima nitida*, *Schumanniohyton magnificum* and *Thomandersia hensii* were tested *in vitro* for their antimalarial activity against the chloroquine-resistant *Plasmodium falciparum* W2 strain. The results showed that the extracts of these plants possessed some antimalarial activity, with the methanol extract of *Picralima nitida* demonstrating the highest activity *in vitro* (14). A slight *in vivo* antiplasmodial activity of the aqueous extract of *Erythrina senegalensis* was observed when tested against *Plasmodium berghei* (54), while the ethanolic stem bark extract showed a good activity against *Plasmodium falciparum*, confirming the antiparasitic potential of this plant (15).

Ten ethanolic (EtOH) and ten dichloromethanic (CH_2Cl_2) extracts from different parts of nine African medicinal plants used in Congolese traditional medicine for the treatment of malaria, were submitted to a pharmacological test to evaluate their effect on *P. falciparum* grown *in vitro*. Of these plant species, 14 (70%) extracts including EtOH and CH_2Cl_2 from *Cassia occidentalis* leaves, *Cryptolepis sanguinolenta* root bark, *Euphorbia hirta* whole plant, *Garcinia kola* stem bark and seeds, *Morinda lucida* leaves and *Phyllanthus niruri* whole plant produced more than 60% inhibition of the parasite growth *in vitro* (at $6 \mu\text{g/ml}$). Extracts from *E. hirta*, *C. sanguinolenta* and *M. morindoides* also showed a significant chemosuppression of parasitaemia in mice infected with *P. berghei* at orally given doses of 100-400 mg/kg per day (55).

Conclusions

Medicinal plants can offer alternative remedies with tremendous opportunities compared to synthetic modern pharmaceuticals. They not only provide access and affordable medicine to poor people; they can also generate income, employment and foreign exchange for developing countries. Many traditional healing herbs and plant parts have been shown to have medicinal value, especially in the rural areas and these can be used to prevent, alleviate or cure several human diseases. The safety of raw medicinal plant materials clearly provides a compelling rationale for both additional scientific study and national health care policies that can address the judicious use of traditional medicines to improve the quality and quantity of these materials and to ensure their efficacy.

This review has shown that many plants from Africa and other continents have been reported to be used to treat malaria in traditional medicine. Modern *in vitro* screens against the *P. falciparum* has confirmed the antimalarial properties for many of these plants and their extracts and clearly given the magnitude of the problem and the national costs due to malaria illness, death and suffering, the use of medicinal plants either in traditional manners and/or as leads for new compounds that may serve in the future for new anti-malarial drugs presents scientific opportunities.

The mosquito repellent properties of essential oils extracted from aromatic plants, also show promising results in the fight against malaria infection by reducing mosquito bites. Traditional medicines could be an important and sustainable source of anti-malarial agents. Given the increasing reports of drug resistance and difficulties in poor areas of being able to afford and access effective anti-malarial drugs, the search for additional strategies continues and in this medicinal plants can and should play an increasingly important role. Clinical trials are needed to incorporate this traditional knowledge in current medical practices and though such trials are always considered costly, the preselection of the most promising herbal or plant based treatments from *in vitro* studies into finely crafted modern clinical trials needs to be considered against the costs that will be incurred by those that would otherwise be infected with malaria.

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Chapter 13

The Golden Roots of *Cryptolepis sanguinolenta*

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The root of the plant *Cryptolepis sanguinolenta* (Lindl.) Schl (Periplocaceae) is used in traditional African medicine to treat a variety of diseases including malaria, jaundice, hepatitis, urinary tract infections, hypertension, inflammatory conditions and stomach ache. Extracts of the roots are also used as a tonic often taken daily for years without evidence of toxicity. Various studies indicate that the crude extracts as well as the isolated alkaloidal constituents of the plant possess a number of interesting pharmacological properties. The focus of this overview is to highlight the potential of *Cryptolepis sanguinolenta* in modern health care.

Description

C. sanguinolenta (Lindl.) Schltr (Periplocaceae) is a shrub that grows in the rainforest and the deciduous belt forest found in West Africa. Related species appear in the east and southern regions of the continent (1). The plant is a slender climber up to 8 meters high; with blood-red exudates; leaves elliptic, oblong-elliptic, or ovate, acutely and shortly acuminate, rounded 2.5 – 7 cm long and 1 - 3 cm broad; flowers greenish-yellow (2). The fruits are paired in linear follicles and are horn-like. The seeds are oblong in shape, small (averaging, 7.4 mm in length and 1.8 mm in the middle), and pinkish, embedded in long silky hairs. Dried *C. sanguinolenta* roots have sweet fragrance and a bitter taste. The surface of the cut dried roots show a bright yellow color (3).

Ethnomedical Use

C. sanguinolenta is one of the important herbs in West African traditional medicine. According to Silva *et al* the root of this plant has been used in Guinea Bissau by Fulani traditional healers for the treatment of jaundice and hepatitis. (4). Kerharo *et al* (as cited in Addy 2003) have also reported that the infusions of the roots are used in the treatment of stomach and intestinal disorders in Zaire and the Casamance district of Senegal (3). In Ghana, the root is a popular antimalarial. The roots *have also been used to treat urinary and upper respiratory tract infections* (5). Unconfirmed claims by some traditional medical practitioners in Ghana suggest that *C. sanguinolenta* root extract enhances sexual performance (6) and it is also effective in the management of diabetic symptoms (7), hemorrhoids (8), breast tumors and related disease (9). An alcoholic extract of the root is also used in Ghana as a tonic to strengthen metabolism (7). Tona *et al* have reported that an extract of *C. sanguinolenta* is used in Congolese traditional medicine for the treatment of diarrhea (10). According to Iwu *et al.*, the plant is mainly used for the treatment of fevers; it is also used to treat urinary tract infections, especially *Candida* (1). Iwu *et al.* has also reported that the plant has other uses which include the treatment of inflammatory conditions, hypertension, microbial infections and stomach ache (1).

Chemistry

Most of the compounds isolated from *C. sanguinolenta* are alkaloids and are analogs of indolo[3,2-*b*]quinoline (11). The major alkaloidal component of the plant is cryptolepine (12). In addition to cryptolepine, comparatively small quantities of a number of related alkaloids have been isolated from the plant. These include 11-hydroxycryptolepine, cryptoheptine, isocryptolepine, quindoline, biscryptolepine, cryptoquindoline, cryptolepicarboline, cryptospirolepine. Quindolinone, cryptotakienine, and cryptomisine (11,13-16) (Figure 1).

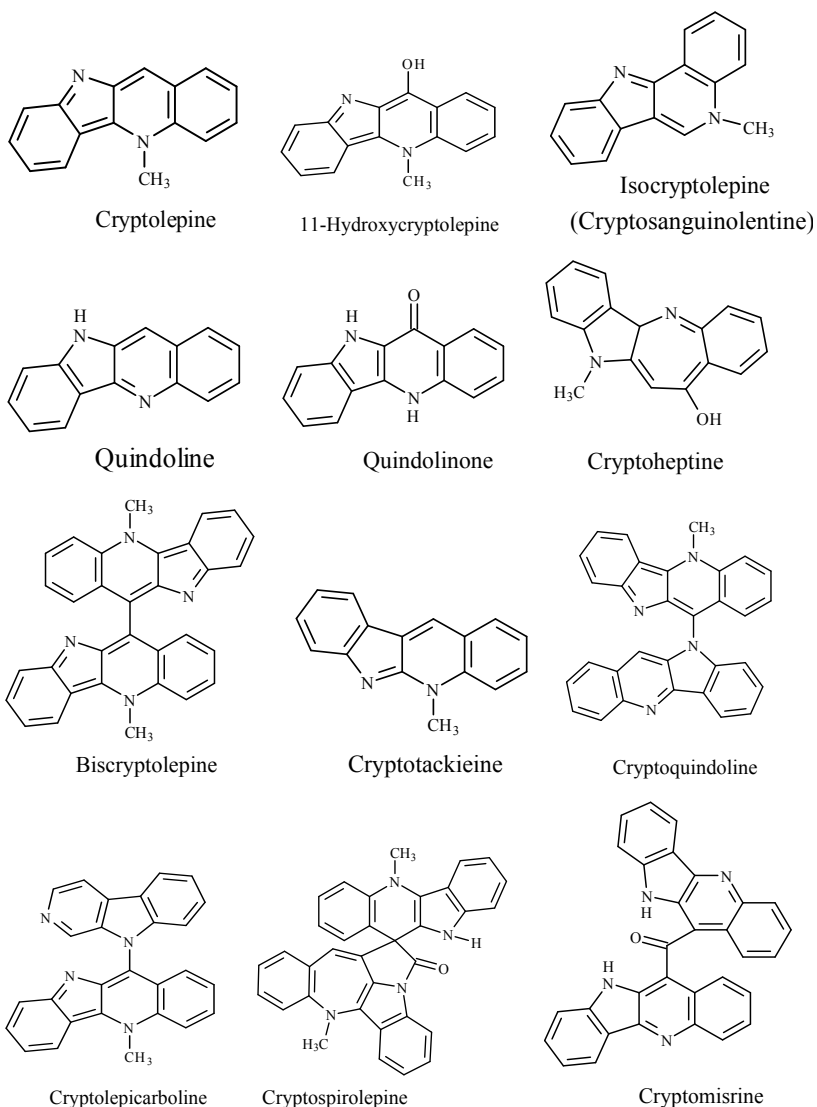


Figure 1. Structures of compounds (alkaloids) isolated from *C. sanguinolenta*.

Pharmacological Properties

Extracts of *C. sanguinolenta* roots and/or cryptolepine have been found to possess several interesting biological/pharmacological properties. These include: anti-plasmodial/anti-malarial (17), antimicrobial (18-23), vasodilatation, vasoconstriction, anti-hypertensive, noradrenoceptor antagonism (24), anti-muscarinic (25), anti-hyperglycemic (26), anti-inflammatory (27), and anti-viral (28) activities.

Cimanga *et al* (17) assessed the anti-plasmodial (anti-malarial) activity of three different extracts and four alkaloids from the root bark of *C. sanguinolenta* *in vitro* against *P. falciparum* D-6 (chloroquine-sensitive strain), K-1, and W-2 (chloroquine-resistant strains).

They observed that cryptolepine, cryptolepine hydrochloride, 11-hydroxycryptolepine, and neocryptolepine showed a strong antiplasmodial activity against *P. falciparum* chloroquine-resistant strains. Quindoline was less active. The highest activity was obtained with cryptolepine. An *in vivo* tests on infected mice showed that cryptolepine, when tested as its hydrochloride, exhibited a significant chemosuppressive effect against *Plasmodium berghei yoelii* and *Plasmodium berghei berghei*, while Cryptolepine had the same effect against *P. berghei yoelii* only. 11-hydroxycryptolepine and Quindoline did not show activity in the *in vivo* test system. In general, they could not deduce any correlation between the *in vitro* and the *in vivo* antiplasmodial activity of the test samples. They were of the view that, although cryptolepine or its hydrochloride is less active than known antimalarials such as mefloquine or artemisinin *in vitro*, and less active than chloroquine *in vivo*, their result provide some rational evidence for the use of *C. sanguinolenta* for the treatment of malaria in traditional medicine in some African countries; and that cryptolepine or related alkaloids may provide useful lead structures for the development of a new class of synthetic antimalarials. Tackie *et al* have patented some antimalarial compounds isolated from *C. sanguinolenta* (29).

As a response to increasing levels of antimalarial resistance, The World Health Organization (WHO) recommends that treatment policies in all countries experiencing resistance of *Plasmodium falciparum* to conventional monotherapies should be combination therapies, preferably those containing artemisinin derivatives. Currently WHO recommends the following therapeutic options: artesunate-sulfadoxine/pyrimethamine, artesunate-amodiaquine, artesunate-mefloquine, sulfadoxine/pyrimethamine-amodiaquine and artemether-lumefantrine (30). The root extracts/isolates of *C. sanguinolenta* may be used in combination therapy of malaria.

Other *in vitro* studies have shown that extracts of *C. sanguinolenta* have anti-microbial activity. Activity against *Staphylococcus aureus*, *E. coli*, *Candida albicans* and enteric pathogens have been reported (4,18-22).

Paulo *et al* (22) determined the Minimum Inhibitory Concentrations (MICs) of the ethanol and the aqueous extracts of *C. sanguinolenta* roots, and that of cryptolepine for 65 strains of *Campylobacter jejuni*, 41 strains of *Campylobacter coli* isolated from sporadic cases of gastroenteritis in Portugal and 86 strains of *Vibrio cholerae* isolated from patients with enteric infections in Angola, Brazil and Portugal. They found that the ethanol extract activity against *Campylobacter* strains (MIC = 25 µg/mL) is higher than that of co-trimoxazole and sulfamethoxazole and *Campylobacter* strains susceptibility for cryptolepine (MIC = 12.5 µg/mL) is about the same as that of ampicillin. The ethanol extract and cryptolepine show some activity against the *Vibrio cholerae* strains, although their activities are lower than that of tetracycline. They concluded that, the roots of *C. sanguinolenta* could be a therapeutic alternative for bacterial etiologic diarrhea.

Gibbons *et al* (23) assessed the activity of cryptolepine hydrochloride against the fast growing mycobacterial species *Mycobacterium fortuitum*, which has been shown to be of use in the evaluation of antitubercular drugs. They observed that the minimum inhibitory concentration (MIC), 16 µg/mL, of the compound was low. This prompted further evaluation against other fast growing mycobacteria namely, *M. phlei*, *M. aurum*, *M. smegmatis*, *M. bovis* BCG and *M. abscessus* and the MICs ranged over 2-32 µg/mL for these species. In conclusion, they stated that, the strong antimycobacterial activity of the compound and the need for new antibiotics with activity against *Mycobacterium tuberculosis*, coupled with the ethnobotanical use of *C. sanguinolenta* extracts to treat infections, highlight the potential of the cryptolepine template for development of antimycobacterial agents.

Bangbose and Noamesi (as cited in Tackie *et al* 1993) reported that cryptolepine possess hypotensive and vasoconstrictive activity. In addition, these same authors observed that cryptolepine hydrochloride is a noradrenoceptor antagonist in the isolated rat vas deferens. Raymond-Hamet (as cited in Tackie *et al* 1993) reported that administration of cryptolepine hydrochloride to dogs at a dosage of 15-30 mg/kg produced marked hypothermia and decreased the hypertensive and renal vasoconstrictive actions of epinephrine; and that iv administration of 5 mg/kg of the alkaloid produced a marked and protracted hypotensive response in the vagotomised dog with a corresponding decrease in renal volume. They inferred that the pronounced vasodilatation produced by the alkaloid was the cause of these effects (24). Cryptolepine seems to have a yohimbine-like activity: a selective α_2 -receptor antagonist. By blocking α_2 -receptors, while sparing α_1 -receptors yohimbine increases noradrenaline release, and produce sympathomimetic effects in some organs. Blockade of postsynaptic α_2 -receptors which occurs in blood vessels and some other organs causes a block of sympathetic responses; so the overall effects are complex. Vasodilatation and a fall in blood pressure usually predominate, and its vasodilator effect has given yohimbine fame as an aphrodisiac (31).

Cryptolepine (3-30 µM) and the alkaloid fraction of *C. sanguinolenta* (3-10 µg/mL) antagonized muscarinic effects at M_1 receptors in rabbit vas deferens, M_2 receptors in guinea-pig atria, and M_3 receptors in guinea-pig ileum. The experiments, using *N*-methylatropine as reference drug, showed a significant antimuscarinic activity for both cryptolepine and the alkaloid fraction, but no appreciable receptor subtype selectivity. Cryptolepine was determined as the antimuscarinic principle of *C. sanguinolenta* (25). The antimuscarinic properties of Cryptolepine suggests that *C. sanguinolenta* may be useful in one or more of the following disease conditions: urinary incontinence, asthma, peptic ulcer, parkinson's disease and motion sickness.

The anti-hyperglycemic property of cryptolepine has been shown as enhanced insulin-mediated glucose disposal in a mouse model of diabetes and in an *in vitro* system using the 3T3-L1 glucose transport assay, indicating an effect on Type 2 diabetes (26). Some hypoglycemic agents isolated from *C. sanguinolenta* have been patented by Luo *et al* (32).

Cryptolepine has been found to possess anti-inflammatory effects in the carrageenan-induced paw edema in rats (27); and Iwu (as cited in Iwu *et al* 1999) has also reported that the compound has shown histamine antagonism (1).

Toxicity

In one of their studies, Ansah *et al* (9) examined the *in vitro* toxicity of the aqueous extract of *C. sanguinolenta* (CSE) and the alkaloid cryptolepine (CLP) using V79 cells, a Chinese hamster lung fibroblast, and a number of organ-specific human cancer cell lines. CSE and CLP caused a dose- and time-dependent reduction in viability of the V79 cell line. In a V79 cell mutation assay (hprt gene), CSE (5–50 µg/mL) only induced mutation at the highest dose employed (mutation frequency 4 and 38 mutant clones per 10⁶ cells for control and CSE, respectively), but CLP (0.5–5.0 µM) was not mutagenic. They observed that the mutagenic dose of CSE was very toxic (less than 15% cell survival) and concluded that the poor genotoxicity of CSE and CLP coupled with their potent cytotoxic action support their anticancer potential. They also indicated that the remarkable similarity in the cytotoxic profiles of the two agents would suggest that CLP is responsible for the activity of CSE, consistent with a previous report that CLP is the major alkaloid of the root extract.

In a study to establish the molecular basis for the diverse biological effects of cryptolepine hydrochloride, Bonjean *et al* (33) observed that DNA is the primary target of cryptolepine. They indicated that the alkaloid binds tightly to DNA and behaves as a typical intercalating agent. Their study also led to the discovery that cryptolepine is a potent topoisomerase II inhibitor and a promising antitumor agent.

Using the fluorescent probe 2', 7'-dichlorofluorescein-diacetate (DCFH-DA) to measure intracellular changes in reactive oxygen species (ROS) Ansah *et al* (34) observed that both the aqueous anti-malarial formulation of *C. sanguinolenta* (CSE) and cryptolepine (CLP) caused a dose-dependent increase in ROS production, which reduced significantly following pre-treatment with N-acetylcysteine, an anti-oxidant. They suggested that reactive oxygen species generation is associated with cryptolepine cytotoxicity. In another study, Cimanga *et al* (35) isolated six of the Cryptolepis alkaloids, identified them spectroscopically as cryptoquindoline, quindoline, neocryptolepine, cryptolepine, 11-hydroxycryptolepine and biscryptolepine, and tested their interaction with the xanthine-xanthine oxidase enzyme system. 11-hydroxycryptolepine was shown to inhibit xanthine oxidase and act as a scavenger of superoxide anions. The other alkaloids were devoid of effect in both assays at the highest test concentration of 100 mM. They concluded that these findings suggest the importance, in the manifestation of both activities, of the hydroxyl group present in 11-hydroxycryptolepine but not in the other alkaloids. This result is interesting because if the major metabolite of *C. sanguinolenta* alkaloids after oral administration happens to be 11-hydroxycryptolepine, it is possible that the extract of the plant may have a net effect as a scavenger of superoxide anions. Its use as a daily tonic may therefore be justified.

Assay

It can be inferred from Appiah's work (36) that the Cryptolepine Base Equivalent (i.e. cryptolepine equivalent of the total alkaloidal content) of *C. sanguinolenta* roots is approximately 0.6%w/w. This means that a dose of Phyto-laria (3) which is a tea-bag containing 2.5g of powdered *C. sanguinolenta* roots is equivalent to 15mg of the total alkaloidal extract. Since this product is recommended to be taken three times daily, it implies that the total daily dose is equivalent to 45mg of *C. sanguinolenta* alkaloids. This works out to a daily dose of 0.6mg *C. sanguinolenta* alkaloids/kg body weight (taking the average body weight to be 70 kg). In another study, Appiah and Sittie (37) found that the ratio of the total alkaloid in a daily dose of four different products of *C. sanguinolenta* roots on the Ghanaian market is 3:4:4:10.

The daily maximum dose of *C. sanguinolenta* alkaloids (in a product of *C. sanguinolenta* root) recommended by herbal practitioners in Ghana is far less than 2.0 mg/kg body weight, and this is normally administered orally in three divided doses. The clinical doses are therefore very low as compared to the concentrations used in various *in vitro/in vivo* experiments including that of the LD₅₀ determination which was found to be 146 ± 49.3 mg of cryptolepine/kg in the male albino mouse after ip injection (38). This is probably one of the reasons why no major adverse effect has been observed over the long period of use of *C. sanguinolenta*. It is noteworthy that Luo *et al.* (as cited in Addy 2003) report the use of *C. sanguinolenta* extract as a tonic, often taken daily for years without evidence of side effects or toxicity (3). For the past 15 years, Gyapong (39), presently about 58 years old, has been taking *C. sanguinolenta* root decoction almost everyday; and he looks younger and healthier than most of his peers.

Unwanted/Adverse effects

The unwanted/adverse effects that might occur following an overdose of *C. sanguinolenta* extract administered orally include blurred vision, dry mouth, constipation, stomach upset, dizziness, postural hypotension and confusion. These effects are generally reversible and are probably due to the vasodilatory and the antimuscarinic effects of the plant extract.

Conclusion

The pharmacological properties of cryptolepine confirm most of the ethnomedical uses of *C. sanguinolenta* by West African natives. Administration of the aqueous extract of *C. sanguinolenta* seems to exert significant and desirable physiological and clinical effects. The plant has a great potential for the development of essential medicines including anti-cancer agents. For instance, taking into account the unwanted side effects of some anti-hypertensive and anti-diabetic agents which include gout, erectile dysfunction and asthma, *C. sanguinolenta* could be developed as an anti-hypertensive and/or

anti-diabetic with a possible desirable side effect as an enhancer of sexual performance.

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Chapter 14

Medicinal and Aromatic Plants of Ghana

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Ghana like most tropical countries abounds in aromatic plants which find applications in various sectors of life including healthcare, the food and beverage as well as the cosmetic industries. A considerable amount of scientific work has been carried out on them. In this review, results of the investigations are highlighted including a brief on the morphological characters, traditional uses, chemical constituents and biological properties to indicate the potentials inherent in these aromatic plants. Seven of these plants are considered, namely: *Chromolaena odorata*, *Canarium schweinfurthii*, *Hyptis suaveolens*, *Monodora myristica*, *Clausena anisata*, *Hoslundia opposita* and *Tetrapleura tetraptera*. The existence of chemo-varieties among African medicinal and aromatic plants with the potential to affect the quality of products has been recognized from this review. It calls for standardization to facilitate the commercialization of these plants.

The use of aromatic plants in various applications dates back to ancient times as evident in the historical records originating from China, Egypt, Greece and Rome. The significant role these plants play in everyday life cannot be over-emphasized. Their use as spices, seasonings, preservatives, medicines and in the dermatological and cosmetic industry can be said to be indispensable.

In Ghana, as in most other developing countries, medicinal and aromatic plants are used widely in traditional healthcare practices as well as the food and beverage sector. The fruits, bulbs and roots of some aromatic plants including *Xylopiya aethiopica* and *Zingiber officinale*, for example, have been included in various widely accepted indigenous soft drinks in Ghana by most people for their aromatic flavors and generally presumed health benefits. It is common to see aromatic plants in most backyard gardens of various homes. Easy accessibility to these plants enable people to fall on them for their medicinal benefits as well as for other domestic uses. Their cultivation and collection for sale to herbalists, and manufacturers of herbal products provide a source of income for many rural women who would be otherwise unemployed.

Medicinal and aromatic plants are invariably linked to Ghana's natural biodiversity and have considerable commercial potential both on the national and international markets. In Ghana, the sources of commercial medicinal and aromatic plants may be categorized into two: those from the wild and those cultivated. However, medicinal and aromatic plants are increasingly being depleted by un-sustainable harvesting methods while environmental degradation problems are threatening the survival of a good number of these plants. Rather unfortunately, traditional and indigenous knowledge on these plants are weakening and, in some instances, may be lost completely where traditional knowledge is still undocumented and only passed on orally from one generation to another. Ghana therefore has the challenge to increase her production to meet the growing demand by supplying high quality medicinal and aromatic plants.

The chemical compositions and the biological properties of aromatic medicinal plants have been documented in a number of scientific reports. In the present review, an attempt has been made to justify the uses of some aromatic plants growing in Ghana with reference to their chemistry, biological activities and economic uses. Seven of these plants more commonly used in Ghana are discussed.

***Chromolaena odorata* (L.) R. M. King & H. Rob.**

Chromolaena odorata (L.) R. M. King & H. Rob. (Syn: *Eupatorium odoratum* L.) (Asteraceae) is a fast growing exotic, perennial and dominant shrub which may have been inadvertently introduced into Ghana (Figure 1). It is commonly referred to as the Busia or Acheampong weed in 'honor' of the two heads of state at the time the plant is believed to have become domesticated in the country (1).

It forms dense tangled bushes, about 2-5 m high; stems are soft and woody, circular, hairy or almost smooth and much branched. The leaves are opposite, measuring 3-9 cm long and 2-5 cm broad; cuneate at the base and acute to acuminate at the apex. The leaves, slightly reddish purple when young, have

dentate margin, with three main veins, and give off a pungent odor when crushed. The flowers are pale mauve, regular, several in stalked capitula. The seeds are borne in the composite flower heads. The traditional uses of the leaf encompasses several medical applications notable among these are its use as a stypic, antimalarial, anticholeretic, and in rural areas where deep freezing facilities are minimal, it is used to temporarily preserve the dead (1, 2).



Figure 1. *Chromolaena odorata* in flower.

Several investigations on the leaves and stems of *C. odorata* have revealed the presence of essential oils (3, 4, 5, 6), steroids (7), triterpenes (8, 9), flavonoids (8, 9, 11, 12, 13, 14, 15, 16, 17, 18, 19). The flowers have also been subjected to investigation for their essential oils (20), fats (21), alkaloids (22) and flavonoids (23).

The essential oils obtained from plants from different parts of the Ivory Coast showed a lot of variation in the composition of the oils (24). The principal constituents of these oils were germacrene D, pregeijerene, *p*-caryophyllene, caryophyllene oxide, geijerene, pinene and β -caryophyllene. Pisutthanan *et al.* (25) reported the presence of pregeijerene, germacrene D, α -pinene, α -caryophyllene, vestitenone, β -pinene, δ -cadinene, geijerene, bulnesol, and *trans*-ocimene as the major components of the Thailand oil. The major constituents of the Chinese oil were *trans*-caryophyllene, δ -cadinene α -copaene, caryophyllene oxide, germacrene-D and α -humulene (26). These apparent differences in the oils of *C. odorata* were not unexpected, and can influence the therapeutic outcomes when the plant is used on account of its essential oil constituents.

Pisutthanan *et al.* (27) isolated six flavonoids out of which three were reported in this plant for the first time. They include 3,5,4'-trihydroxy-7-methoxyflavanone; 5,7,3'-trihydroxy-5'-methoxyflavanone and 3,5,7-trihydroxy-4'-methoxyflavanone. Ling *et al.* (28) also isolated a total of eleven flavonoids from the leaf extract and identified them as eriodictyol 7,4'-dimethyl ether, quercetin 7,4'-dimethyl ether, naringenin 4'-methyl ether, kaempferol 4'-methyl ether, kaempferol 3-*O*-rutinoside, taxifolin 4'-methyl ether, taxifolin 7-methyl ether and quercetin 4'-methyl ether. Suksamrarn *et al.* (23) isolated the flavanones, isosakuranetin (5,7-dihydroxy-4'-methoxyflavanone), persicogenin (5,3'-dihydroxy-7,4'-dimethoxyflavanone), 5,6,7,4'-tetramethoxyflavanone and 4'-hydroxy-5,6,7-trimethoxyflavanone, the chalcones, 2'-hydroxy-4,4',5',6'-tetramethoxychalcone and 4,2'-dihydroxy-4',5',6'-trimethoxychalcone, and the flavones, acacetin (5,7-dihydroxy-4'-methoxyflavone) and luteolin (5,7,3',4'-tetrahydroxyflavone) from the flowers.

Biller *et al.* (22) isolated the pyrrolizidine alkaloids 7- and 9-angeloylretronecine, intermedine, rinderine and 3'-acetyl rinderine. The concentrations of these hepatotoxic alkaloids were high in the roots and mature flower heads, but negligible in the leaves and stems. This finding is noteworthy since the leaves are mostly used in traditional medicine. Phenolic acids including protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, ferulic and vanillic acids have also been isolated from the plant (29).

The antimicrobial, anti-inflammatory, wound healing and antioxidant activities of the plant have been documented (26, 29, 30). Various extracts and isolated compounds showed antimicrobial activity. Irobi (31) showed that the ethanolic extract of the plant had bactericidal effect on reference microbial strains and hospital isolates including *Bacillus thuringiensis* (var *israeli*), *Bacillus stearothermophilus* (NCTC 10339), *Staphylococcus aureus* (NCTC 6571), *Escherichia coli* (NCTC 11699), *Pseudomonas sp.*, *Streptococcus faecalis* and *Klebsiella sp.* Isosakuranetin, 4'-hydroxy-5,6,7-trimethoxyflavanone, acacetin and luteolin isolated from the flowers, exhibited various degrees of activity against *Mycobacterium tuberculosis* (23). The oil from the aerial parts also showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The dichloromethane/water extract of the aerial part of the plant showed potent anti-HSV-1 activity ($IC_{50} = 1.74(1)/(4)$ g/ml) but moderate activity against *Mycobacterium tuberculosis in vitro*. The extract of the subterranean part of the plant also showed anti malarial activity against *Plasmodium falciparum* K1 strain with the EC_{50} of 9.39 (1)/(4)g/mL. The aqueous alcohol extract of the leaves inhibited *in vitro* growth of *Cryptococcus neoformans*, *Microsporium gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum* (27). The minimum inhibitory concentration ranged from 62.5 to 500 μ g/ml for the extract and from 25 to 100 μ g/ml for fractions of the extract (32). The possible site of action for the antibacterial activity appears to be the cell wall of the susceptible organisms this has been shown to be affected by alkaline pH and heat (31).

Acacetin and luteolin also showed moderate cytotoxicity against human small cell lung cancer (NCI-H187) (23).

The anti-inflammatory and wound healing activities of the plant have been linked to the flavonoid compounds eriodictyol 7,4'-dimethyl ether, quercetin

7,4'-dimethyl ether, naringenin 4'-methyl ether, kaempferol 4'-methyl ether, kaempferol 3-*O*-rutinoside, taxifolin 4'-methyl ether, taxifolin 7-methyl ether and quercetin 4'-methyl ether as the anti-inflammatory agents present in the leaf (33). The plant showed analgesic, anti-inflammatory and antipyretic activities which were attributed to the presence of flavonoids in the plant (34). Extracts from the leaves of *C. odorata* have been shown to be beneficial for treatment of wounds. Eupolin ointment, prepared from the leaves of *C. odorata* in Vietnam has been shown to promote the healing of soft tissue wounds and burns (35, 36, 37, 38).

Canarium schweinfurthii Engl.

Canarium schweinfurthii Engl. (Burseraceae) (Syn.: *C. chevalieri* Guillaum., *C. occidentale* A. Chev. and *C. velutinum* Guillaum) is a large forest tree which is widely distributed throughout the evergreen and deciduous forests of tropical Africa from Senegal to west Cameroon and extending to Ethiopia, Tanzania and Angola (39, 40). In Ghana, it is called Bediwunua (Twi), Kandangunuu (Brosa) and Eyele (Nzema). It is commonly called the bush-candle tree or the incense tree (39).

C. schweinfurthii is a large forest tree growing up to 36.5 m high and 3.7 m in girth, with its crown reaching to the upper canopy of the forest. It has a long clean, straight and cylindrical bole exceeding 27.4 m. Diameter above the heavy root swellings can be up to 4.5 m. The bark is thick; in the young tree it is fairly smooth, becoming increasingly scaly and fissured with age. It is rough grey to greenish orange. The slash is light yellow to red or brown and fragrant, exuding a heavy, sticky sulphur-yellow oleoresin and which solidifies like paraffin. The gum is burned and the residue is used in tattooing. It is used as a fumigant to drive away mosquitoes and as incense. It is used in deodorizing shea butter and used by women as fragrant pomade. The fresh bark is used for colic and piles; the bark decoction is used as for dysentery, cough and chest pains, and diabetes (2, 39, 40, 41).

C. schweinfurthii is characterized by its essential oil and resin. The oil obtained from the oleo-resin by hydrodistillation afforded octylacetate (60%) and nerolidol (14%) as the major constituents. This oil showed significant analgesic effect but was unable to reduce inflammatory process in experimental animals (42). Helene *et al.* (43) isolated from the seeds the novel phenylpropanoid schweinfurthinol and the known *p*-hydroxy-benzaldehyde, *p*-hydroxybenzoic acid, *p*-hydroxycinnamaldehyde, coniferaldehyde, ligballinol and the flavonoid amentoflavone. The fruit is said to be similar to olives but is rarely used. Georges *et al.* (44) showed that the pulp oil has low iodine, peroxide and carotene values, with a fatty acid composition showing a high content of oleic (89.4%) and stearic (67.7-84.0%) acids in the liquid, semi-solid and solid forms of the oil. The content of these two acids is much higher in *C. schweinfurthii* oil than in any other vegetable oil. According to Eromosele *et al.* (45) the seed oil is a non-drying oil which may be suitable as animal feed. Onimawo and Adukwu (46) determined the fatty oil from the fruit pulp to have an acid value, 0.68; saponification value, 196.35; iodine value, 89.83 and

peroxide value 7.80. Abayeh *et al.* (47) examined the quality characteristics of the fatty oils obtained by solvent extraction from the mesocarp and endocarp using hexane, followed by chloroform-methanol mixture and then water saturated with butanol. Analysis of the fatty acid composition of the first hexane extracts indicated that the oils were primarily C16 and C18s. The mesocarp contained 31.7% hexadecanoic acid, 30.0% 9-octadecenoic acid, 30.1% 6,9-octadecadienoic acid and 8.2% 9,12,15-octadecatrienoic acid, while the endocarp, contained 31.2% hexadecanoic acid, 28.9% 9-octadecenoic acid and 31.3% 6,9-octadecadienoic acid. Ajiwe *et al.* (48) showed that the fatty oil from the seed is semi-drying with relatively high saponification value and could be used for making alkyl resins which could be used for making paints and the oil from the pulp used for the production of polish and solid soap.

Kamtchouing *et al.* (41) have established the hypoglycemic effect of the stem bark extract in male rats. The extract reversed hyperglycemia, polyphagia and polydipsia provoked by streptozotocin.

Hyptis suaveolens (L.) Poit

Hyptis suaveolens (L.) Poit (Lamiaceae) is a weed found in waste places and cultivated lands in Ghana and from Senegal to Nigeria (1). *H. suaveolens* is a strong -scented herb which grows up to 1.5 m high with quadrate hairy stems. The leaves are hairy, ovate to obovate in shape, about 3-5 cm long and 2-4 cm wide and serrulate margins.

It is commonly referred to as Bush Tea-Bush. In Ghana it is called Opea (Twi), Suruwie (Ga), Awusakadji (Ewe), Kumkum (Nzema) and Beaba (Dabgani). It is native to Tropical America, but now widespread as a weed throughout the tropics. The plant is used as an appetizing agent, to combat indigestion, stomach pain, nausea, flatulence, colds, and infection of the gall bladder. Steam bath of the leaves is used against fevers. A poultice of the leaves is used to dress sores and swellings. A decoction of the leaves is used to preserve dead bodies. The fresh plant is placed in rooms to repel mosquitoes. Instillation of the fresh extract into the nostrils is used to revive persons who are fainting (1, 2, 39).

The chemical constituents of *H. suaveolens* comprise of essential oils and a few triterpenes (49, 50, 51, 52). Iwu *et al.* (53) identified in the steam distilled oil 32 terpenoid compounds. The major compounds were: limonene; thujane, α -pinene, α -phellandrine, 3-cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-3-cyclohexen-1-ol, 3-cyclohexen-1-carboxyaldehyde, elemene, 4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene, octahydro-1,4-dimethylazulene, 5 α -, 8 β -,H-9 β -,H-10 α .-labd-14-ene; 5 α .-androst-9(11)-en-12-one and 5 α .-androst-2,11-dione. Fun *et al.* (54) showed by GC analysis that the oil has high contents of 1,8-cineole (27-38%) and sabinene (12-18%). Analysis of the oil by Thoppil and Jose (55) showed the presence of citronellyl acetate, β -caryophyllene, piperitone oxide and geranyl acetate. Zollo *et al.* (56) reported that the most abundant compounds in the oil collected from Cameroon were hydrocarbons (> 58%). Tchoumboungang *et al.* (57) reported the presence of sabinene (20.6%), β -caryophyllene (17.5%) and bergamotol (10.9%) as the

principal constituents of the oil from Cameroon. Sidibel *et al.* (58) reported that the oil was composed mainly of sabinene (31-38%), with also β -caryophyllene and *trans*- α -bergamotene as major constituents. Azevedo *et al.* (59) analysed oil samples collected from 11 localities in Brazil and found sabinene, limonene, bicyclogermacrene, β -phellandrene and 1,8-cineole as the principal constituents. They also found that samples collected from lower latitudes produced sesquiterpene-rich oil. A further analysis of 9 oils from different geographical sources in Brazil showed spathulenol, 1,8-cineole and (*E*)-caryophyllene as the principal constituents (60). Campos *et al.* (61) investigated oils of samples in various vegetative and fruiting stages, and collected from different localities of Brazilian Cerrado, and found sabinene, 1,8-cineole, spathulenol, (*E*)-caryophyllene and bicyclogermacrene as the principal constituents. Silva *et al.* (62) analyzed steam-distilled oils from leaves, stems and inflorescences of greenhouse specimens and obtained a total of 38 compounds which were mainly monoterpenes (16) and sesquiterpenes (13). The sesquiterpenes β -caryophyllene (10,39%) and spathulenol (13,30%) were present in higher yields in the leaves and stems, respectively, while the monoterpene 1,8-cineole (27,47%) was the major compound present in the inflorescence oil. The inflorescence produced more oil than the leaves and stems. Oliveira *et al.* (63) analyzed the chemical composition of the essential oils of seven populations of *H. suaveolens* in vegetative, flowering and fruiting stages by GC-MS and confirmed that monoterpene hydrocarbons were mainly produced in plants from sites located in higher latitudes and altitudes regardless of the phase of growth, while sesquiterpenes were mainly produced in fruiting samples grown at lower altitudes. Eshilokun *et al.* (64) analyzed oils obtained from samples from two university campuses in Nigeria and reported that α -pinene (13.6%), sabinene (13.2%), *p*-cymene (11.7%), terpinen-4-ol (9.8%) and terpinolene (6.3%) were the major monoterpenes in the oil from the campus of Lagos State University, while sabinene (30.0%), terpinen-4-ol (11.4%), terpinolene (5.6%), 1,8-cineole (5.2%), beta-pinene (4.4%) and α -terpinene (4.2%) were the main monoterpenes oil sample from Obafemi Awolowo University. Both samples showed the presence of β -caryophyllene (5.1-5.9%) and *trans*- α -bergamotene (1.6-5.2%) as the major sesquiterpenes. Martins *et al.* (65) showed that when cultivated in greenhouse, *H. suaveolens* yields oil rich in oxygenated sesquiterpenes. The major constituents found in the study were spathulenol, lobulol, dehydroabietol, α -cadinol and *p*-phellandrene. Koba *et al.* (66) found sabinene (28.0%) and *p*-caryophyllene (25.8%) as the major constituents of the oil from Togo.

Rao *et al.* (52) isolated the novel triterpenoid, hyptadienic acid from the aerial part of *H. suaveolens*. This was the first report of a naturally occurring A-ring contracted triterpene outside the lupane series. Triguna *et al.* (67) isolated the new natural triterpenoid, 3 β -hydroxylup-12-en-28-oic acid, from the roots of *H. suaveolens* in addition to α - and β -amyrin.

Mandal *et al.* (68) have reported on the antimicrobial activity of the leaf extract of *H. suaveolens*. The steam-distilled oil, petroleum ether and ethanol extracts exhibited broad-spectrum antibacterial and antifungal activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Micrococcus luteus*, and the fungi *Fusarium oxysporum*,

Aspergillus niger, *Helminthosporium oryzae*. The oil showed the highest antibacterial activity on *Aspergillus niger* and *Micrococcus luteus* (69).

***Monodora myristica* (Gaertn.) Dunal**

Monodora myristica is a tree widely distributed in the evergreen and deciduous forests along the west coast and central Africa (40) (Figure 2). It is locally referred to as Awerewa (Twi), Ayerew-amba (Fanti), Avonoba (Nzema) and Maalai (Ga). The plant grows up to 24 m high and 1 m in girth. It has a clear bole; the bark is thin and fairly smooth. It gives a white slash. The leaves which alternate, are obovate-oblong to obovate-elliptic in shape. The flowers are large and fragrant. The sepals are red-spotted, crisped and have wavy edges. There are 6 petals; the outer petals measuring up to 10 cm long and having crisped margins and spotted red, yellow and green; the inner petals form a white to yellow cone-like structure at the centre and spotted red outside and green inside. The green fruits which bear the seeds are up to 20 x 15 cm are suspended on long stalks. The seeds are numerous and are embedded in sweet scented pulp. They are oblong in shape, about 9 cm long, brown when fresh. The seed has a thin seed coat and hard kernel (40).



Figure 2. *Monodora myristica* (Gaertn.) Dunal in flower

The seeds are aromatic and are sold all over West Africa as spice (Figure 3). It is a common ingredient in soups, snuffs, pomades and traditional medicines. The seeds are also used for treating constipation and migraine, and are applied to sores and swellings (2, 40).

The chemical constituents that have been identified in this plant are mainly essential oils and alkaloids. The seeds on steam distillation yielded an oil composed of 75 % monoterpene hydrocarbons, a few sesquiterpene hydrocarbons (3 %) and oxygenated compounds (70). The major constituents included α -phellandrene (50.4 %), α -pinene (5.5 %) and myrcene (4.35 %), germacrene-D-4-ol (9.5 %). Other compounds which were found were α -pinene, δ -2-carene, β -caryophyllene, valencene, γ -muurolol and carvacryl acetate. Ekundayo and Hammerschmidt (71) identified fifty three constituents of the essential oil from the seeds and found the principal constituent as *p*-cymene and linalool. Boyom *et al.* (72) have suggested that the leaves also produce essential oil made up of mostly sesquiterpenes with β -caryophyllene as the major constituent. However, the apparently odorless leaves, stems and roots of the plant did not yield essential oils on steam distillation in our laboratory (unpublished data). Cimanga *et al.* (73) have shown that the essential oil from the seed is antibacterial.



Figure 3. *Monodora myristica* (Gaertn.) Dunal seeds as sold in Ghana.

The alkaloids encountered in *M. myristica* are prenylated indoles isolated from the seeds. These were the first to be isolated from a higher plant. So far only two have been reported including the simple 6-(3,3-dimethylallyl)-indole and the dimeric 6-(γ,γ -dimethylallyl)-indoles (74, 75).

The crude ether extract significantly inhibited oviposition by the seed beetle, *Callosobruchus maculatus* (Bruchidae), on legume seeds and exhibited ovicidal and larvicidal action. Ofuya *et al.* (76). Oluwafemi and Taiwo (77) have recently shown that the toxigenic effects of crystalline aflatoxin B-1 produced by *Aspergillus flavus* in cockerels, can be reversed by administration of alcoholic extract of the seeds of *M. myristica*.

***Clausena anisata* (Willd.) J.D. Hook ex. Benth**

Clausena anisata (Willd.) J. D. Hook ex. Benth (Rutaceae) is a small tree that grows in the Guinea-Congolian belt and it is widespread in tropical Africa (Figure 4). It is a shrub or small tree which grows up to 6 m high. The leaves are pinnate and highly fragrant; the leaflet, about 15 or more, are pellucid-dotted and rather pubescent below especially on the nerves. The leaflets alternate, are variable and measure 7.5 x 5 cm. They are obliquely ovate to ovate-lanceolate in shape and have entire margin. The flowers are white or cream and numerous. They are small, in inflorescence and are up to 22.5 cm long. The fruits are blue-black drupes, ellipsoid in shape, and measure 0.8 cm diameter (40).



Figure 4. *Clausena anisata* (Willd.) J.D. Hook ex. Benth in fruit.

C. anisata is Sesadua (Akyem), Samanobere (Ashanti), Tonton tso (Ga) and Ayira (Eve). The leaves are used for snake-bites and in nasal drops as an analgesic and antiseptic for bronchial troubles, headaches, and sinusitis. The twigs are used as chewing sticks. The root preparation is given to children for stomach troubles. They are also used in mouth-washes, for toothache, headache, constipation, fever and dysentery. The leaves are hung in houses to repel mosquitoes (2, 40, 78).

Gundidza *et al.* (79) studied the essential oil from the leaf and found sabinene, terpinen-4-ol, α - β -ocimene, germacrene and germacrene-B as the principal constituents. Ekundayo *et al.* (80) reported on the constituents of the essential oil of *C. anisata* leaves and found estragole as the major compound present in the oil.

Other constituents of *C. anisata* include carbazole alkaloids, coumarins. Okorie (81) reported the isolation of new carbazoles from the roots. Mesteri and

Reisch (82) isolated the new optically inactive carbazole mupamine from the root bark. Ngadjui *et al.* (83) isolated from a combined stem and root extracts six alkaloids including heptaphylline, girinimbine, ekeberginine and 3-methylcarbazole, 1-methyl-3,4-dimethoxy-2-quinolone and 3-formyl-1-hydroxycarbazole. Chakraborty *et al.* (84) isolated from the alcoholic extract of the stem bark the two novel carbazoles clausenol and clausenine. Ito *et al.* (85) isolated three new carbazole alkaloids from *C. anisata* collected from Thailand. These carbazoles namely clausamine-A, -B and -C are characterized by 1-oxygenated 3,4-disubstituted structures with a lactone moiety.

A total of twenty coumarins isolated from the leaves, stem-bark, roots and fruits of this plant have been discussed. Okorie (81), Reisch and Wickramasinghe (86), and Ngadjui *et al.* (87) reported the isolation of new coumarins from the roots. Lakshmi *et al.* (88) isolated monoterpene furanocoumarin lactones from *C. anisata*. Ngadjui *et al.* (83) isolated from a combined stem bark and roots, anisocoumarins A, B, C and D. Ngadjui *et al.* (1991) isolated two geranyl coumarins from the leaves and identified them as anisocoumarins I and J.

In considering the biological activities, Adesina and Adewunmi (89) reported the isolation of the coumarins heliottin and imperatorin from the roots, and their molluscicidal properties. Adewunmi and Delle Monache (90) later reported the molluscicidal activity of the coumarins from *C. anisata* and showed that this activity is related to the nature of the substituents and the ring system. Lakshmi *et al.* (91) showed that the 50% alcoholic extract has spasmolytic activity in the guinea pig ileum. This activity was attributed to the presence of furanocoumarins present in the plant. Anisolactone was found to be the most potent compound and was confirmed by *in vivo* tests. Clausenol has shown activity against Gram-positive and Gram-negative bacteria and fungi (84). Okunde and Olaifa (92) reported on the acute toxicity of estragole, the major constituent of the leaf oil. Makanju (93) reported that the aqueous extract of the root bark caused depression of the CNS and also showed mild anticonvulsant activity in mice.

***Hoslundia opposita* Vahl**

Hoslundia opposita Vahl (Lamiaceae) is a scrambling weak shrub that grows in secondary, fringing and savannah forests of tropical Africa. It is widely distributed from Senegal through East Africa to South Africa (40). In Ghana it is called Abrewa aninsu, Nunumerewa (Twi), Nunum nini (Ashanti), Alomazobe (Nzema) and Akotadzometsi (Ewe).

H. opposita grows up to 4.6 m high, with square stems, brown and fibrous bark. The leaves occur in twos or threes and measure 10 cm in length and 3.8 cm in width. The leaves are narrowly ovate-lanceolate to acuminate shaped with cuneate base and serrate margin. The flowers are small, lilac-white in color and occur in lax terminal panicles (40).

The leaves, stems and twigs are used for constipation, wounds, skin diseases, snake bite, venereal diseases, conjunctivitis, vertigo and epilepsy. The

roots are used for sores, colds, sore throats and abdominal pains. The decoction of the whole plant is drunk for stomach troubles and gonorrhoea (2, 40, 78).

The essential oil from the plant collected from Nigeria and Rwanda has been reported on (94). Analysis of the leaf oil showed the major constituents to be largely the sesquiterpenes and sesquiterpene alcohols (95). Ayedoun *et al.* (96) reported on the chemical composition of the oil from specimens collected from Benin and Cameroon. In the Benin oil, only β -caryophyllene (16.2%) and germacrene D (27.2%) were present in amounts above 15%. The oil from Cameroon was more complex, and showed the presence of α -copaene (12.3%) and δ -cadinene (10.5%), β -caryophyllene (10.3%) and germacrene D (15.1%). Chagonda and Chalchat (97) analyzed the hydro-distilled oil of wild and cultivated samples collected from Zimbabwe and found both wild and cultivated plants had eugenol (55.1-76.2%) and β -caryophyllene (6.5-8.5%) as the major components. Oils from two wild sources confirmed two chemotypes: those containing (Z)- β -ocimene (3.5-4.3%) and germacrene-D (2.1-41.6%) and those containing α -humulene (6.4-7.6%) and (E)- β -ocimene (2.1-2.5%). Caryophyllene oxide (1.6-2.9%), *trans*- α -bergamotene (2.0-2.3%), β -bisabolene (1.1-1.6%), geraniol (0.7-2.3%) and 1,8-cineole (2.6-4.4%) were important minor components in the oils. Tonzibo *et al.* (98) also analyzed the oils collected from different localities in Ivory Coast. The results demonstrated apparent variability in the chemical composition of the oils, and identified four clear chemotypes: oils rich in germacrene D (31.1%), oils containing β -caryophyllene (13.3%), germacrene D (24.1%) and benzyl benzoate (30.1%), oils containing as major constituents menthol (10.9%), thymol (13.9%), β -caryophyllene (20.5%) and germacrene D (10.7%), and oils containing β -caryophyllene (24.8%) and germacrene D (24.1%).

Ngadjui *et al.* (87) isolated three novel flavonoids namely hoslundin, hoslundal and hoslundiol, from the twigs. They further isolated the flavonoids oppositin, 5-*O*-methylhoslundin, tectochrysin, hoslundiol and hoslundin from the methanol extract of the twigs. The isolation of oppositin and 5-*O*-methylhoslundin was the first report of the two pyrone-substituted flavonoids in nature. Another novel pyrone-substituted flavonoid hosloppin was isolated from the methanolic extract of the leaves (99).

Achenbach *et al.* (100) isolated four new abietane-type esters namely 3-*O*-benzoylhosloppone, 3-*O*-cinnamoylhosloppone, 3-*O*-benzoylhinokiol and 3-*O*-benzoylhosloquinone from the root bark of the plant.

Gundidza *et al.* (95) have shown that the essential oil from the leaves has significant activity against *Aspergillus niger*, *Acinetobacter calcoacetic*, *Brochothrix thermosphacta* and *Flavobacterium suaveolens*. Mujovo *et al.* (101) isolated three known compounds, 5,7-dimethoxy-6-methylflavone, hoslundiol and euscaphic acid. 5,7-dimethoxy-6-methylflavone inhibited the HIV-1 reverse transcriptase enzyme by 91, 53 and 52%, respectively, at 100 $\mu\text{g ml}^{-1}$.

Euscaphic acid was active against a drug-sensitive strain of *M. tuberculosis* with a minimum inhibitory concentration of 50 $\mu\text{g ml}^{-1}$ (101).

3-*O*-Benzoylhosloppone inhibited the growth of the multidrug resistant strain K1 of *Plasmodium falciparum in vitro* with an IC_{50} -value of 0.4 $\mu\text{g ml}^{-1}$ (100).

Muchuweti *et al.* (102) analyzed the 70% ethanolic extract for its antioxidant activity and their total phenolic content. The antioxidant activity calculated as 5.31 +/- 4.47%, while the total phenolics in the extract estimated as tannic acid equivalent (TAE) was calculated as 0.024 +/- 0.006 TAE.

***Tetrapleura tetraptera* (Schum. et Thonn.) Taub.**

Tetrapleura tetraptera (Schum. et Thonn.) (Mimosaceae) is a tree that grows in the tropical deciduous forest of West Africa, up to 24.3 m high and 1.2 m in girth, extending from Senegal to Cameroon, and it is found in Sudan, Uganda and Zaire (103) (Figure 5). In Ghana it is called Prekese (Twi), Esem (Fanti) and Aprekese (Nzema).

T. tetraptera is a tall tree growing in the deciduous, secondary and fringing forests with sharp buttresses, and silvery grey to reddish smooth thin bark. The leaves which are sensitive to touch, are bipinnate. They are made up of 8-12 pairs of alternating leaflets, and each pinna is made up of 6-8 opposite pairs. The leaflets are oblong to elliptic in shape and rounded at the apex. The fruits have four longitudinal wing-like ridges which are perpendicular to each other. Two of these ridges are woody; the other two are filled with soft sugary pulp. They are oily and aromatic. The fruits are usually brown in color, shining and glabrous, and bearing small, black, hard seeds (green internally) which rattle in the pods (40) (Figure 6).



Figure 5. *Tetrapleura tetraptera* (Schum. et Thonn.) Taub.



Figure 6. *Tetrapleura tetraptera* (Schum. et Thonn.) Taub. fruit

Generally in West Africa, the fruit is widely used in traditional remedies for the treatment of disease conditions including convulsion, gastric ulcer, rheumatism, fevers, whitlow, skin rashes, smallpox, malaria and dysentery (2, 39, 40, 104). It is also used to manage diabetes and cardiovascular diseases like hypertension and stroke in some parts of Ghana (Amoako-Atta, CBUD Director, KNUST, personal communication). The fruits are used to flavor foods and alcoholic beverages (2, 40).

Essential oil, flavonoids and triterpenoids. have been isolated from *T. tetraptera*. The triterpenes isolated from the fruits and bark are oleanane-type saponins (105, 106, 107, 108). They include the monodesmosidic diglycoside of the rare sapogenin 27-hydroxyolean-12(13)-en-28-oic acid (109). aridanin, 3-*O*-(2'-acetamido-2'-deoxy-beta-D-glucopyranosyl) echinocystic acid, 3-*O*-[beta-D-glucopyranosyl-(1"-6')-2'-acetamido-2'-deoxy-beta-D-glucopyranosyl]olean-12-en-28-oic acid and acid-3-*O*-sodium sulfate echinocystic acid-3-*O*-sodium sulfate (108). Some of these saponins were reported for the first time as natural products and unique with the potential as natural molluscicides (109, 110, 111). Other compounds isolated from the plant include umbelliferone from the leaves, ferulic acid from branches and scopoletin from the fruits (112). Fleischer *et al.* (113) isolated three known flavonoids: butein, isoliquiritigenin and naringenin from the fruits. The presence of these compounds which were reported for the plant for the first time, and have proven antidiabetic and antihypertensive actions confirms the traditional use of the fruit in the management of these conditions, as reported by Ojewole and Adewumni (114).

The ethanolic extract of the fruit showed significant blood schizonticidal activity in *Plasmodium berghei* infected mice and anti inflammatory activity (114, 115). Nwiauwa *et al.* (116) have reported on the anticonvulsant properties of the essential oil of the fruit.

Concluding Remarks

There is widespread occurrence of aromatic plants in Ghana and Africa in general and these have extensive applications in the life and culture of the people (Table I). Apart from their use as spices in cuisines, they are used in preparation of various traditional remedies for the treatment of several diseases. More often than not an herbal preparation contains one or more aromatic plant part (s); though the method of storage of the plant parts or the preparation of the herbal product by boiling causes the loss of most of the aromatic constituents.

There is a plethora of chemical constituents produced by the aromatic medicinal plants with great diversity in their structure and biological activities. It is clear that the variety of biological activities shown by the plant extracts and sometimes their isolates provide scientific support for the traditional applications of these medicinal plants. However, there is a clear lack of clinical evidence, which is needed to promote the use of such medicinal and aromatic plants as potential therapeutic agents in evidence-based healthcare practice.

From this review, it is evident that chemo-varieties exist among the medicinal and aromatic plants. These variations may be due to various factors including climatic and edaphic factors. This calls for collaborative studies towards the standardization of these plants to allow commercialization of African medicinal and aromatic plant products which are generally low on the international market.

Chromolaena odorata, *Canarium schwenfurthii*, *Hyptis suaveolens*, *Monodora myristica*, *Clausena anisata*, *Hoslundia opposita* and *Tetrapleura tetraptera* are aromatic medicinal plants which are commonly used in Ghana and other parts of West Africa where environmental degradation and unsustainable harvesting practices threaten their survival. The issues relating to conservation of these potential economic and therapeutic agents must also be taken seriously.

Table I. Summary of aromatic medicinal plants reviewed.

<i>Name of plant</i>	<i>Common English (and tribal Bioac. names)</i>	<i>Com.</i>	<i>Major uses, and parts used</i>	<i>Mode preparation</i>	<i>of Ref.</i>
<i>Chromolaena odorata</i> (Asteraceae)	Siam weed (Twi)	(Busiam)	Styptic. Wound healing Leaves, stems (Aerial parts).	Antimalarial. Poultryce. Aqueous/ alcoholic extracts. EO	23, 25, 31, 32, 35-38
<i>Canarium schweinfurthii</i> (Bursaceae)	Bush-candle tree. African elemi (Bediunua (Twi), Kandangunuu (Brosa), Eyele (Nzema)	Incense tree. EO	Perfumery. Antidiabetic. Stem, bark.	Oleo-resin. EO. aqueous extract.	Stem 41, 42
<i>Hyptis suaveolens</i> (Lamiaceae)	Pignut (Opea (Twi), Suruwie (Ga), Awusakadji (Ewe), kumkum (Nzema) and Beaba (Dabgani).	EO	Infection of the gall bladder. Mosquito repellent. stimulant. Indigestion. Stomach Flatulence. Nausea. Leaves and twigs,	Cold. EO. Appetite Fumigation (Whole plant)	61-66, 68- 69
<i>Monodora myristica</i> (Annonaceae)	Calabash nutmeg (Awerewa Ayerew-amba (Fanti), Avonoba (Nzema) and Maalai (Ga)	nutmeg. Jamaica EO (Twi), Avonoba	Spice. Constipation and Migraine. Sores and Swellings. Seeds.	Whole seeds. Seed extracts	71, 73

Table I. Continued.

<i>Name of plant names)</i>	<i>Common English (and tribal Bioac. Com.)</i>	<i>Major uses, and parts used</i>	<i>Mode preparation</i>	<i>of Ref.</i>
<i>Clausena anisata</i> (Rutaceae)	Unknown (Sesadua (Akyem), Samanobere (Ashanti), (Ga)and Ayira (Ewe)	EO, C-ALK, CO	Pain, Nasal drops and Bronchial and Mosquito repellent Antisnake-venin. Leaves, roots.	Extracts from 79-80, 83, 85, 87, 89-91
<i>Hostundia opposita</i> (Lamiaceae)	Orange bird gooseberry (Abrewa aninsu, Nunumerewa (Twi), (Ashanti), Alomazobe and Akotadzometsi (Ewe).	Bird EO, FL	Constipation. Wounds, colds. Skin diseases, diseases. Conjunctivitis. Epilepsy. Snake bite. Leaves, Twigs, Roots	94-102
<i>Tetrapleura tetraptera</i> (Mimosaceae)	Unknown (Prekese (Fanti) and Aprekese (Esem (Twi), Nzema)	EO, ALK, SAP	Convulsion. Gastric ulcer. Rheumatism. Malaria. Whitlow. Dysentery. Diabetes. Hypertension. stem, bark.	102, 105-106, 108-109, 111, 113-116

NOTE: Bioac. Com., Bioactive Components, EO, essential oil, ALK; alkaloids, FL, Flavonoids, C, Carbazole, CO, coumarins

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Chapter 15

Nicosan: Phytomedicinal Treatment for Sickle Cell Disease

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Nicosan (formerly called Niprisan) is a multiple herbal drug that has been approved in Nigeria for the treatment of sickle cell disease. This phytomedicinal-based drug was developed by traditional healers in Nigeria and has recently been the subject of *in vitro* studies and clinical trials. Nicosan is an extract mixture of four plants, *Piper guineenses*, *Pterocarpus osun*, *Eugenia caryophyllum*, and *Sorghum bicolor*, which grow wild or are cultivated in West Africa. In this chapter, we will discuss the development history of this herbal drug and its chemical properties.

Sickle cell disease is an autosomal recessive genetic disease that is of major significance in Africa. Sickle cell disease has been intensely studied since it was first recognized in the early 1900s (1) and several recent reviews are available (2-5). Here, we present a brief summary of the biology of the disease.

Hemoglobin A is a tetrameric protein formed by noncovalent association of 2 β -globin and 2 α -globin protein chains. A heme molecule is associated with each of the four protein chains. In adults, hemoglobin A is the major oxygen transport molecule in the blood. Sickle cell disease results when an individual inherits at least one allele of the sickle hemoglobin (*Hb S*) variant of the β -globin gene and another abnormal globin gene. The most common form of sickle cell disease is sickle cell anemia where individuals are homozygous for the *Hb S* allele. In the *Hb S* variant there is a single nucleotide substitution in the gene, which results in a single amino acid substitution, a valine instead of a

glutamic acid, as the sixth amino acid of the β -globin protein. This position is on the surface of the β -globin protein and the substitution of the nonpolar amino acid valine for the polar amino acid glutamate reduces the solubility of the deoxygenated form of hemoglobin S. In the deoxygenated form, the surface region containing the valine substitution in a hemoglobin S molecule binds to another deoxyhemoglobin S molecule ultimately resulting in polymeric tubular fibers that distort the red blood cell, producing the sickled shape from which the disease gets its name. Many of the clinical symptoms of sickle cell disease are caused by the adhesion of the sickled cells to the vascular endothelium along with aggregate formation with other cells, both of which interfere with normal blood flow in the small blood vessels.

Sickle cell anemia occurs in individuals that are homozygous for the *Hb S* gene. Individuals that are heterozygous, having one *Hb S* gene and one normal gene, have the sickle cell trait and, under most circumstances, do not have symptoms of the disease. The sickle cell trait occurs in 10-30% of individuals in Equatorial Africa as well as in some Mediterranean countries and some regions of India (4). The high incidence of sickle cell trait in some populations where malaria is prevalent led Allison (6) to propose that there was a selective advantage conferred on the heterozygote individuals. Numerous studies have since confirmed this and have shown that the *Plasmodium falciparum* parasites can accelerate sickling of infected cells where the parasites then have lower survivability (7). Individuals with sickle cell trait do become infected with the malaria parasites, but are less likely to die from the infection than are individuals homozygous for the normal β -globin gene (8).

Magnitude of the Sickle Cell Disease Problem

Although sickle cell disease was among the first genetic diseases to be understood at the molecular level, there is still no simple non-toxic treatment to offer individuals afflicted with the condition. In the United States, sickle cell disease is the most common form of genetic blood disorder and the National Heart, Lung and Blood Institute estimates there are about 70,000 individuals with the disease (http://www.nhlbi.nih.gov/resources/docs/scd_program.htm). Approximately 8% of African Americans are heterozygote carriers of the *Hb S* gene (9). Worldwide there are approximately 10 million sufferers, and in Nigeria, one of the most severely affected nations, it is estimated that more than 2 million individuals suffer from sickle cell disease and 25 million are carriers (10). In Africa, it is estimated that 300,000 children are born annually with the disease and 50% of them die by age five (www.sicklecelldisease.net/cbo/documents/update_20050621/2005june.pdf; 11).

The cost to society of sickle cell disease is high in terms of human suffering and in the financial burden of providing treatment to alleviate the pain and symptoms of the afflicted individuals and the loss of income to the individuals and their families and communities. Health problems due to sickle cell disease include chronic anemia, vaso-occlusive crises, splenic sequestration, acute chest syndrome, stroke, splenic and renal dysfunction, and susceptibility to bacterial infections (2,5,12). In Africa, treatments for sickle cell disease are limited

because of cost and lack of basic knowledge about the disease (13). In North America, Europe and the Caribbean, treatments are more readily available and the average life expectancy has increased from 14.3 years in 1973 to 45-55 currently (11,14,15). However, this is still considerably below the overall life expectancy in the United States of 77.5 years (16). The increase in life expectancy for individuals with sickle cell disease is of course accompanied by a high cost of medical treatment. Nietert et al. (17) reviewed several studies that examined the health care costs of sickle cell disease in the United States. The authors concluded that patients with sickle cell disease require numerous visits to healthcare providers and account for a disproportionately large share of healthcare costs. One study estimated that in the United States annual direct costs of hospitalizations for sickle cell disease totaled \$475 million, in 1996 values (17).

Clearly sickle cell disease is a major health concern in the United States. It is of course a far bigger concern in Africa where millions of individuals suffer because of a lack of resources and the historically low priority of sickle cell disease among public health decision makers (13,18). In response to the seriousness of the problem in many developing nations, the World Health Organization and the United Nations Educational, Scientific and Cultural Organization (UNESCO) launched several initiatives to enhance community education and training of health care professionals in affected areas (18,19).

Current Global Therapeutic Options for Sickle Cell Disease and the Development of Nicosan

There is no cure for sickle cell disease and current therapies are aimed at managing the numerous complications associated with the condition. These therapies include penicillin treatment, immunization against *Streptococcus pneumoniae*, blood transfusions, and hydroxyurea (12,20). Hydroxyurea is the only drug therapy specific for sickle cell disease. Hydroxyurea appears to act through several mechanisms, including increased production of fetal hemoglobin, reduced adhesion of blood cells to endothelial cells, improved erythrocyte hydration, and reduced neutrophil counts (21). Hydroxyurea has proven effective but there are some concerns with toxicity and not all patients respond to the treatment (21-23). There is clearly a need for development of additional therapies.

In Nigeria, Rev. P.O. Ogunyale developed an herbal-based remedy for sickle cell disease, which was scientifically investigated by researchers at the Nigerian National Institute for Pharmaceutical Research and Development (NIPRD). This herbal medicine is a dried extract of four herbs including the seeds of *Piper guineenses* (common name climbing Black Pepper or Benin Pepper), stems of *Pterocarpus osun* (common name Camwood), the fruit of *Eugenia caryophyllum* (common name Clove), and the leaves of *Sorghum bicolor*, also known as sorghum (10). The formulation and use of this herbal extract for the treatment of sickle cell disease was patented by NIPRD and is protected under United States Patent 5800819.

In early tests of the drug, it was found to have no toxicity at high doses in rats and it delayed polymerization of hemoglobin S *in vitro* (24,25). Phase I and Phase II clinical trials in Nigeria indicated that Nicosan was safe and significantly reduced the number of sickle cell disease associated crises experienced by the patients (10,26).

This clinical trial was independently evaluated by Cordeiro and Oniyangi (27) who concluded “This Phase IIB (pivotal) trial suggests that a phytomedicine, NIPRISAN[®], was effective in reducing episodes of SCD crisis associated with severe pain over a six-month period. NIPRISAN[®] did not appear to affect the risk of severe complications or the level of anaemia. No serious adverse effects were reported.”

The *in vitro* antisickling effects of Nicosan were extensively investigated by Iyamu et al. (28). Erythrocyte sickling was inhibited 50% by 0.05 mg/ml of Nicosan and 0.05 µg/ml delayed deoxy-Hb S polymerization by six-fold. Based on the low doses of the drug required to elicit these effects it was suggested that the drug interacted directly with the hemoglobin S molecules. Results from *in vivo* studies on transgenic sickle cell mice showed that the survival time under severe acute hypoxic conditions was extended from 10 min in the control mice to 60 min in mice administered 50 mg/kg Nicosan (29).

Taken together, these laboratory and clinical studies indicate that Nicosan is a safe and effective treatment for sickle cell disease. In 2002, the Nigerian National Institute for Pharmaceutical Research and Development granted Xechem International exclusive rights for development, production and marketing of Nicosan. In 2003, the Food and Drug Administration (FDA) of the United States of America granted orphan drug status to Nicosan for the treatment of sickle cell disease. This is the first herbal drug to receive this recognition from the FDA. In 2005, the European Medicine Evaluation Agency (EMA) also granted orphan drug status to Nicosan. The Nigerian regulatory agency, National Agency for Food and Drug Administration and Control (NAFDAC), more recently, has approved Nicosan and it was launched for commercial sale as a new drug in Nigeria on July 6, 2006.

Botanical Components of Nicosan

Nicosan is an extract of four plants, all which are used as herbal remedies for other various conditions in parts of Africa. Each species is briefly described below.

Piper guineense Schum & Thonn.

Benin Pepper or climbing Black Pepper, *P. guineense*, is a climbing vine of the family Piperaceae native to central and western Africa. The dried fruit of *P. guineense* has long been used as a spice in western Africa and can be obtained commercially. Benin pepper is closely related to the Black Pepper, *Piper nigrum* fruit of global commerce and is used in many of the same ways in food preparation. In Nigeria the leaves are also used for flavoring. The dried fruit

was one of the commodities, along with elephant tusks and palm oil, traded by the former Kingdom of Benin (now southern Nigeria) with the early European explorers (30,31). In addition to its use as a spice, in regions of Africa the fruits and leaves are used as a treatment for vomiting, worms, tonsillitis, rheumatism and stomach aches (32-34). Extracts of the seeds and roots have also been shown to have insecticidal activity (35). The seeds are used in Nicosan.

Pterocarpus osun Craib

Camwood, *P. osun*, is a nitrogen fixing legume tree of the family Fabaceae indigenous to West Africa. As a forest tree, it is used for timber, as well as a source of tannins for the leather industry (36). Hollowed logs of *P. osun* were used for making traditional drums used for long-distance communication in West Africa (37). The heartwood of *P. osun* contains red pigments that are used in traditional West African cosmetics (36) and as a histochemical stain (38). The red pigments have been identified as the condensed biflavonoids, santalin A and santarubins A and B (39). The seeds are sometimes used as food (40). The leaves and stems are used to treat skin diseases, which may be because of their antimicrobial activity (41,42). The ground leaves are also used in a Nigerian traditional soap (43). The stems are used in Nicosan.

Eugenia caryophyllum

Clovetree, *E. caryophyllum*, is a member of the family Myrtaceae native to Indonesia and naturalized throughout much of the tropics. The species had formerly been referred to as *Eugenia caryophyllus* (Spreng.) Bullock & S.G. Harrison, *Eugenia caryophyllata* Thunb., and *Caryophyllus aromaticus* L. The currently accepted name is *Syzygium aromaticum* (L.) Merr. & L.M. Perry (USDA Germplasm Resources Information Network, <http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?16153>; Integrated Taxonomic Information System, <http://www.itis.gov/index.html>; 44). Cloves have been part of the world's spice trade for thousands of years, valued for both flavoring and medicinal uses (30). The plant part used as a spice is the dried flower bud and the main contributor to the aroma and flavor is the phenylpropanoid eugenol, found as the major constituent in the bud's essential oil. Clove oil is commonly used to treat toothache, due to the anaesthetic and antiseptic properties of eugenol (45,46). In Nigeria, the clovetree fruit is used to treat toothache and mouth infections (47). The fruit is used in Nicosan.

Sorghum bicolor (L.) Moench

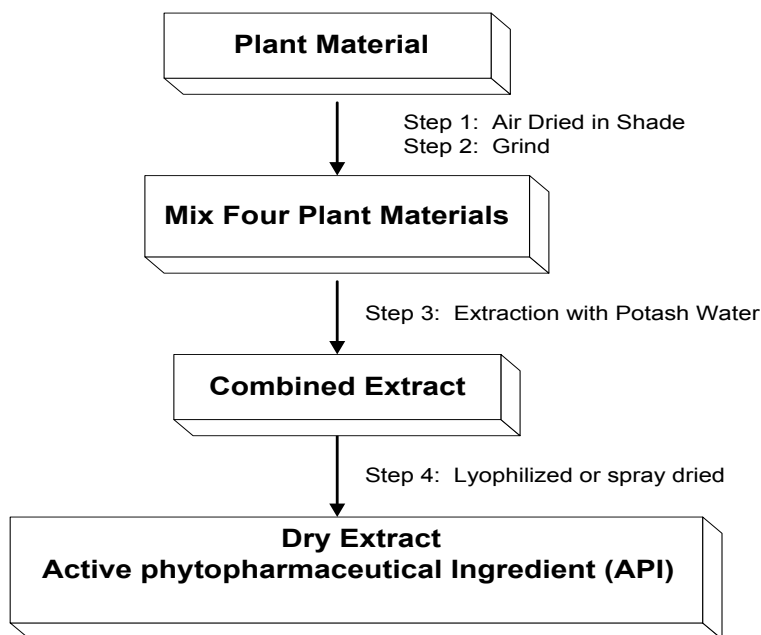
Sorghum, *S. bicolor*, is a member of the grass family Poaceae. Believed to have evolved about 11.9 million years ago (48) in Africa, there is evidence this plant was one of our earliest cultivated crops, cultivated as early as 5,000 years ago (49). The grain is used for food and in brewing. Worldwide, sorghum is the

fifth most important cereal crop and it is particularly important in Africa because it has good tolerance to heat and drought (50,51). Aqueous extracts of dried sorghum leaf sheaths are used by traditional healers in Nigeria to treat anemia (52,53). The leaves are used in Nicosan.

Preparation and Quality Control Testing of Nicosan

Nicosan is prepared from a cold-water extract of the plants described above. The composition of Nicosan is described in the patent (United States Patent 5800819) as follows:

“Generally the initial mixture from which the extraction product is prepared will include from about 12 to about 17 parts by weight of *Piper guineenses* seeds, from about 15 to about 19 parts by weight of *Pterocarpus osum* stem, from about 12 to about 18 parts by weight of *Eugenia caryophyllum* fruit, from about 25 to about 32 parts by weight of *Sorghum bicolor* leaves, and from about 15 to about 22 parts by weight of said potash.”



(Analytical & Biological evaluation)

Figure 1. Steps in the preparation of Nicosan

Nicosan is produced by Xechem Nigeria in Abuja, Nigeria. The steps in the preparation of Nicosan are illustrated in Figure 1. In practice, each of the four plants is first dried outdoors in the shade, and then ground separately by machine. The ground materials are further dried until the weight reaches a stable minimum. Each of the four ground plant materials are then mixed by weight. The active components are extracted in water

containing potash at room temperature for 8 hrs. The extract is then filtered and spray dried. Each of the steps has been optimized to establish a consistent method of preparation of Nicosan that has the same level of antisickling activity in different lots.

Each batch of Nicosan is then tested for *in vitro* antisickling activity and is also analyzed by HPLC. Blood samples obtained from sickle cell patients are used to assess the antisickling activity of Nicosan. The antisickling activity can be seen in the morphology of the homozygous *HbS* (SS) cells treated with Nicosan. SS cells that had been incubated in the presence or absence of Nicosan (0.5 mg/ml) under low oxygen conditions (4% O₂) at 37°C for 4 hrs are shown in Figure 2. The low oxygen conditions promote sickling of the SS cells and under these conditions most of the cells not treated with Nicosan were sickled. The antisickling activity of Nicosan is evident since most of the treated cells had the normal shape of red blood cells.

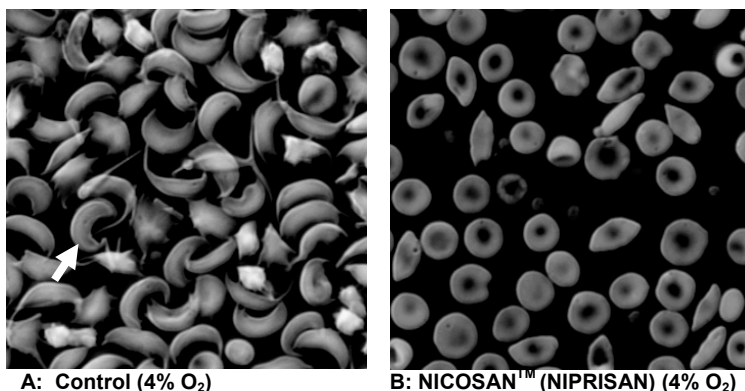


Figure 2. Comparison of morphology of SS cells after incubation in the absence (A) or presence (B) of a high concentration (0.5 mg/ml) of Nicosan under 4% oxygen for 4 hours. The arrow points out a sickled cell in the control sample (A)

From a natural products standardization perspective, the HPLC profiles of three different products batches of Nicosan are illustrated in Figure 3. Here, the HPLC patterns of the three batches are nearly identical as needed. The presence and/or absence and relative ratio and total content of selected targeted compounds are used in the standardization and quality control process. Some of the compounds present in the extract have been identified and are discussed more below.

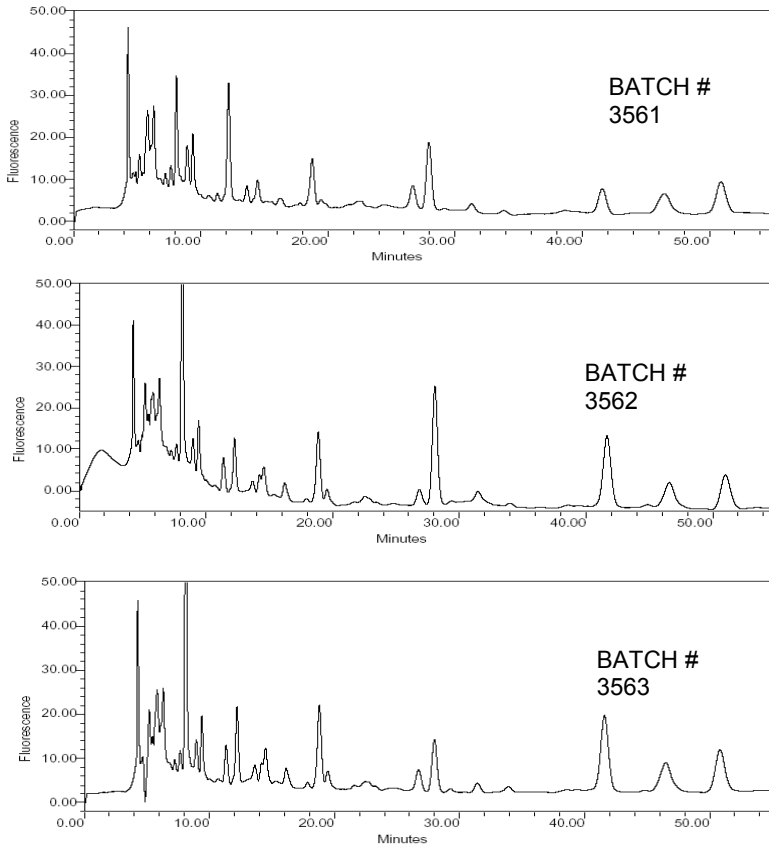


Figure 3. HPLC profiles of three batches of Nicosan

A portion of each of the three different batches used for the HPLC analysis shown in Figure 3 was sent to the National Heart, Lung and Blood Institute Sickle Cell Disease Center at Children's Hospital of Philadelphia, Philadelphia, Pennsylvania for determination of the level of antisickling activity. In these experiments, a low concentration (0.05 mg/ml) of Nicosan product was incubated with SS cells for 1 hr instead of the normal 3-4 hrs so that any differences could be seen more clearly. The levels of anti-sickling activity of the 3 different batches were nearly identical (Figure 4).

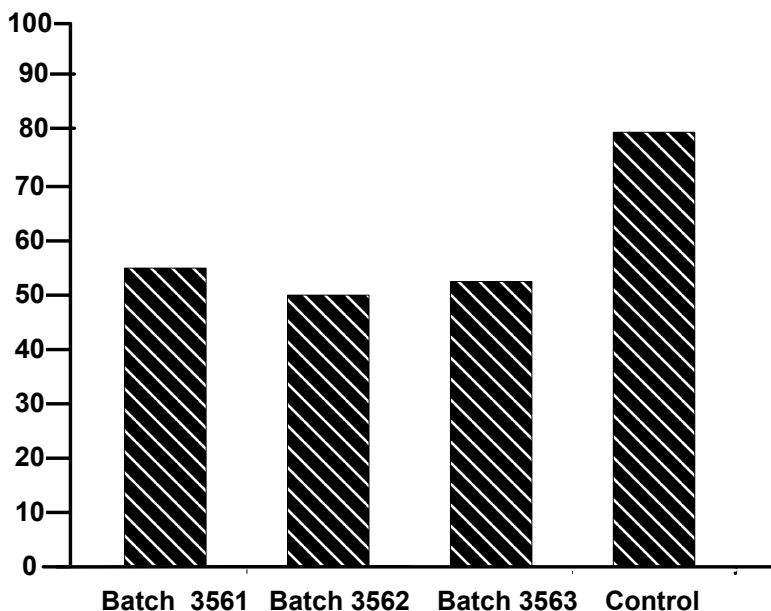


Figure 4. Anti-sickling activity of three batches of Nicosan. The SS cells were incubated with 0.05 mg/ml Nicosan at 4% oxygen for 1 hour

Chemical Constituents of Nicosan

The mode of action of Nicosan is not yet known but some known antisickling compounds in the final product have been identified. Based on the results of the studies with transgenic sickle cell mice, Iyamu et al. (29) hypothesized that Nicosan specifically interacted with Hb S since it was effective at low doses in preventing sickling. A series of *in vitro* studies have revealed that aromatic aldehydes have antisickling activity. Several aromatic aldehydes were reported to form Schiff base adducts with Hb A (normal hemoglobin) resulting in increases in oxygen affinity (54). With blood from sickle cell patients, salicylaldehyde and *o*-vanillin exhibited *in vitro* antisickling activity, through the formation of adducts with HbS (54). X-ray crystallography of the vanillin adduct with HbA revealed that a secondary binding site for vanillin was between His 116 β and His 117 β (55). His 116 β is believed to be a contact residue in polymer formation between Hb S molecules. Covalent binding of vanillin at this site may inhibit polymer formation and this may be the underlying mechanism for the observed *in vitro* antisickling activity. Although vanillin is an effective antisickling compound *in vitro*, it is not effective when taken orally because it is broken down in the digestive tract to vanillic acid, which does not have anti-sickling activity (56).

Other anti-sickling aldehydes, such as 5-hydroxymethyl-2 furfural (5HMF), furfural, 5-methyl-2-furfural and 5-ethyl-2-furfural, also bind covalently to hemoglobin, but at a different position, the *N*-terminal α Val1 position (57). Of these compounds, 5HMF was the most effective in inhibiting sickling of SS

cells and was 3.5 times more effective than vanillin. The authors proposed that the mechanism of anti-sickling activity was that the binding of the compound to the *N*-terminal α Val1 stabilized the hemoglobin molecule in the oxygenated state, and/or destabilized the deoxy conformation, which is the state that is prone to polymerization leading to sickling (57).

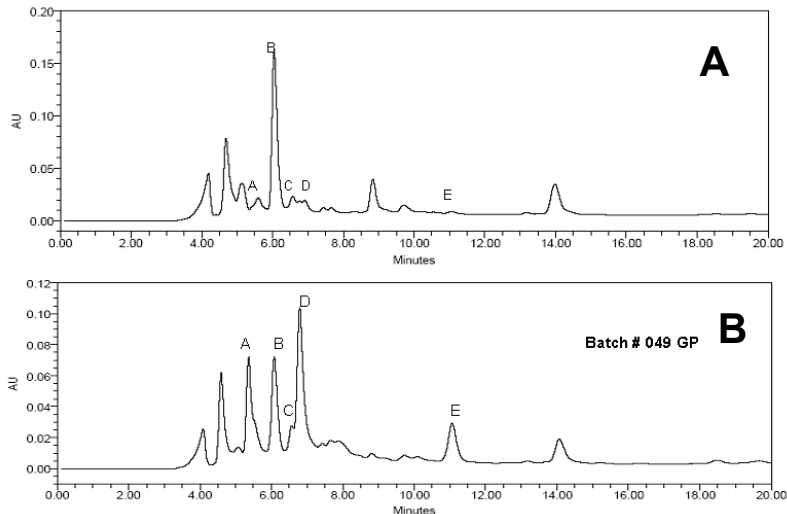
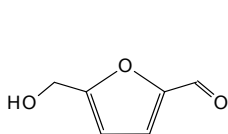


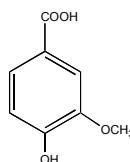
Figure 5. HPLC profiles of Nicosan before (A) and after (B) spiking with five aromatic aldehydes. The structures of the aldehydes are shown in Figure 6. The elution positions of the pure compounds, A-E, correspond to the designations in Figure 6.

Abdulmalik et al. (58) confirmed the *in vitro* anti-sickling activity of 5HMF.

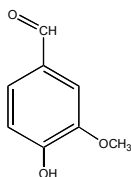
They also reported that 5HMF administered orally to transgenic sickle cell mice was not destroyed in the gastrointestinal tract, was transported into red blood cells, and was bound to HbS molecules. Oral administration of 5HMF to the transgenic sickle cell mice protected them from the effects of severe hypoxia. When exposed to 5% oxygen, the untreated transgenic sickle cell mice died within 15 min due to pulmonary sequestration by sickled cells. However, 87% of the 5HMF-treated transgenic sickle cell mice survived the full 60 min treatment period.



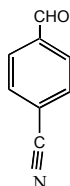
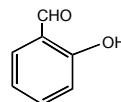
A: 5HMF (5-Hydroxy Methyl furfural)



B: Vanillic Acid



C: Vanillin

D: 4-Cyano
benzaldehyde

E: Salicylaldehyde

Figure 6. Structures of the aromatic aldehydes used for the spiking of Nicosan shown in Figure 5.

Nicosan contains several aromatic aldehydes including 5HMF, which, as described above, has been shown to have anti-sickling activity when administered orally. Other aromatic aldehydes present in Nicosan, including vanillin, 4-cyanobenzaldehyde and salicylaldehyde, have been shown to have anti-sickling activity *in vitro* (54). The HPLC profile of a Nicosan extract in the region of elution of most aromatic aldehydes is illustrated in Figure 5A. In Figure 5B, the HPLC chromatogram of the same extract spiked with five aromatic aldehydes is shown. The structures of the aldehydes are shown in Figure 6. The labels on the HPLC peaks correspond to the labels of the aldehydes in Figure 6, based on the known elution position of the pure compounds. These data suggest the presence of some of the known anti-sickling aromatic aldehydes in Nicosan. However, when the aromatic aldehydes were eluted from the HPLC column and combined, the level of anti-sickling activity of the combination was lower than the anti-sickling activity of Nicosan itself. This suggests there may be additional anti-sickling compounds present in Nicosan. Current research is aimed at mass spectral analysis of other HPLC peaks to confirm the identification of the other compounds in Nicosan and to address how these compounds are metabolized and determine their bioavailability. Whether additional compounds are derived from the parent molecules that provide anti-sickling benefit is also not yet known.

Conclusion

Nicosan is a new multi-component herbal remedy that has been shown both *in vitro* and *in vivo* to inhibit sickling of homozygous HbS cells. It has been clinically tested in sickle cell patients in Nigeria and was found to reduce the

number of painful crises experienced by the patients. Nicosan is a unique example of an herbal treatment developed by a traditional healer in Africa that has been successfully subjected to extensive scientific testing, and has reached the bar of safety and efficacy for it to now be registered as a pharmaceutical drug for use in Nigeria

Acknowledgment

We would like to thank Dr. Robert Swift of Xechem International, Inc. for encouraging this chapter and Dr. Toshio Asakura, Children's Hospital of Philadelphia, for his leading research efforts in studying this disease and the *in vitro* antisickling assays. We also wish to acknowledge Dr. Wambebe, former director of Niprid, Nigeria for his initial groundbreaking research on the antisickling effects of the original herbal formula which was shown effective in reducing sickle cell anemia in the original human clinical trials. We also recognize the pioneering work of Dr. Ramesh Pandey, former CEO of XECHEM INC and his tenacity to validate using modern science this traditional herbal remedy which allowed its development as a duly registered pharmaceutical product in Nigeria for the treatment of sickle cell anemia. The commercialization and registration of NICOSAN as a pharmaceutical and procurement of the ORPHAN DRUG status in the USA may be the first time in Africa such a traditional herbal remedy has received such pharmaceutical status and we acknowledge all those involved.

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Chapter 16

Rooibos and Honeybush: Recent Advances in Chemistry, Biological Activity and Pharmacognosy

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Rooibos (*Aspalathus linearis* (Brum.f.) Dahlg.) and honeybush (*Cyclopia* Vent. species) have a long history of traditional uses in South Africa as indigenous herbal health beverages or tisanes. These herbal teas are rich in several unique polyphenolic compounds that not only differ from that of *Camellia sinensis* teas, but also from one another. The bioactivity of both includes *in vitro* and *in vivo* antimutagenic, antioxidant, cancer modulating, cardiovascular and other activities. The first clinical controlled study which showed that rooibos decreased oxidative lipid damage and increased the redox status in plasma is discussed while human studies on honeybush have not yet been studied to date.

Rooibos, *Aspalathus linearis* (Brum.f) Dahlg. (Family Fabaceae; tribe Crotalariaeae) indigenous to the Cedarberg and neighboring mountains of the Western Cape Province of South Africa has been used as a herbal beverage by the indigenous Khoi people since the late 1700s (1). A Russian immigrant, Benjamin Ginsberg started commercial rooibos trading in 1904. Today rooibos is cultivated on a large scale (more than 12 000 metric tons, <http://sarooibos.co.za/content/view>) to serve both local and increasing international market demands. Aside from the tea after infusing creating a deep natural red color with a full flavor and body, this herbal tea is caffeine free (2), has a low tannin content – estimated at 3% in the leaves (3,4), is high in antioxidant activity and bioactive phytochemicals. Scientific studies have shown via *in vitro* and animal laboratory based experiments and newly conducted human studies that this herbal tea will promote good health. Together, all these factors have contributed to the popularity of this herbal tea as a health beverage. Traditionally, rooibos and honeybush are consumed as a hot brew where one cup of freshly boiled water contains one tea bag (~2.4g) with an infusion time of 2-5 min. This brew is then either consumed hot with the addition of milk and/or sugar or cold with the addition of lemon juice and honey.

Unlike rooibos being an established South African crop in the industry, honeybush (*Cyclopia* species) is still in its infancy of developing into a sustainable crop for commercial use, with a current annual production of <300 tons. The species grow within the coastal and mountainous areas of the Western and Eastern Cape Provinces (5) being part of the Fynbos biome. More than 20 species have been identified of this woody legume, but the product known as honeybush herbal tea is made from mainly two species, *Cyclopia intermedia* E. May and *C. subternata* Vogel (6). Like rooibos, honeybush does not contain caffeine and has a low-tannin content rendering it well suited for a night time beverage and for those experiencing nervousness (7,8).

Both rooibos and honeybush species belong to the Fynbos biome, which accounts for more than 80% of the plant species in the Cape Floral Kingdom, the smallest but highest in biodiversity of the six plant kingdoms in the world.

Phytochemistry

During manufacturing, two forms of rooibos are produced, traditional/fermented and “green”/unfermented rooibos. With traditional rooibos the freshly cut leaves and stems are placed purposefully in piles and allowed to ferment as it is during the fermentation process that most of the aromas of the herbal tea as well as the final color develop. The fermented leaves are then dried and it is the art of drying that can significantly impact the quality (time and temperature). In contrast, and similar to the processing of traditional Chinese green tea, in the processing of green rooibos herbal tea, oxidative changes are kept to a minimum with a quick drying of the plant material (9). Similar to rooibos, honeybush also needs to be fermented and more recently has also become available in a ‘honeybush green tea’ form as well. It is during fermentation that the rich red color and unique flavor are developed that is so unique to traditional rooibos, also referred to as “red” tea or “red bush” tea. The

“green” rooibos does not have this color nor flavor but rather has a green/yellow color with a “grassy” nose.

The phenolic constituents of traditional and green rooibos differ from that of *Camellia sinensis* teas and are unique in that it contains aspalathin, a C-C linked dihydrochalcone glucoside (10) and also the recently discovered cyclic dihydrochalcone, aspalalinin (11). To date, rooibos is the only source of aspalathin which allows this compound to be used as a chemical marker for quality control in the case of green rooibos and authentication of plant material. Another rare compound, nothofagin, a 3-dehydroxy dihydrochalcone glucoside, previously shown to be found in the heartwood of *Nothofagus fusca* (12) is also present in the rooibos teas (13). During fermentation (an oxidative environment) the dihydrochalcone content of rooibos decreases substantially with less than 7% of the original aspalathin content remaining in traditional rooibos (14) as aspalathin is oxidized to dihydro-iso-orientin (15). A recent report showed that during degradation of aspalathin under oxidative conditions, the diastereomeric mixtures of dihydro-iso-orientin and dihydro-orientin are formed as the major and minor products respectively, with maximum concentrations of iso-orientin and orientin occurring after 6 h (16). Several other phenolic compounds are present in rooibos and include the C-C linked β -D-glucopyranosides such as the flavones orientin, iso-orientin, isovitexin and vitexin, and the flavanones dihydro-orientin, dihydro-iso-orientin and hemiphlorin (16) which are also degraded during fermentation but to a lesser extent. Other flavonols present in rooibos include hyperoside, quercetin, quercetin-3-roninobioside, rutin, iso-quercitrin and the flavones luteolin, luteolin-7-O-glucoside and chrysoeriol (13). The flavanols, (+)-catechin and (-)-epicatechin form the chain extending units of the rooibos procyanidin-type tannin with (+)-catechin as terminal unit (17). Variations in the polyphenolic content of rooibos may occur due to genetic variation in plant material when seeds are used for propagation, wild compared to cultivated populations of *A. linearis* and differences in the manufacturing process (18,19,20).

The polyphenolic composition of honeybush differs from that of rooibos as well as *Camellia sinensis* teas. The three major constituents in the leaves of the more than 20 *Cyclopia* species investigated by De Nysschen and co-workers (6) included the xanthone mangiferin and glycosides of the flavanones hesperetin and isosakuranetin. Combinations of these three compounds in varying quantities are seen as a unique character for *Cyclopia* species, although a recent study using LC-MS to identify polyphenols, could not report on any detectable quantities of isosakuranetin in many of the *Cyclopia* species (21). Later studies also identified other phenolic metabolites with potential health benefits such as flavonols, flavonones, isoflavones, coumestans, flavones, xanthones, cinnamic acids and (+)-pinitol, with mangiferin, isomangiferin and hesperedin present in all species analyzed to date (21,22,23). The total polyphenol, flavanol/flavone, and flavanol/proanthocyanidin content of a cup (200 mL) of traditional/fermented and green/unfermented rooibos and honeybush herbal teas is presented in Table I (data obtained from Analytical Laboratory, Oxidative Stress Research Centre, CPUT, South Africa). The herbal teas were provided by Rooibos Limited (Mr A Redelinghuys, Clanwilliam, South Africa).

Table I Total Polyphenol, Flavonol and Flavanol Content of a Single Cup¹ of Rooibos and Honeybush Herbal Teas.

<i>Antioxidant content/capacity</i>	<i>Traditional² rooibos</i>	<i>Green rooibos</i>	<i>Traditional² honeybush</i>	<i>Green honeybush</i>
Total polyphenols ^b	73.4 ± 1.8	106.5 ± 2.1	23 ± 0.2	74.0 ± 1.9
Total flavonols/ Flavones ^c	33.4 ± 0.4	26.9 ± 0.3	2.2 ± 0.06	16.4 ± 0.3
Total flavanols/ pro- anthocyanidins ^d	2.6 ± 0.02	6.0 ± 0.1	0.6 ± 0.01	4.3 ± 0.1

NOTE: ¹A single cup = 200 mL, prepared by steeping one tea bag in 200 mL of freshly boiled water for 5 min, ^bthe Folin–Ciocalteu method was used to determine the total polyphenol content and is expressed as mg GAE (gallic acid equivalents), ^c mg of quercetin (360 nm method), ^d mg of catechin DMACA method. ²Traditional = Fermented; Values in columns are means ± SD of 10 samples done in triplicate.

The presence of phenolic acids in rooibos such as caffeic acid, ferulic acid, p-coumaric acid, p-hydroxybenzoic acid, vanillic acid and protocatechuic acid have also been reported (13). Recently, a study reported on the enhancement of the antioxidant yield and soluble solid matter from fermented rooibos with the addition of a fungal cocktail of hydrolyzing enzymes (24). When using green rooibos material, a resultant semi-fermented rooibos is produced with a higher aspalathin content. The influence of the enzyme treatment on the unique aroma of rooibos still needs to be investigated. Previously 99 and 218 volatile components were reported in the vacuum steam distillate and headspace vapor of traditional rooibos (15), respectively. Guaiacol (24%), 6-methyl-3,5-heptadien-2 one isomer (5.2%), damascenone (5%), geranylacetone (4.2%), β-phenylethyl alcohol (4.1%) and 6-methyl-5-hepten-2-one (4%) comprises the major volatile components (25). A later study reported on a similar characterization of the volatile fraction of traditional/fermented rooibos and concluded that the dichloromethane extraction from a brewed extract consisted of 50 components while the steam distillation and extraction fraction consisted of 123 components, with 42 components were identified as new rooibos volatiles (26).

The aroma volatiles of honeybush have also been reported by Wang *et al* (27). The aroma components were dominated by monoterpene alcohols, of which α-terpineol (28%) was the major component, with minor amounts of linalool (7%), nerol (2%) and geraniol (8%). These monoterpenes are responsible for the sweet, floral and fruity notes of the tea, while other components such as phenylethyl alcohol (3%) and 5-methylfurfural (2.1%) imparted also sweet and honey notes. Other volatiles such as eugenol (6%), linalool oxides (7%), and methyl-heptenol (3%) were also detected. With both honeybush and rooibos, the exact nature of the aromas and flavor will depend significantly on the species collected, time of collection, drying, fermentation and processing (27).

Rooibos and honeybush also contain essential micro- and macro elements, with the traditional/fermented forms containing lower amounts of elements than the “green”/unfermented forms (28). The levels of Al and Ni in the rooibos and

honeybush infusions were shown to be significantly lower when compared to that of tea and coffee, while no significant differences in element quantities in rooibos and honeybush infusions could be reported. The elemental content in the raw plant material (1g plant material was decomposed at 500 °C for 16 h and ash heated at 100 °C for 5-10 min in 3 mL *Aqua regia* made up to final volume of 20 mL) and in hot water infusions of traditional/fermented and green rooibos and honeybush (50 mL of boiled distilled water was used to extract 1g rooibos material for 15 min) is shown in Table II.

Table II. Mineral Content of Traditional/Fermented and Green Rooibos and Honeybush Plant Material (mg/kg) and Infusions (mg/L).

	<i>Al</i>	<i>B</i>	<i>Ca</i>	<i>Cu</i>	<i>Fe</i>	<i>K</i>	<i>Mg</i>	<i>Mn</i>	<i>Ni</i>	<i>P</i>	<i>Zn</i>
^a Rf-raw	172	20	1926	11	120	3628	1687	51	1.3	325	8
^b Rg-raw	90	13	4598	6	36	2104	2226	42	0.5	438	5
^c Hf-raw	80	29	2166	10	62	5272	1146	42	1.3	868	9
^d Hg-raw	131	38	1206	13	86	2702	495	65	2	1399	20
^a Rf-inf	0.2	0.4	7	0.2	0.2	58	10	0.2	0.0	2	0.0
^b Rg-inf	0.2	0.4	19	0.2	0.0 ^e	38	20	0.6	0.0	8	0.1
^c Hf-inf	0.2	0.2	9	0.1	0.1	93	11	0.2	0.0	12	0.1
^d Hg-inf	0.2	0.3	15	0.1	0.1	105	16	0.4	0.0	12	0.2

NOTE: ^aRf-raw/inf: fermented rooibos plant material/infusion; ^bRg-raw/inf: green rooibos plant material/infusion; ^cHf-raw/inf: fermented honeybush plant material/infusion; ^dHg-raw/inf: green honeybush plant material/infusion. e 0.0 less than 0.1 All plant materials were bought in a market in Czech Republic.

SOURCE: modified from Malik et al., 2008 (28).

Bio-activity

Traditionally, rooibos has a long history of medicinal use with anecdotal evidence linking the consumption to relief of digestive disorders, skin allergies, insomnia, nervous tension and mild depression (1,29). In 1968, a young South African mother, Annetjie Theron, found that rooibos appeared to alleviate her baby's symptoms of colic. She documented all these effects in a book and started to communicate her observations through the press and other public forums (30). Since that time, rooibos has been recommended for many other ailments and Annetjie Theron established her own cosmetic and toiletry business in South Africa, distributing worldwide with rooibos being a key ingredient.

Japanese and South African researchers were the first to scientifically investigate the possible health promoting properties of rooibos which led to publications reporting on various biological activities (31-37). A few studies regarding the *in vitro*, *in vivo* and *ex vivo* biological activities of honeybush has been published by mainly South African researchers and are mostly limited to the exported species, *Cyclopia intermedia*. These studies will be discussed.

Honeybush has traditionally been used as an expectorant, a stimulator of milk production in lactating women and to treat various digestive disorders (29,38,39) but no clinical trials have been conducted yet.

Antioxidant Properties

Flavonoids have been shown to exhibit powerful antioxidant activities with mechanisms involving free radical scavenging, metal chelation and singlet oxygen quenching with the inhibition of enzyme activity. As previously mentioned, rooibos is rich in flavonoids. Numerous studies have reported on the antioxidant activity of rooibos using various types of extracts of rooibos in a number of different assay systems (33,34,40-44). In one of the earliest studies published it was shown that aqueous extracts of traditional rooibos effectively scavenged the superoxide anion radical ($O_2^{\cdot-}$) and hydroxyl radical ($\cdot OH$) when using electron spin resonance spectrometry (33). Recently a review was published where the antioxidant activities of hot water extracts from traditional/fermented and green/unfermented rooibos were compared using $O_2^{\cdot-}$, DPPH \cdot (2,2-diphenyl-1-picrylhydrazyl radical), ABTS $^{++}$ (2,2'-azino-di-3-ethylbenzothiazoline-6-sulfonic) and FRAP (ferric reducing antioxidant potential) assay systems (20). These results clearly show that the antioxidant potential decreases as a result of fermentation and could be attributed to a decrease in total polyphenol content. Results from a previously published study confirmed that there is a strong correlation ($R^2=0.812$) between the aspalathin content in green/unfermented rooibos and the total antioxidant activity as determined by the ABTS $^{++}$ scavenging method was shown (45).

In vitro antioxidant activity for the various *Cyclopia* species can be impacted by processing (46,47). The antioxidant activities of a cup of fermented and green rooibos and honeybush (source: Rooibos Ltd., Clanwilliam, South Africa) using the oxygen radical absorbance capacity (ORAC) assay (unpublished data, Dr JL Marnewick and Mr F Rautenbach, Analytical Laboratory, Oxidative Stress Research Centre, CPUT, South Africa) shows that green rooibos and green honeybush exhibit greater antioxidant activity than the same lot fermented (Table III).

Table III Antioxidant activity of a cup^a of rooibos and honeybush herbal teas

<i>Antioxidant capacity</i>	<i>Traditional^c rooibos</i>	<i>Green rooibos</i>	<i>Traditional^c honeybush</i>	<i>Green honeybush</i>
ORAC ($\mu\text{mole TE}^b$)	1537.6 \pm 27	2093.6 \pm 50	780.1 \pm 20	1620.5 \pm 37

NOTE: ^aA cup = 200 mL, prepared by steeping one tea bag in 200 mL of freshly boiled water for 5 min; ^bAntioxidant activity is expressed as $\mu\text{mole trolox equivalents (TE)}$ per cup, using the fluorescein method; ^cTraditional = Fermented; Values in columns mean \pm SD of 10 samples done in triplicate.

The use of natural antioxidants in supplements can also exhibit pro-oxidant activity under certain conditions such as the concentration and nature of the polyphenolic compounds causing oxidative damage to important cellular components (48). Aqueous extracts and crude polyphenolic fractions of both traditional and green rooibos were evaluated for possible pro-oxidant activity using a Fenton reaction model system containing $FeCl_3$ -EDTA and H_2O_2 for the generation of hydroxyl radicals. Pro-oxidant activity was shown for pure aspalathin while the dihydrochalcone and flavonoid contents of the enriched

rooibos extracts correlated ($R^2 = 0.977$ and 0.971) with the pro-oxidant activity, but the total polyphenol content did not show any correlation (48).

All these studies mostly demonstrated *in vitro* antioxidant activities, but recently *ex vivo* and *in vivo* antioxidant capacity of rooibos and honeybush were also shown in various experimental animal studies (37,49-52). Being known for its good antioxidant potential *in vitro*, the effect of chronic administration of traditional rooibos on lipid peroxidation in rat brain led to positive results (37). After consuming rooibos for 21 months damage to the central nervous system was assessed by measuring the accumulated lipidperoxides and TBARS (thiobarbituric acids reactive substances) in the brains of these female rats. The MRI (magnetic resonance imaging) scans showed a significant lower content of TBARS, comparable to that of young five week old control rats. Of importance is the fact that aging induces deterioration of the CNS (central nervous system) that is partly due to the cytotoxic effect of reactive oxygen species (ROS) generated in the brain, and rooibos suppressed the accumulation of lipidperoxides associated with the aging process (37). A recent study used Japanese quails as a model for ageing and reported that the consumption of either a rooibos extract or milled rooibos plant material reduced the decrease in egg production by aged quail hens, thereby prolonging their productive period (53), with a possible explanation that rooibos might exhibit estrogenic activity, but this work needs to be reconfirmed or verified by others. These studies do however, suggest and provide a rationale that rooibos herbal tea as viewed by the Japanese are an anti-ageing herbal beverage.

Although, long term consumption of traditional rooibos by Japanese quails did not alter the fragility of erythrocytes to H_2O_2 -induced hemolysis, a decreased *ex vivo* peroxide-induced hemolysis of their red blood cells was shown (49). When traditional and green rooibos and honeybush were consumed by male Fischer rats for 10 weeks at a concentration customarily used for human consumption (2% w/v), interesting results were obtained in terms of the hepatic oxidative status and drug metabolizing enzymes (50). Although none of the rooibos or honeybush herbal teas had any significant effect on the hepatic ORAC (oxygen radical absorbance capacity) values, both rooibos and green honeybush improved the oxidative status in the liver as shown by a significant increase in the ratio of reduced to oxidized glutathione (GSH:GSSG), an indicator used for cellular oxidative stress (Figure 1).

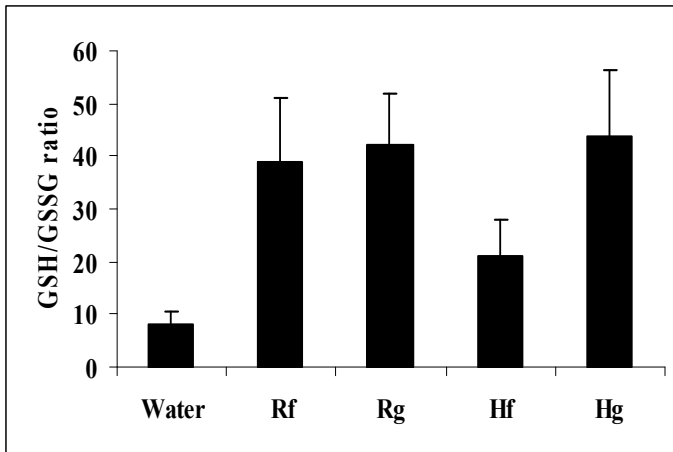


Figure 1 Effect of fermented and green rooibos (Rf, Rg) and honeybush (Hf, Hg) on the hepatic redox status (reduced to oxidized glutathione ratio) of rats consuming the various herbal teas for 10 weeks. The control group consumed water (Data are from reference 50)

Rooibos and honeybush also enhanced the activities of the phase II cytosolic enzyme, glutathione S-transferase alpha, while the green rooibos and honeybush teas also increased the activity of the phase II microsomal enzyme UDP-glucuronosyl transferase. Modulation of the oxidative status and phase II enzyme activity were suggested to be important events in the protection against adverse effects related to oxidative damage. Similarly, two other studies also reported on the hepatoprotective effect of traditional rooibos in rats that have been exposed to a potent liver pro-oxidant, carbon tetrachloride (CCl₄), with rooibos preventing the induction of lipidperoxidation by CCl₄ (51) and the prevention of oxidative stress in streptozotocin-induced diabetic rats with rooibos being recommended as an adjuvant support for the prevention and therapy of diabetic vascular complications (52). When considering the inhibition of lipidperoxidation, a few studies have reported on the activity of both traditional and green rooibos to inhibit/decrease such oxidative damage (54,55) *in vitro* as well as *in vivo* (52).

When comparing the antioxidant potencies of both the traditional/fermented and green/unfermented rooibos and honeybush, evidence is difficult to select for the best one, since the activity is highly dependent on the assay used, the nature of the raw material, and type of samples (aqueous extracts, enriched-extracts or solvent extracts) used.

Chemopreventive/Antimutagenic Properties

Initial studies demonstrated the *in vitro* antimutagenic properties of both traditional and green rooibos and honeybush extracts using various test systems. These systems included the *Salmonella* mutagenicity assay (56), COMET assay (57), chromosomal aberration assay using Chinese hamster ovary cells (35) and X-ray-induced oncogenic transformation using mouse embryo fibroblast cells (36). In general, all these studies reported positively on the *in vitro* protection/modulation of genetic damage/cell proliferation by various extracts of rooibos.

Subsequently, the *in vitro* antimutagenic findings have also been substantiated using experimental animal models. One of these studies conducted included a 10 week rooibos feeding in male Fischer rats (58) to evaluate possible *ex vivo* antimutagenic activity of brewed traditional and green rooibos and honeybush herbal teas. Novel findings from this study included that hepatic cytosolic fractions from rats consuming the green rooibos brew for 10 weeks significantly protected against 2-acetylaminofluorene (2-AAF)-induced mutagenesis in the *Salmonella* mutagenicity assay with tester strain TA 98, using Aroclor 1254-induced microsomes for mutagenic activation, while green honeybush showed a marginal protective effect. When using aflatoxin B₁ (AFB₁) as mutagen both the traditional and green rooibos and honeybush significantly protected against the induced mutagenicity. The activation potential of hepatic microsomal preparations from rats consuming the rooibos teas were also evaluated and both rooibos teas and green honeybush reduced the activation of AFB₁ but not that of 2-AAF. Presumably, the rooibos and honeybush modulate different isoforms of the phase I drug metabolizing enzyme, cytochrome P450 directing the metabolism away from the formation of the putative mutagenic metabolite (58).

The cancer modulating properties of rooibos and honeybush were subsequently reported on and these studies provided the first evidence for the *in vivo* modulation of tumor promotion (54,59,60). In a 7,12-dimethylbenz[a]anthracene-initiated, 12-O-tetra-decanoylphorbol-13-acetate promoted two-stage skin carcinogenesis model, methanolic fractions from traditional and green rooibos and honeybush were topically applied to the skin of ICR mice and significantly reduced the mean number as well as the size of tumors on these mice (54). In the skin tumor development model, the honeybush extracts “performed better” than the rooibos, as the honeybush extracts showing the highest inhibition (90% and 84.2% for the green and fermented extracts) (Fig. 2). This again suggests that one health beverage should not be artificially described as “better” than another based on any single test or *in vitro* antioxidant activity alone.

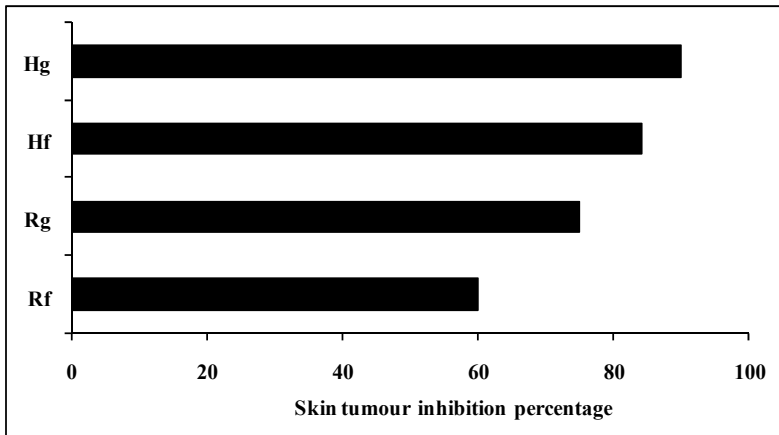


Figure 2 Inhibitory effect of topical application of rooibos and honeybush extracts on skin tumor development over a 20 week period in a two-stage mouse skin carcinogenesis model (Data are from reference 54)

The type of phytochemicals, their ratios and combination in addition to the specific assay systems chosen play a major role in the outcome. A recent study investigated the *in vitro* transport of aspalathin, the main rooibos polyphenol, as well as a green rooibos extract across the skin and intestinal epithelium (61). Only 0.07% and 0.08% of the initial aspalathin dose for the green rooibos extract and pure aspalathin solution penetrated the different layers of the skin, while 100% and 79% transported across Caco-2 cell monolayers, suggesting that the presence of other phytochemicals in the green rooibos extract may assist higher transport. When the cancer modulating properties of hot water rooibos and honeybush extracts were monitored in a liver carcinogenesis model it was the green rooibos that showed protection against fumonisin B₁-induced (FB₁) cancer promotion (59), while the other herbal extracts decreased either the smaller or larger sized foci, but not the total number of foci in the liver. Diethylnitrosamine (DEN) was used as cancer initiator, while the number and size of pre-neoplastic lesions staining positive for the placental form of gamma glutamyl transferase (GSTP) were scored. Green rooibos significantly reduced the number of pre-neoplastic foci in the liver (Fig. 3), presumably by arresting their growth (59). Recently a similar effect was reported using a site specific carcinogen, N-methylbenzyl nitrosamine to induce esophageal cancer in male Fischer rats. Rats consumed a hot water extract of traditional and green rooibos for 25 weeks where after the number and size of esophageal papillomas were scored. Green rooibos and honeybush significantly inhibited the development of larger (>10 and <20 mm³) papillomas as well as reduced the number and size of papillomas (60). Results from these studies could prove important in the development of cancer prevention strategies.

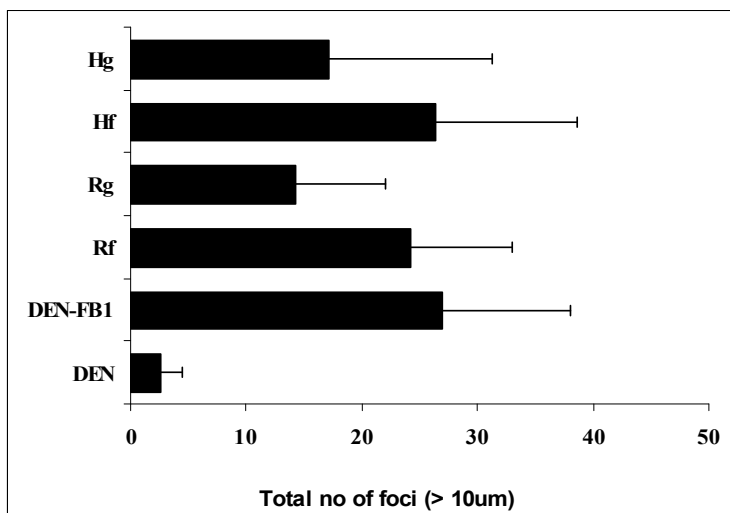


Figure 3 Effect of green and fermented rooibos (Rg, Rf) and honeybush (Hg, Hf) on the induction of GSTP⁺ foci by combined treatment of DEN and FB₁ (Data from reference 59)

Other Bioactive Properties

Extracts of both rooibos and honeybush species have been shown to possess estrogenic activity *in vitro* (62-65). Some of these studies reported on the binding of certain phenolic compounds such as phyto-estrogens present in rooibos and honeybush, to the estrogen receptor using various cell lines. These results suggest intra and inter *Cyclopia* species variability with regards to estrogenic potency exists. Using experimental animal and *in vitro* models an aqueous extract of presumably fermented rooibos (15% w/v) produced a dose-dependent decrease in the mean arterial blood pressure of rats, as well as cause an antispasmodic effect mediated predominantly through K_{ATP} channel activation (66). This study also reported that the aqueous rooibos extract also possesses smooth muscle relaxing effect. The selective bronchodilatory effect of the tea extract was shared by one of its known flavonoid compounds, chrysoeriol, while orientin was found selective for its inhibitory effect on the gut. The investigators suggested that these observations may explain the medicinal use of rooibos tea in hyperactive gastrointestinal, respiratory and cardiovascular diseases with the potential to be developed as a remedy for the congestive airway disorders (66). As impaired immune responses is known to be the cause of many allergies, the effect of rooibos on the immune response has been examined in many cell cultures or experimental animal studies and showed that rooibos increased antibody responses and improved cell survival through the stimulation of interleukin-2 (IL-2) in splenocytes primed with ovalbumin (OVA) and CD3 (67). Recently the same group also reported that an aqueous rooibos fraction (mainly consisting of oligosaccharides and polysaccharides) increased immunoglobulin M production in anti-OVA-stimulated murine

splenocytes, associated with the production of IL-10. A comment was made that this extract could be of clinical use (68). Antimicrobial activity of rooibos and honeybush extracts has been reported in a few publications. Aqueous extracts of both fermented and green rooibos were shown to inhibit the growth of *Staphylococcus aureus*, *Bacillus cereus*, *Listeria monocytogenes*, *Streptococcus mutans*, *Saccharomyces cerevisiae* and *Escherichia coli*, with the fermented extracts being more effective (69). In the case of *E. coli* the author suggested a bacteriostatic mechanism to be involved in the growth inhibition when using liquid cultures (69). Although these results could not be confirmed when an aqueous extract of rooibos was tested against *E. coli* using a solid medium zone inhibition method, antimicrobial activity against *Bacillus cereus*, *Micrococcus luteus* and *Candida albicans* were shown. (70). Recently extracts of green rooibos and honeybush (*C. subternata*, *C. genistoides*) showed growth inhibitory effects against *E. coli* using liquid cultures, with green rooibos and green *C. subternata* (100 mg/mL) also reducing spore germination of the plant pathogen, *Botrytis cinerea* (71). Alkaline extracts (1% sodium carbonate) of fermented rooibos (containing an acidic polysaccharide) were reported to suppress the cytopathic effects of HIV *in vitro* (72) using infected MT-4 cells. It was suggested by the authors that the extracts possibly inhibited the virus to bind to the CD4 receptor extracts from rooibos tea.

Pharmacocnosy

Few human studies or clinical trials examining the potential health and/or therapeutic applications of rooibos have been reported. During the early 1980s, a small (n=7) study was conducted in humans suffering from asthma or hay fever. Neither the ingestion of traditional rooibos nor the topical application of a rooibos poultice exhibited any antihistaminic effects (73). Nearly a decade later, another human study was published that showed a positive effect an infusion of rooibos had on patients with atopic dermatitis and *herpes simplex* viral infection (74). A decreased itching and induced-inflammation was reported as well as a decreased incidence of *herpes simplex*. As yet, no human studies on immune responses have been published.

Another small (n=10) human study in the late 1970s reported on the effect of rooibos on iron absorption (32). No detrimental effect on iron absorption was shown after the subjects had consumed traditional/fermented rooibos (200 mL containing milk and sugar) when compared with the control group consuming water. Subsequently, a more recent study published in 2005 confirmed these results that the intake of 200 mL of traditional rooibos (with milk and sugar) per day for 16 weeks by school children did not have any adverse effects on their iron status (75).

An eight week randomized placebo-controlled intervention study reported on the antioxidant status of lead factory workers drinking traditional/fermented rooibos (76). The modulation of oxidative status of these workers was shown by a decreased level of lipid peroxidation (measured as malondialdehyde in the plasma) and increased level of blood glutathione (GSH). The authors suggested that rooibos could play a beneficial preventive role in occupationally exposed

workers. Although not reporting on the consumption of an aqueous brew of traditional rooibos, another study was conducted using an aspalathin-enriched extract of green rooibos (77). Subjects were given such a tablet (containing 15% aspalathin) twice daily for 2 weeks where after various blood parameters including antioxidant status biomarkers were monitored. No changes could be seen except for a minor decrease in the antioxidant status of these subjects when using a xanthine/xanthine oxidase test system (77).

Only recently the first human intervention study was completed in the Western Cape Province of South Africa to monitor the modulation of oxidative stress by traditional rooibos in adults at risk for developing heart disease (<http://sarooibos.co.za/content/view>). This study also served as the first scientific proof for human safety when considering the various clinical pathology results (Table IV).

Table IV. Effect of Traditional Rooibos Consumption on Selected Blood Clinical Pathology Markers Related to Liver and Kidney Function.

Analyte	Baseline	Wash out	Rooibos	Control
AST (U/L)	24.5 ± 10.8	21.1 ± 7.2	24.1 ± 14.2	17.3 ± 7.3
ALT (U/L)	26.4 ± 15.9	23.3 ± 12.5	21.4 ± 14.0	15.0 ± 8.8
GGT (U/L)	32.4 ± 21.2	31.5 ± 15.9	30.6 ± 17.6	27.9 ± 15.1
ALP (U/L)	81.6 ± 35.5	85.5 ± 27.8	79.0 ± 25.0	64.4 ± 22.6
LDH (U/L)	155.0 ± 51.8	150.7 ± 31.7	179.9 ± 44.2	182.8 ± 68.5
Total protein (g/L)	76.2 ± 31.3	80.4 ± 22.2	66.6 ± 4.4	65.9 ± 18.5
Urea (mmol/L)	5.3 ± 1.6	5.3 ± 1.7	4.1 ± 1.1	4.3 ± 1.3
Creatinine (µmol/L)	98.4 ± 45.5	93.5 ± 22.5	78.0 ± 19.1	68.5 ± 28.0
D Bilirubin (µmol/L)	3.5 ± 1.8	3.0 ± 1.5	3.6 ± 2.1	8.2 ± 11.2
T Bilirubin (µmol/L)	10.1 ± 3.8	10.3 ± 5.9	9.9 ± 3.9	15.6 ± 12.0
Glucose(mmol/L)	5.4 ± 1.3	5.9 ± 2.7	4.8 ± 0.6	5.5 ± 1.7
Total iron (µmol/L)	16.0 ± 5.4	17.1 ± 4.7	15.7 ± 4.8	13.9 ± 3.9

NOTE: All samples (n=40) were done in triplicate and values in columns represent means ± SD. Abbreviations: aspartate aminotransferase (AST), alanine aminotransferase (ALT); gamma glutamyl transferase (GGT); Alkaline phosphatase (ALP); lactate dehydrogenase (LDH); Conjugated bilirubin (D bilirubin); total bilirubin (T bilirubin).

SOURCE: Unpublished data (Dr JL Marnewick, Oxidative Stress Research Centre, Cape Peninsula University of Technology, Bellville, South Africa).

Forty adults (male and female) between the ages of 30 and 65 were required to consume 6 cups of traditional rooibos daily for 6 weeks. No adverse effects were reported by any of the participants during the study period as also shown by the various liver (AST, ALT, GGT, ALP, LDH) and kidney (creatinine total protein, urea) parameters measured in the serum of the participants. Preliminary results obtained from this study indicated that the consumption of rooibos protected the body against oxidative damage as shown by a decrease in two lipid peroxidation biomarkers, conjugated dienes, a primary oxidation product, as well as malondialdehyde, a secondary oxidation product. An increase in the redox status of the participants as seen by an increase in circulating GSH levels, decrease in GSSG levels with a resultant increase in GSH/GSSG ratio was also reported (unpublished data, Dr JL Marnewick, Oxidative Stress research Centre, Cape Peninsula University of Technology, South Africa). When compared to the control (water) phase, the levels of circulating conjugated dienes after completion of the rooibos intervention phase decreased by nearly 35%. When considering MDA, the levels of TBARS decreased with 52% after completion of the rooibos intervention when compared to the control (water intervention) phase. Lipid peroxidation is associated with cellular injury and that the byproducts are present at increased levels in oxidative damage. This study thus suggested that rooibos protects the body against oxidative damage which is in line with the findings of the study by Nikolova and co-workers (76). Results from this study will be finalized and a paper will be submitted to a medical journal for publication. Further evidence from human studies is required before

strong conclusions can be made regarding the association between rooibos and heart disease. No published reports describing the effects of honeybush in humans have appeared in any English peer-reviewed journals to date.

Conclusions

Humans are constantly seeking to advance their health and alleviate various ailments with herbal remedies. In this review, which reflects an integrated approach reporting on different *in vitro*, *ex vivo*, experimental animal test systems and few human clinical studies the roles rooibos and honeybush can play in the improvement of human health was discussed. Results from the body of evidence relative to the chemistry, biochemistry, and biological activity coupled to initial clinical studies clearly suggest that both of these South African teas provide healthy and beneficial herbal teas which warrant further controlled clinical investigations into the potential health modulating properties of these two promising natural products.

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Chapter 17

Umckaloabo: From a Patent Remedy to a Modern Herbal Pharmaceutical based on *Pelargonium sidoides* with Clinically Proven Efficacy

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Pelargonium sidoides has a long-standing tradition in the treatment of diseases, starting with ethnobotanical records from the mid 19th century. *Pelargonium sidoides* is native to the coastal regions of South Africa. In the first half of the 20th century, a product made from the root (Umckaloabo) was successfully used in Europe for the treatment of tuberculosis. Various metabolites including phenolic and cinnamic acids, tannins, flavonoids and coumarins were characterized in *P. sidoides*. In the late 20th century Umckaloabo was developed into a safe and efficacious herbal pharmaceutical for the treatment of upper respiratory tract infections, specifically acute bronchitis in children. EPs® 7630 (Umckaloabo®), an ethanolic root extract (1:9-11) of *P. sidoides* roots, showed antibacterial, antiviral, and immunomodulatory properties in a vast number of investigations. These activities seem to account for its therapeutic effect. In Germany, Umckaloabo is a fully licensed medicinal product ranging among the most widely bought self-medications. Numerous clinical trials with EPs® 7630 have confirmed its efficacy and safety.

This species is native to the coastal regions of South Africa. The plant is notable for its narrow, deep red flowers and its large, heart-shaped leaves. Along with the closely related *P. reniforme* Curt, the root has been used for centuries in South African traditional medicine to treat coughs, upper respiratory tract irritations, tuberculosis, and gastrointestinal complaints. In the first half of the 20th century, a product made from the root (Umckaloabo) was used in Europe for the treatment of tuberculosis. In the late 20th century Umckaloabo was developed into a safe and efficacious herbal pharmaceutical for the treatment of upper respiratory tract infections, specifically acute bronchitis in children. Various metabolites including phenolic and cinnamic acids, tannins, flavonoids and coumarins were characterized in *P. sidoides*. Activities against pathogens which are primarily responsible for respiratory tract infections and the immunomodulatory potential provide the rational basis for its therapeutic use. Numerous clinical trials with an ethanolic root extract (1:9-11) of *P. sidoides* roots, referred to as EPs® 7630 (Umckaloabo®) have confirmed its efficacy and safety.

Umckaloabo, the patent remedy

Ethnobotany

The ethnobotany of South African *Pelargonium* species and specifically *P. sidoides* has been extensively reviewed (1). Early recordings of the traditional uses of *Pelargonium* species refer predominantly to indications such as diarrhoea and dysentery owing to the astringent nature of their roots (2-8). Harvey and Sonder (9), in their treatment of *P. sidoides* [as *P. reniforme* Curtis var. *sidaefolium* (Thunb.) Harv.], stated the species as useful as an astringent in dysentery. The most detailed account of the value and uses of *P. sidoides* is that of Smith (10). Phillips (11) reported that the roots of are used to treat colic in Lesotho. Watt and Breyer-Brandwijk (12) reviewed ethnobotanical data for several *Pelargonium* species. The claim that the roots of *P. sidoides*/*P. reniforme* have been utilized for the therapy of tuberculosis in traditional medicine of South Africa (13,14) could not be substantiated by other sources.

Etymology of 'Umckaloabo'

The etymology of "Umckaloabo" has been discussed in detail in (1). "Umckaloabo" is the name introduced by Charles Henry Stevens for his tuberculosis medicine. Attempts to explain the origin of the word *umckaloabo* remain hitherto inconclusive. One interpretation was offered by Bladt (13) claiming it to be a derivation from isiZulu *umKhulane*, a term for various ailments with symptoms like fever, cough etc., and *uHlabo*, stinging breast pain. Doke and Vilakazi (15) refer to *umkhuhlana* (sic!) as a collective term for naturally occurring diseases accompanied by fever such as colds, influenza, pneumonia, pleurisy and malaria. According to Callaway (16) the term *uhlabo* is

derived from *isiZulu / isiXhosa ukuhlaba*, to stab or a stabbing pain, referring to pleurodynia or pleurisy. The legitimacy for quoting the *isiZulu / isiXhosa* words, however, is questionable, as the ethnicity of Stevens' healer and hence the language he used is uncertain. The term *Umckaloabo* may thus be an invention of Stevens.

History of commercialization

In 1897, the then 17-year old Charles Henry Stevens from Birmingham, UK, was diagnosed with pulmonary tuberculosis and sent by his doctor to South Africa to recover. There he met a local healer, Mike Kijitse, who prescribed a root concoction which drove his condition into complete remission within three months. On his arrival back in the UK he was pronounced cured. Over the next 10 years Stevens returned to South Africa and involved himself in various unsuccessful ventures to commercialize his "discovery". In 1907 he brought quantities of the root back to England and established the "Stevens Co." in London, UK, in order to market his cure in England. Not without success, his company accounts for 1908 reveal takings of £4,415 (17, 18).

The industrialization of the 19th century signalled a dramatic increase in urban population, particularly in England. Cramped unhealthy conditions and inhuman working hours and inadequate nutrition were the result. The infectious and resistant Tuberculosis (TB) bacteria had found ideal breeding grounds as the exhausted, haggard bodies of the industrial proletariat had nothing to fight back with. Under these circumstances, the "white plague" was responsible for claiming the lives of hundreds of thousands. Every fifth death was due to TB. The only cures the medical establishment in Europe had come up with to date were fresh air, rest and good food, i.e. an illusion for most of its victims. Robert Koch discovered the bacterium responsible for TB in 1882 but the development and widespread application of useful medication would take another 60 years.

The medical establishment of the time was not oblivious to Stevens' activities, the founder being a layperson and the disease in question the no. 1 national epidemic. Stevens (and others selling various "suspicious" treatments) did not have to wait long for the BMA's (British Medical Association's) response: In 1909 it published "Secret Remedies – what they contain and what they cost", a book in which "Stevens' Consumption Cure" was denounced as "quackery" and the origin of the plant used called into question (19-22).

While Stevens carried on selling his remedy, he felt an increasing impact of the BMA publication, impairing his sales in the UK and his attempts to take his remedy abroad. In 1912 he brought libel action against the BMA, an unprecedented step. While in the first trial the jury could not agree on a verdict, he was defeated in 1914, when despite numerous expert witnesses for Stevens, the jury found in favour of the BMA (supported by a recently published government report), the case was dismissed and Stevens ordered to pay the costs of £2,000. Both trials were extensively covered in both national and international press (23-31). An appeal in 1915 was refused.

War intervened. Stevens served with distinction, being promoted to the rank of major (19). After the war production and marketing of *Umckaloabo* (or

"Stevens' Cure") continued and Stevens' business extended as far as the US and India.

In January 1920, the Swiss physician Dr Adrien Secheyay heard about Stevens Consumption Cure from a patient. He began gathering information about it to use the treatment on his TB patients. After curing a young woman, he went on to treat some 800 patients in the next nine years (and further into the 1960s). He frequently reports to the Medical Society on his successes and eventually publishes a selection of case reports concluding the cure to be an advance in the treatment of tuberculosis (17,32-40).

As part of his "marketing strategy", Stevens published (or had published) numerous polemics and books of case reports. These books were published by Fraser & Co. in London, a publisher who – located conveniently close to Stevens' business - never published anything but 'Umckaloabo literature' and thus may well have been run or at least 'sponsored' by Stevens (41-43). By 1931, Stevens employed 50 people manufacturing and distributing his remedy (lozenges, an extract and capsules) (44). The US authorities, meanwhile, debarred the remedy from the mails (45).

Stevens' lobbying kept the public interest up and the authorities busy. Even though he never received any official recognition for his remedy, there are numerous records for attempts being made by the medical establishment to identify it (46). Publicity also helped maintaining the success of his business (47). Then war disrupted supply routes. Stevens died aged 62 in 1942. Secheyay continued treating tuberculosis patients with Umckaloabo until well into the 1960s (48-54).

As early as the 1930 Stevens' remedy had attracted attention throughout central Europe ("his" books were translated into German, Italian, Rumanian etc., cf. (55)). When the arrival of modern antibiotics resulted in the interest in the remedy waning, Stevens' son eventually sold the business to a drug manufacturer in Germany. There it appeared unchanged in the German National Formulary (Rote Liste), but without being actively marketed. Despite repeated attempts (56-58), it took until well into the 1970s for the plant ingredient of the remedy finally to be identified, when the mystery was finally resolved by Dr. Sabine Bladt, a pharmacist of Munich University (13,14,59-62). At this point the drug received renewed interest and pharmacological research was initiated and an ethanolic root extract (1:9-11) of *Pelargonium* (EPs® 7630) developed.

Marketing of the remedy as a treatment for bronchitis and symptoms of common cold started in the 1990s while research into the compounds and their mechanisms of action continued. Studies were initially observational, but later developed into fully fledged clinical trials (63-77).

In December 2005, the Federal Institute for Drugs and Medical Devices (BfArM, Bonn) approved a new license for the use of Umckaloabo (EPs® 7630) as a drug (78,79). It is a fully licensed liquid herbal medicine on the German market (€80,000,000 turnover in 2006). The drug is listed in the European Pharmacopoeia (80,81).

A preparation of *P. sidoides* mother tincture is marketed in the Ukraine, Russia and Latvia as Umkalor. A further preparation - meanwhile registered under the Traditional Herbal Products Directive - is available in the United Kingdom of Great Britain and Northern Ireland (82). Various liquid and solid

preparations are available as herbal supplements in North America and Mexico. A variety of liquid and dry forms of mono-preparations and herbal combinations that include *P. sidoides* are available in South Africa and adjacent countries, e.g. Linctagon, Phyto Nova Cough Syrup and Natura Pentagen.

Proprietary extracts of *Pelargonium sidoides* and their preparations, as well as the use thereof, are to date protected by a total of seven patents in various countries (83-89).

Umckaloabo, the pharmaceutical

Botanical identity and habitat

Taxonomy and description of *P. sidoides* vs. *P. reniforme* have been reviewed (1). Confusion about the correct identity of *P. sidoides* stems largely from Harvey and Sonder (9) including it as a variety within a broader concept of *P. reniforme*. Whether or not the name *P. reniforme* includes *P. sidoides* is thus not always clear. This is also reflected in the fact that into the late 1990s EPs® 7630 was referred to as an extract of *P. sidoides/P. reniforme* (90). This does, however, not necessarily imply use of both species as the taxonomy of *P. sidoides* vs. *P. reniforme* remained doubtful until revision (91,92). The correct scientific name of the species is *P. sidoides* DC. Dreyer and Marais (92) summarised the diagnostic differences between the two species.

The dried product can easily be adulterated with the very similar-looking *P. reniforme*, as morphological distinction of the dried product is extremely difficult. Chemical analysis, however, provides a reliable identification method, as *P. sidoides* contains umckalin and its 7-O-Methylether, which are absent in *P. reniforme*.

P. sidoides occurs in the Republic of South Africa throughout the Eastern Cape, Lesotho, Free State and southern and south-western Gauteng. *P. sidoides* is found at altitudes ranging from near sea level to 2300m in Lesotho. Most of the material is still wild-crafted in the Eastern Cape Province, but significant quantities of raw material will soon be produced from cultivated, seed-propagated plants.

Chemistry

A comprehensive study of all the chemical constituents of both underground and areal parts of *P. sidoides* and *P. reniforme* was done by Kolodziej (93). He also summarised earlier publications (67,72,94-103,172). However, further research may be warranted as these investigations were based on single samples of *P. sidoides* and *P. reniforme*, thus not taking into account possible geographical, phenotypical and genotypical patterns. A summary of main constituents in the roots and herb including constituents shown to be contained in Umckaloabo, an aqueous ethanolic extract of *P. sidoides* roots

(EPs® 7630) is given in Table I. A detailed account of the constituents of EPs® 7630 has been presented (104,105).

A yield of 0.52% (of dry weight) essential oil was obtained from the leaves of *P. sidoides* by hydrodistillation (106) and 102 components could be identified by GLC and GC-MS analyses.

Table I. Main constituents of *Pelargonium sidoides* root, herb and of EPs 7630

<i>Compound</i>	<i>Roots</i>	<i>Herb</i>	<i>EPs® 7630</i>
Phenolic acids, phenylpropanoids and derivatives			
Gallic acid	+	+	+
Gallic acid methyl ester	+	+	+
Gallic acid ethyl ester		+	
Shikimic acid			+
Shikimic acid 3-0-gallate		+	+
Glucogallin		+	
Protocatechuic acid		+	
Coumarins, coumarin glycosides and coumarin sulfates			
7-Hydroxy-6-methoxycoumarin (Scopoletin)	+	+	+
6,7,8-Trihydroxycoumarin	+		+
6,8-Dihydroxy-7-methoxycoumarin	+		+
7-Hydroxy-5,6-dimethoxycoumarin (Umckalin)	+	+	+
7,8-Dihydroxy-6-methoxycoumarin (Fraxetin)	+	+	+
8-Hydroxy-5,6,7-trimethoxycoumarin	+		+
7-Acetoxy-5,6-dimethoxycoumarin	+		
5,6,7-Trimethoxycoumarin	+		+
6,8-Dihydroxy-5,7-dimethoxycoumarin	+	+	+
5,6,7,8-Tetramethoxycoumarin (Artelin)	+		+
Umckalin-7-β-D-glucoside	+		+
Fraxetin-7-β-D-glucoside		+	
Magnolioside	+	+	
Isofraxoside	+		
6,7-Dihydroxycoumarin-8-sulfate	+	+	
5,6-Dimethoxycoumarin 7-sulfate	+		+
6-Hydroxy-5,7-dimethoxycoumarin 8-sulphate	+		
8-Hydroxy-5,7-dimethoxycoumarin 6-sulphate	+		

Source: Modified from (93)

Table I. continued

<i>Compound</i>	<i>Roots</i>	<i>Herb</i>	<i>EPs® 7630</i>
<i>Flavonoids</i>			
Isoorientin		+	
Isoorientin 2"-O-gallate		+	
Isovitexin		+	
Isovitexin 2"-O-gallate		+	
Quercetin		+	
Taxifolin 3-O-β-D-glucoside		+	
Orientin		+	
Orientin 2"-O-gallate		+	
Dihydrokaempferol 3-O-β-D-glucoside		+	
Luteolin 7-O-β-D-glucoside		+	
Vitexin		+	
Vitexin 2"-O-gallate		+	
Epigallocatechin-3-O-gallate		+	
<i>Flavan-3-ols / Hydrolysable Tannins</i>			
Catechin	+		
Gallocatechin	+		
Proanthocyanidins	+		+
<i>Others</i>			
β-Sitosterol	+		
(+)-Cyclolariciresinol-2a-β-D-glucoside		+	
4,6-Dihydroxyacetophenone 2-O-β-D-glucoside		+	
4-Allyl-2,5-dimethoxyphenol-1-β-D-glucoside		+	

Pharmacology

A detailed account of the pharmacological properties of *P. sidoides* and/or EPs® 7630 has been given (1). The following overview summarizes this information.

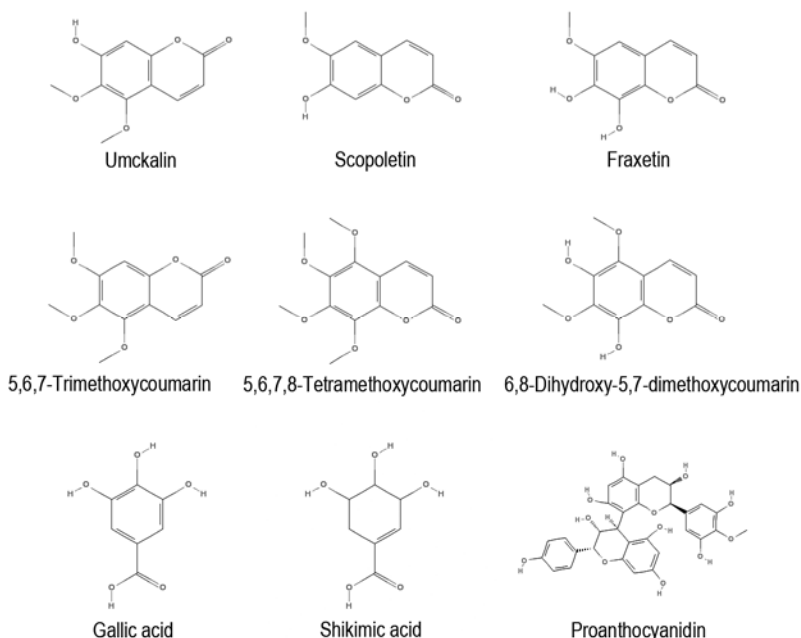


Figure 1. Selected constituents of *P. sidoides* and EPs® 7630 (drawings PubChem)

Antibacterial properties

Antibacterial activity of extracts and isolated constituents of *P. sidoides* was evaluated against three gram-positive and five gram-negative bacteria (107). Most compounds exhibited antibacterial activities. Further investigations complement these findings (108).

EPs® 7630 shows a synergistic indirect antibacterial effect in group A-streptococci through inhibition of bacterial adhesion to human epithelial cells as well as induction of bacterial adhesion to buccal epithelial cells (109-111). The antiadhesive effect of EPs® 7630 was confirmed for *Helicobacter pylori* growth and adhesion to gastric epithelial cells (112,113). Umckaloabo significantly stimulated phagocytosis and oxidative burst. Intracellular killing was also enhanced (114-120).

Modulation of epithelial cell-bacteria interaction through EPs® 7630 may protect mucous membranes from microorganisms evading host defence mechanisms. This provides a rationale for the treatment of upper respiratory tract infections with EPs® 7630 (121).

Anti-mycobacterial activity for hexane extracts of roots of *P. reniforme* and *P. sidoides* was established (122-124). As no significant effect on the bacterial growth of two strains of mycobacteria by extracts and fractions of *P. sidoides* could be shown (101), it was postulated that an antitubercular effect may be achieved indirectly by stimulation of immune response. This assumption was supported by Mativandlela et al. (125,126) as none of the compounds

isolated from *P. sidoides* showed any significant activity against *M. tuberculosis*.

Immunomodulatory properties

Extracts and isolated constituents of *P. sidoides* were investigated for their effects on nonspecific immune functions in various bioassays (98,107,127). Results imply indirect activity, possibly through activation of macrophage functions. Activation was confirmed through the presence of tumour necrosis factor (TNF-alpha) and inorganic nitric oxides (iNOS). TNF-inducing potencies for EPs® 7630 as well as interferon-like activities were also observed (128,129). Interferon (IFN)-beta production increased and natural killer cell mediated cytotoxicity was enhanced in MG-63 human osteosarcoma cells preincubated with Umckaloabo (130).

Kolodziej et al. (131,132) investigated polyphenol-containing extracts of *P. sidoides* and simple phenols, flavan-3-ols, proanthocyanidins and hydrolysable tannins for gene expressions (iNOS, IL-1, IL-10, IL-12, IL-18, TNF-alpha, IFN-alpha/gamma). All extracts and compounds were capable of enhancing the iNOS and cytokine mRNA levels in parasitized cells. EPs® 7630 induced low mRNA levels in non-infected cells, but considerably up-regulated transcript expressions in infected cells (133). Production of IFN-gamma mRNA was also stimulated. Similar profiles were obtained for the methanol-insoluble fraction and gallic acid. Significant immunomodulatory properties for EPs® 7630, extracts and isolated constituents of *P. sidoides* were demonstrated in various functional bioassays (134).

EPs® 7630 stimulates host defence through enhancing the release of antimicrobial peptides (135).

Effects on the mucociliary system

EPs® 7630 significantly increased ciliary beat frequency in a dose-dependent manner (136,137).

Effects on symptoms of sickness behaviour

EPs® 7630 showed inhibition of sickness behaviour in a dose-dependent manner (138,139).

Antiviral properties

An inhibitory effect of an aqueous root extract of *P. sidoides* against herpes simplex virus type 1 and 2 was shown in a plaque reduction assay and high antiviral activity against both herpes viruses could be demonstrated in viral suspension tests (140).

Toxicology, adverse effects, precautions, contraindications, interactions

Toxicity of coumarins present in the crude drug and/or extracts can be considered negligible (NOEL >750 mg/kg body weight). No hepatotoxic activity could be established for coumarins present in EPs® 7630 (72,103,141,142). In controlled clinical trials with over 7,200 adults and children mild adverse events, consisting of gastrointestinal complaints and skin rashes, occurred in <15% of subjects (79,143). A total of 34 hypersensitivity reactions have been recorded through the World Health Organisation (WHO) international pharmacovigilance programme and may be associated with EPs® 7630 (144).

Roots et al. (145) investigated a potential interaction of a *P. sidoides* extract (EPs® 7630) with penicillin V in a placebo-controlled, double-blind trial with 28 healthy humans. No interaction was shown. Interaction with anticoagulants and antiplatelet drugs could be ruled out as the coumarins so far identified in EPs® 7630 do not appear to possess anticoagulant characteristics (146).

The extract of *P. sidoides* root (EPs® 7630) is contraindicated during pregnancy and lactation as no specific data on its effect on pregnant or lactating women is available.

Clinical evidence of efficacy

Results for a total of 20 clinical trials have thus far been published, 7 of which were observational studies, the remaining 13 were randomized, double-blind and placebo-controlled. For 6 of those 13 trials, only preliminary results have been published. For more detailed information see Table II. In several instances, more than one publication presents the results for one and the same trial/study; Table II summarizes and combines the various references. All trials have been carried out using a *P. sidoides* extract (EPs® 7630) in liquid or solid forms.

The data derived from these trials has been evaluated in two reviews (147,148). Akbabiaka et al. (147) focused on *Pelargonium sidoides* for acute bronchitis and identified 6 studies (90,149-151) and two unpublished trials referred to as "Kieser (2007a)" and "Kieser (2007b)". Results for the latter two were initially presented by Kamin (152) and are thus referred to this reference for the purpose of this overview) which met their inclusion criteria (randomized trials against a control intervention, excluding trials which admitted patients with pre-existing chronic bronchitis and/or infectious diseases), two of which were/are unpublished. Methodological quality of the trials reviewed was good; a Jadad score of 5 was calculated for three trials. Akbabiaka et al. (147) concluded that there was encouraging evidence for the efficacy of *P. sidoides* in the treatment of acute bronchitis vs. placebo.

Timmer et al. (148) reviewed the efficacy of *P. sidoides* extract for acute respiratory tract infections and identified 12 trials meeting their inclusion criteria (randomized trials against a control intervention with complete resolution of symptoms as primary outcome criteria). Four trials were excluded from the analyses due to high risk of bias. They reviewed eight trials

(150,151,152*,153**,154). *(referred to as Anonymous 1-4), **(referred to as Bachert unpublished).

While the evidence from trials with acceptable methodology was considered limited and more trials are called for, both reviews concluded treatment with *P. sidoides* offered relief from the symptoms of acute respiratory tract infection, and specifically from acute bronchitis in children. Taking into account additional data from observational studies and post-market surveillance, results support the use of Umckaloabo (EPs® 7630) as a possible alternative to antibiotics for the acute treatment of these conditions. Overall safety and a very low incidence of side effects have been confirmed.

Table II. List of clinical trials

		<i>Results (References)</i>
<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	
Upper respiratory tract infections, acute or chronic	Observational study 166	166 children and adolescents aged 1-19 were treated for up to three weeks, median 7 days. Primary outcome criteria were change in symptoms and evaluation of efficacy and tolerability on a four point scale after completion of the treatment. Improvement of all related symptoms (cough n=62, fever n=47, with complete remission in 50% and 85% respectively) could be observed, in 2/3 of patients no further treatment was required. Tolerability was described as "good" or "very good" in more than 90%. Adverse effects were not reported. (64)
Upper respiratory tract infections, acute or chronic	Observational study 641	641 patients were included aged <10->60 and treated for up to >14 days, median 12 days. Significant improvement of symptoms were observed after 7 days (n=240) and 14 days (n=305) respectively. In 85% treatment could be concluded after 2 weeks. In 88.9% efficacy was assessed as "good" or "very good". Tolerability was described as "good" or "very good" in 639 cases. Adverse effects were not reported, in 7 cases new or aggravated symptoms were reported. (65)
Bronchitis, acute	Observational study 259	259 children aged up to 12 years were included and treated for up to three weeks, median 14 days. Cough, expectoration, rattling and chest pain were assessed in a 5-level, verbal rating scale. Overall success of therapy was also assessed in a 5-level rating scale. A remission or improvement of >80% of symptoms was observed after conclusion of the treatment. Overall remission could be observed in 57.9%, overall improvement in 32%. Tolerability was described as "good" or "very good" in 96.5%. Adverse effects were reported in 6 cases and included mild exanthema and gastrointestinal complaints. (71)

Table II. Continued.

<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	<i>Results (References)</i>
Bronchitis, acute	Observational study 742	742 children (0 to 12 years) were included and treated according to age (0-2, 2-6, >6) with 15/30/60 drops of Umckaloabo for up to 14 days. Overall outcome measure was the sum of five Bronchitis Severity Score symptoms. Bronchitis Severity Score decreased from 6.0+/-3.0 points at baseline to 2.7+/-2.5 points after 7 days and to 1.4+/-2.1 points after 14 days. Complete or partial remission of symptoms was observed in 90.2%. Tolerability was described as "very good" in 94.9%, 8 patients experienced non-serious adverse effects including aggravation of symptoms, mild exanthema, gastrointestinal complaints, unrest and dyspnoea. (70,155)
Bronchitis, acute	Randomized controlled trial 60 (30/30)	60 children aged 5-14 were included and treated for 7 days with Umckaloabo (30), or with acetylcysteine (30). Changes in Bronchitis Severity Score were evaluated. Score base value was 7 (+/-3) points and decreased in the Umckaloabo group by 7 (+/-2) points, in the acetylcysteine group by 6 (+/-3) points. Total remission of symptoms after 7 days was observed in 76.7% (Umckaloabo) and 56.7% (acetylcysteine). Tolerability was assessed as "very good" by 53.3% (Umckaloabo) and 46.7 (acetylcysteine). Adverse effects were not reported. (90)
Angina catarrhalis	Randomized controlled trial 60 (30/30)	60 children aged 6-10 were included, 30 of which were treated with Umckaloabo, 30 received symptomatic treatment (Priessnitz' cure and gargling with apple vinegar) over a period of 10 days. Target criteria were change in angina-related symptoms and tolerability. By day 4, improvement of symptoms had reached the response criteria in 23 cases in the Umckaloabo group vs. 9 cases with symptomatic treatment. Tolerability was assessed as "good" or "very good" by 100% in the Umckaloabo group after 10 days vs. 63.4% in the symptomatic treatment group. Adverse effects were not reported. (156)

Table II. Continued.

<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	<i>Results (References)</i>
Non-GABHS tonsillopharyngitis in children	Randomized, placebo-controlled double-blind trial 143 (73/70)	143 children aged 6-10 years with a Tonsillopharyngitis Severity Score \geq 8 points were included. EPs® 7630 or placebo were given (20 drops 3 times daily) for 6 days. Score decreased from baseline to day 4 7.1 +/- 2.1 points with EPs® 7630, and 2.5 +/- 3.6 points with placebo. The 95% confidence interval for the difference between the groups was [2.7; 4.9] and showed significant superiority in efficacy of EPs® 7630 ($P < 0.0001$). 4 and 43 withdrawals for reasons other than complete recovery were reported for the Umckaloabo and placebo groups respectively. Tolerability was assessed as "good" or "very good" for almost all cases in both groups. Adverse events were reported for 15 patients (EPs® 7630 1 patient, placebo 14), however, were neither classified as serious nor related to the medication. (157,158)
Bronchitis, acute	Randomized, placebo-controlled double-blind trial 468 (233/235)	468 adults with Bronchitis Severity Score \geq 5 points were included. EPs® 7630 or placebo were given (30 drops 3 times daily) for 7 days. Decrease from baseline to day 7 was 5.9 +/- 2.9 points with EPs® 7630, and 3.2 +/- 4.1 points with placebo. The 95% confidence interval for the difference of effects between the treatment groups showed significant superiority in efficacy of EPs® 7630 on day 7 ($p < 0.0001$). Tolerability was assessed as "good" or "very good" by 96.1% in the EPs 7630 group vs. 88.1% in the placebo group. Non-serious adverse events occurred in 36 patients (EPs® 7630 20 patients, placebo 16 patients). Most commonly reported were ear and labyrinth disorders and gastrointestinal disorders. (149)

Table II. Continued.

<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	<i>Results (References)</i>
Bronchitis, acute	Randomized, placebo-controlled double-blind trial 124 (64/60)	124 adults with Bronchitis Severity Score >5 were included. 64 patients received 30 drops EPs® 7630, 60 patients received placebo three times daily for 7 days. Decrease of Bronchitis Severity Score from baseline to day 7 was 7.2 +/- 3.1 points with EPs® 7630 vs. 4.9 +/- 2.7 points with placebo. The 95% confidence interval for the difference of effects between treatment groups showed a significant superiority of EPs® 7630 (P < .0001). Treatment effect was recognized in 68.8% of patients in the EPs® 7630 group vs. 33.3% of patients in the placebo group (P < .0001) within the first 4 days. 3 and 4 withdrawals were recorded for the verum and placebo group, respectively. Tolerability was assessed as "good" or "very good" by 98.4% in the EPs 7630 group vs. 96.7% in the placebo group. Non-serious adverse events occurred in 25 of 124 patients (EPs® 7630 15 patients, placebo 10 patients). (78,150,159,160)
Sinusitis, acute bacterial maxillary	Randomized, placebo-controlled double-blind trial 272	272 patients with radiologically confirmed acute bacterial maxillary sinusitis were treated with 3x 60 drops EPs® 7630 or placebo, respectively, for a maximum of 3 weeks. Primary outcome criteria were sinusitis symptoms. Symptoms decreased from 14.4 +/-1.8 points by 7.0 +/-3.2 points in the verum group, while the baseline value of 13.9 +/-1.7 in the placebo group remained unchanged (p<0.0001). No serious adverse events were recorded. (161)
Sinusitis, acute bacterial maxillary	Randomized, placebo-controlled double-blind trial 103 (51/52)	103 patients with radiologically confirmed acute bacterial maxillary sinusitis were treated with 3x 60 drops EPs® 7630 or placebo, respectively, for a maximum of 22 days. Primary outcome criteria were the change in Sinusitis Severity Score after 7 days. Score decreased by 5.5 points in the verum group, and 2.5 points in the placebo group (p<0.00001). Complete recovery at treatment end was observed in 32 and 4 patients in the verum and placebo group, respectively. No serious adverse events were recorded. (153,161)

Table II. Continued.

		<i>Results (References)</i>
<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	
Sinusitis	Observational study 361	361 patients aged 1-94 were included and treated with EPs® 7630 (days 1 and 2 30 drops 12 times daily (children 20 drops), thereafter 30 drops 3 times daily (children 20 drops) for 28 days, patients with chronic sinusitis received a prophylactic treatment for another 8 weeks of 30/20 drops two times daily (adults/children). Within 4 weeks 80.9% of the patients became symptom-free or experienced improvement in symptoms. Success rate was over 90% for each individual symptom. Tolerability was assessed as "good" or "very good" by 95.8%. 17 patients reported non-serious adverse events, mostly gastrointestinal complaints. (162)
Bronchitis, acute	Observational study 205	205 patients with a mean age of 42 +/-16 were included and treated with 3x 30 drops EPs® 7630 daily for 7 days. Change in the total score of 5 Bronchitis Severity Score symptoms was the outcome measure. Total score of bronchitis symptoms was 6.1+/-2.8 points at baseline and decreased by 3.3+/-3.8 points to 2.8+/-2.6 points by the final examination on day 7. 60.5% of the patients assessed their health as much improved at the end of the study. Non-serious adverse events occurred in a total of 16 patients, mostly gastrointestinal complaints. (163)
Bronchitis, acute	Observational study 2099	2099 patients (0-93 years) were treated with an age-dependent dose of EPs® 7630 for 14 days. Change of the Bronchitis Severity Score was the primary outcome criterion. Score decreased from 7.1+/-2.9 points at baseline to 1.0+/-1.9 points at patients' individual last visit. Children showed decrease of score from 6.3+/-2.8 points to 0.9+/-1.8 points. Children <3 years showed a decrease of score from 5.2+/-2.5 points to 1.2+/-2.1 points. Compliance was assessed as "good" in 96.4%. Mostly non-serious adverse events occurred in 26 patients, mostly gastrointestinal complaints. (164)

Table II. Continued.

<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	<i>Results (References)</i>
Bronchitis, acute	Randomized, placebo-controlled double-blind trial 217 (108/109)	217 patients (18 to 66 years) were included. 108 (109) patients received 30 drops of EPs® 7630-solution (placebo) 3x times daily for 7 days. Bronchitis Severity Score decreased by 7.6 +/- 2.2 points in the EPs® 7630 group and by 5.3 +/- 3.2 points in the placebo group. Time to remission was significantly shortened in the verum group. The 95% confidence interval for difference between effects showed highly significant superiority of EPs® 7630 ($p < 0.0001$). 47 non-serious adverse events were recorded. (151,165-167)
Bronchitis, acute	Randomized, placebo-controlled double-blind trials 399 (298/101)	399 children aged 6-18 are included and treated with 30, 60 and 90mg EPs® 7630 tablets and placebo, respectively. Primary outcome criterion is the mean change of the Bronchitis Severity Score. The two higher dosages showed significant improvement vs. placebo (score decreased -3.6/-4.4/-5.0 vs. -3.3, $p < 0.001$ for 60/90mg. Non-serious adverse events are reported in 18 patients, mostly gastrointestinal complaints. (147*,148**,152,168) *referred to as Kieser (2007b), **referred to as Anonymous 2
Bronchitis, acute	Randomized, placebo-controlled double-blind trials 405 (101,101,101,102)	405 patients aged > 18 , mean 40, were included. Patients were divided up into 4 groups receiving 3 times daily one tablet of 10, 20 or 30mg EPs® 7630 or placebo for 7 days. Decrease of Bronchitis Severity Score and typical symptoms was observed. Decrease was significant in all 3 verum groups vs. placebo (score decreased -4.3/-6.1/-6.3 vs. -2.7, $p < 0.001$ for 30/60/90mg. 3 times 20mg daily appears to correspond to 3 times 30 drops, dose increase beyond 60mg daily does not appear to increase efficacy. Non-serious adverse events are reported in 18 patients, mostly gastrointestinal complaints. (147*,148**,152,169,170) *referred to as Kieser (2007a), **referred to as Anonymous 3

Table II. Continued.

<i>Indication</i>	<i>Trial type/Size (verum / placebo)</i>	<i>Results (References)</i>
Bronchitis, acute	Randomized, placebo- controlled double- blind trials 200	200 children and adolescents aged 1-18 were included. Patients received EPs® 7630 solution or placebo three times daily 10/20/30 drops (age groups 1-6/>6-12/>12-18) for 7 days. Decrease in Bronchitis Severity Score and typical symptoms were observed. Difference of reduction in score and other symptoms was significant (p<0.0001) in verum (-3.4) vs. placebo (-1.2). Non-serious adverse events related to verum were recorded in 31 cases (6 possibly related to therapy), mostly gastrointestinal complaints. (148*,152,168,171) *referred to as Anonymous 1
Bronchitis, acute	Randomized, placebo- controlled double- blind trials 220	220 children and adolescents aged 1-18 were included. Patients received EPs® 7630 solution or placebo three times daily 10/20/30 drops (age groups 1-6/>6-12/>12-18) for 7 days. Decrease in Bronchitis Severity Score and typical symptoms were observed. Difference of reduction in score and other symptoms was significant (p<0.0001) in verum (-4.4) vs. placebo (-2.9). Non-serious adverse events related to verum were recorded in 2 cases (unrelated to therapy). (148*,152,168,171) *referred to as Anonymous 4
Cold, common	Randomized, placebo- controlled double- blind trial 103 (52/51)	103 adult patients aged 18-55 with cold symptoms (maximum symptom score of 40 points) were included. Patients received either 30 drops EPs® 7630 or placebo 3 times daily for 10 days. Outcome criterion was the sum of symptom intensity differences of the cold intensity score from day 1-5. From baseline to day five, the mean score improved by 14.6 +/- 5.3 points with EPs® compared to 7.6 +/- 7.5 points with placebo (p < .0001). 8 patients were withdrawn (4/4). Tolerability was assessed as "good" to "very good" in 94.2% for verum vs. 82.4% for placebo. Non-serious adverse events occurred in 3 patients (2/1), one possibly related to verum (mild epistaxis). (154)

Conclusions

Pelargonium sidoides has a long-standing tradition in the treatment of diseases, starting with ethnobotanical records from the mid 19th century and followed by the enthusiastic perseverance of Charles Henry Stevens and Adrien Sechehaye in the first half of the 20th century.

In Germany, a fully licensed medicinal product containing a special extract of *P. sidoides* root is now among the most widely bought self-medication products. EPs® 7630 (Umckaloabo®), showed in vitro antibacterial, antiviral, and immunomodulatory properties in several studies. These activities seem to account for its therapeutic effect in patients suffering from acute bronchitis, tonsillopharyngitis, sinusitis and symptoms of the common cold. Efficacy and safety have been proved in numerous clinical trials.

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Chapter 18

African Psychoactive Plants

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Psychoactive plants have been used by humans for recreational, spiritual, and therapeutic purposes for millennia. Africa possesses an ancient tradition of medicinal plant use and has a rich tradition of using its indigenous plants for these purposes. Given African's high floristic diversity, and the strong connection between plants and the many African cultures and societies, relatively few African psychoactive plants have been investigated in detail when compared to the Americas. This review examines the traditional uses of plants from sub-Saharan Africa that exhibit an effect on the central nervous system. The use, chemistry and pharmacology of well researched and documented plants such as *Catha edulis*, *Cola* species, *Datura* species, *Pausinystalia yohimbe* (= *Corynanth yohimbe*) and *Tabernanthe iboga* are reviewed. Newer discoveries are highlighted as well as particular difficulties that are encountered when investigating African psychoactive plants.

Introduction

“Whatever deceives men seems to produce a magical enchantment”
Plato 427-347 BC, Greek Philosopher

Among the plants used by humans, those able to alter the consciousness and the senses have drawn particular consideration. Frequently surrounded by mystery, superstitions, magical thoughts and religious rituals, they have often been revered and attributed supernatural qualities. This is a phenomenon that can still be observed today, in many tribal shamanistic societies. This is made evident from an African example, Eboka. To quote Schultes and Hofmann's popular book, *Plants of the Gods* (1);

“Zame ye Mebege [the last of the creator gods in Bwiti culture, of Gabon and the Congo] gave us *Eboka* [also known as *iboga*; *Tabernanthe iboga* root]. One day ... he saw ... the Pygmy Bitamu, high in an *Atanga* tree, gathering fruit. He made him fall. He died, and Zame brought his spirit to him. Zame cut off the little fingers and the little toes of the cadaver of the pigmy and planted them in various parts of the forest. They grew into the *Eboka* bush (1).”

Tabernanthe iboga is used in Bwiti culture to this day to seek information from the ‘spirit (ancestral) world’, and induces hallucinations accompanied by strong stimulation of the central nervous system (CNS). More recently, anecdotal as well as several scientific reports in Western scientific literature indicate possible anti-addictive effects of *T. iboga* (2).

Today, much is known about the chemistry and biological activity of *T. iboga* and a handful of other African psychoactive plants, but the majority of African psychoactive plants have only been investigated superficially or not at all. Until recently most of the research on psychoactive plants has focused on the New World, in particular the Americas (3). It is widely accepted that more plants are utilized culturally as hallucinogens (sometimes referred to as entheogens when associated with cultural and religious use) in the New World than the Old (1). Of 97 reviewed plants with proven or alleged hallucinogenic potential only eight species are of African origin (1). This is contrary to the fact that African traditional cultures and indeed African traditional medicine are the oldest and possible most diverse of all medicinal systems.

Initial explanations for this apparently low occurrence of African psychoactives may be that indigenous Americans retained the fundamental shamanistic characteristics of hunting societies and thus more actively sought mystic visionary experiences by means of hallucinogenic plants than many peoples of the Old World (4). However, this may not be the case in Africa, as a large proportion of the population still use and practice traditional ‘shamanistic’ medicine which often involves ‘communication’ with ancestral spirits.

A more likely reason suggested by De Smet (3) is simply that most of the research, up until recently, on this subject has been conducted in the Americas and Europe. Africa and Australia have largely been neglected. Unfortunately, much of this information may be lost with the erosion of traditional knowledge and the rapid onset of modernization. The introduction of Christianity and Islam to sub-Saharan Africa has also possibly contributed to the loss of traditional knowledge of the psychoactive plants and associated rituals in particular. The uses of plants to induce altered states of consciousness for spiritual and religious purposes were probably not encouraged by these ‘newly introduced religions’. In addition, there was a reluctance by many researchers at in the 1980s and 90s

to document and work in areas concerning rituals not of their own beliefs and substances of 'abuse' (5).

Psychoactive plants have been used by humans for recreational, spiritual, and therapeutic purposes for millennia (6). It is evident from the African plants that have received the most attention, such as *T. iboga* and *Catha edulis* that research has been focused on those plants most commonly used for spiritual or recreational purposes. This is understandable as these plants are often more obvious because of their cultural importance and consequently more noticeable, in particular hallucinogens. The more subtle, often more therapeutically important plants, such as mild stimulants, sedatives, those used to treat convulsions and epilepsy have been sadly overlooked. The CNS-related mode of action of many plants may not immediately be obvious, for example nausea and vomiting are associated with the gastrointestinal system but is often treated via the CNS with scopolamine. The hunger-suppressant (anorectic) activities of South African succulent plants of the species *Hoodia* (7) are another example.

Scope of This Review

Since Louis Lewin's *Phantastica* (6) the first comprehensive survey on the use and abuse of mind-altering plants, a large body of information has accumulated on plants that have an effect on the CNS. A thorough treatment of such plants and their active constituents falls outside of the scope of this review they can be found in many recent reviews (8-11).

The focus of this paper is to review and identify those psychoactive plant species of sub-Saharan Africa. The biological and cultural diversity of Africa is immense (there are over 2,000 languages represented in sub-Saharan Africa). However, these ancient medicinal systems, usually based on oral traditions, are poorly documented even to this day. In contrast, North Africa and the Middle East have a relatively well documented traditional medicine (12-14). The Babylonians, Assyrians and Sumerians recorded herbal remedies in cuneiform on clay tables as long ago as 4000 BC. Not only can we attribute the origins of civilization to North Africa and the Middle East but also possibly the most important psychoactive plant, *Papaver somniferum* (opium poppy), from which the first alkaloid and psychoactive chemical was isolated. Morphine was first isolated by the German pharmacist Sertuner in 1803 (15).

This paper will review some well documented psychotropic genera. Recent studies have shown that members of the Amaryllidaceae, notably *Boophone*, *Crinum* and *Pancreatium* have similar alkaloid chemistry to European species. One such alkaloid, galanthamine (named after *Galanthus* the European snowdrop) is currently used to treat the symptoms of Alzheimer's disease (AD) due to its ability to inhibit acetylcholinesterase. The chemistry and biological activity, of the Amaryllidaceae will be discussed in more detail in another review chapter in this book (16). Of particular interest, in addition to the potent acetylcholinesterase inhibitors are the Amaryllidaceae alkaloids that bind to the serotonin re-uptake transporter, an important target in recent antidepressant therapy. Recent investigations of *in vivo* and *in vitro* CNS-related biological activity of African medicinal plants are highlighted below. Very few of the

active constituents are known, an area which needs to be tackled in future research if we are to make meaningful progress. This chapter addresses the interface of two very different fields of research, African traditional medicine and neurobiology. Brief introductions to these two fields will be given in the next section to support the discussions that follow.

The Central Nervous System (CNS)

This section reviews basic principles of brain anatomy and function to provide a framework within which to discuss the effect of plants on the CNS. The human nervous system is exceptionally complex, it is the body's major communication system, and is divided into central and peripheral regions. The central nervous system consists of the brain and spinal cord, and the peripheral nervous system consists of all other nerves. Although thought processes and reason are most commonly associated with the CNS, almost every aspect of physiological function is affected by CNS activity. After all, 'Brain death' is widely accepted as the definition of the end of human life (10). The follow description of CNS anatomy and physiology can be found in most text books on the and reviews on the subject (11, 17).

The spinal cord controls reflex actions, and relays sensory and motor information between the body and the brain, so that the organism can respond appropriately to its environment. The region of the brain where it meets the spinal cord is called the hindbrain (or rhombencephalon), and is composed of the medulla (myelencephalon), pons and cerebellum (metencephalon). The medulla is vital to sustaining life, and controls processes such as breathing, heartbeat and blood flow. The medulla contains receptors for the opioid drugs, such as heroin and morphine, which is why these drugs can cause respiratory depression and death (11, 17). The pons is a 'relay station' for signals being carried from the cortex to the cerebellum, which is involved in body movements and coordination, another function affected by opioid drugs.

Above the hindbrain is the midbrain (mesencephalon), which contains two areas that are very important with respect to psychotropic plant use, in particular substance dependence. The ventral tegmental area (VTA) has dopamine rich cell bodies, and projects to the limbic system and forebrain regions (11, 17). The VTA is involved in signalling the importance of stimuli that are critical to survival such as those associated with feeding and reproduction. However, many psychoactive drugs also have powerful effects on the VTA, which contributes to the development of dependence by signalling to the brain that psychoactive substances are very important from a motivational perspective. The dopaminergic projection from the VTA to the nucleus accumbens is known as the mesolimbic dopamine system, and is the neurotransmitter system that is most strongly implicated in the dependence-producing potential of psychoactive drugs (18,19). Consistent with a relationship among drug sensitization, mesolimbic dopamine, and drug-seeking behavior, alkaloids from the reported anti-addictive *T. iboga* also blocked the sensitized dopamine responses to morphine and cocaine in the nucleus accumbens (2).

Another important midbrain structure is the substantia nigra, which also has dopaminergic projections to the forebrain, but these pathways are involved in coordinating and executing movements of the body. Degeneration of neurons in the substantia nigra leads to the characteristic symptoms of Parkinson disease (10, 17).

The forebrain (prosencephalon) is composed of the diencephalon and the telencephalon (cerebral hemispheres). Important areas of the diencephalon are the thalamus, the hypothalamus, and the posterior lobe of the pituitary gland. The hypothalamus is critical for regulating hormonal signals and basic bodily functions – concerning, for example, water balance (osmolality), metabolism, body temperature and reproductive hormones – as well as responding to changes in these functions (17). The hypothalamus also secretes hormones that travel to the nearby posterior lobe of the pituitary gland. A steroidal glycoside with anorectic activity, isolated from the South African plant *Hoodia gordonii* (20) increases the content of ATP by 50–150% in hypothalamic neurons (7). With evidence of metabolic or nutrient-sensing by the hypothalamus, it is suggested by MacLean and Luo (7) that ATP may be the currency of energy sensing, thus changes in ATP levels in the hypothalamus may prompt the appropriate neural, endocrine and appetitive responses. This would be similar to other fundamental hypothalamic homeostatic centers, such as those for temperature and osmolality (7).

The thalamus functions as a relay station for sensory and motor information going to and from the cortex to other areas of the brain and body. The outermost layer of the brain is the cortex, which is made up of layers of nerve cells or neurons, and has a highly folded organization that increases its surface area and the number of neurons that it contains (11, 17). The cortex is involved in many aspects of psychotropic plant use, from the primary effects of psychoactive drugs on sensations and perceptions, to the complex behaviours and thoughts involved in drug craving and uncontrolled use (17).

Beneath the cortex are several other important structures. The basal ganglia are structures involved in voluntary motor behaviour and consist of the caudate, putamen, globus pallidus and amygdale. The caudate and putamen together are known as the striatum. Degeneration of these structures in some diseases, for example Parkinson's disease, may cause cognitive and motor impairment (11, 17).

Neurons and Synapses

Communication in the brain takes place between nerve cells or neurons. Psychoactive substances alter many aspects of communication between neurons, as will be discussed below. Neurons are highly specialized cells that exist in many shapes, sizes and varieties. However, they share the following basic structural regions: cell body or soma, dendrites, axon, and terminal buttons. The cell body, or soma, is the metabolic centre of the neuron, and contains the nucleus and other structures that sustain the neuron. The nucleus plays a role in mature neurons, where it is used to synthesize proteins in response to a wide variety of stimuli (11, 17).

Psychoactive substances can affect the expression of DNA, resulting in short-term or long-term changes in neuronal function, and ultimately, behaviour. Dendrites are highly branched processes extending from the cell body of the neuron, which receive chemical messages from other neurons. This branching, and the presence of dendritic spines (small swellings on the surface of a dendrite with which a terminal button from another neuron forms a synapse), allows many different neurons to converge on a single nerve cell, facilitating the coordination and integration of many complex messages. The number of dendritic spines can increase or decrease following exposure to psychoactive substances, thus altering communication between neurons, and most likely contributing to the behavioural and neurological effects of these substances (11, 17).

The simplified architecture of a synapse is illustrated in Figure 1. The presynaptic terminal contains vesicles, which are filled with neurotransmitters. Presynapse and postsynapse are separated by a narrow synaptic cleft into which the neurotransmitters are released from the vesicles via exocytosis(11, 17). Transmitters diffuse across the synaptic cleft and, after a lag period of about 0.5 milliseconds, bind to a receptor on the postsynaptic cell. The ion permeability of the postsynaptic membrane is changed causing a sudden change in the corresponding membrane potential. In neurons within the brain, this electric disturbance can induce an action potential, which will result in a change of mental state.

Many nerves are excitatory, however, the binding of neurotransmitters to inhibitory receptors on the postsynaptic membrane causes the opening of K^+ and Cl^- ion channels that hyperpolarise the membrane and thus blocks the generation of an action potential. Neuroreceptors are found at the post- and presynaptic membrane. Activation of presynaptic receptors usually leads to an inhibition of neurotransmitter release, whereas their inhibition results in an enhanced release of neurotransmitters.

Many types of neurotransmitters have been discovered so far, but in general there are three major broad categories: amino acid neurotransmitters, amino acid-derived neurotransmitters, and peptides (chains of amino acids). The amino acid transmitters include glutamate, GABA, glycine and aspartate (11, 17). The monoamines, norepinephrine and dopamine (catecholamines) and serotonin (indoleamine) are derived from amino acids. Large molecule peptide neurotransmitters are generally synthesized in the cell body, and transported along the axons to the synapse. Small molecule neurotransmitters can be synthesized in the terminals. The neurotransmitters will be discussed in more detail later with the plants and plant compounds with which they interact.

There are distinct regions of the brain where cell bodies for a specific neurotransmitter exist, and other regions or 'projections' where the axons from those cell bodies project to, and where the neurotransmitter is ultimately released. Thus, not every neurotransmitter is released in every area of the brain compartmentalizing specific regions of the brain to perform specific functions. The neurotransmitters and neuroreceptors are the basic elements for signal transduction in the synapses of the central nervous system and are therefore important targets of psychoactive compounds (17).

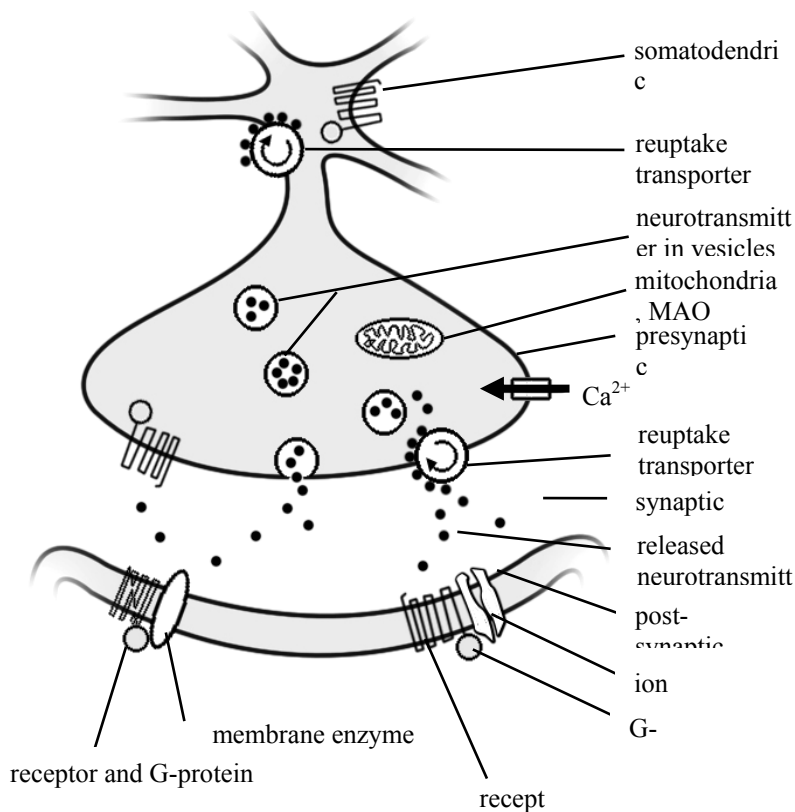


Figure 1. A simplified synapse

Numerous diseases or conditions are a result of this chemical neurotransmission process malfunctioning. The CNS is complex in both structure and function, making it particularly vulnerable to factors causing it to malfunction. This important and complex system within animals represents an important target area which is ideal for interference by defense chemicals. Indeed, many secondary metabolites in both plants and animals are known to effect neurotransmission and signal transduction (11, 17). Traditional medicine and many modern pharmaceuticals utilize these properties in an attempt to treat ailments of the CNS.

Psychoactive plants of African origin

African views of illness and treatment

Culture is the lens and guide we use in constructing, defining, and interpreting the world around us. Thus, people from different cultural contexts and traditions define and experience events (i.e. illness) in different ways. This

is particularly true of views about mental disorders and subsequently their diagnosis and treatment since these cannot be separated from cultural experiences. To determine which plants used in African traditional medicine may act on the CNS, an understanding of indigenous or traditional African views of illness and treatments are required. A brief interpretation follows, however more thorough treatments on African culture as a whole and specific regions are available (21-32).

In many traditional cultures, illness is believed to be caused by psychological conflicts or disturbed social relationships that upset an equilibrium which is expressed in the form of physical or mental problems (33). Although Africa has a diverse culture, there is a common thread that follows that the disruption of this equilibrium, what we call illness, may be caused by external psychological or spiritual factors, or both, that relate to African cosmology and "threaten the intactness of the person" (34). In traditional cultures, then, healing involves restoring this equilibrium.

In general, many African cultures group illnesses into three categories (32). Illnesses which have no discernible moral or social cause; these tend to be minor ailments such as rashes and colds. This is the only class of illness that occurs by chance, and for which causes are not sought (30). Some illnesses are considered 'modern diseases' which can be contracted by people anywhere in the world, and which are believed to be first introduced into Africa by European settlers (27). Lastly, there is the belief that there are diseases which only African people can contract, and to which all African people are vulnerable (23). A large majority of mental disorders fall under this last category. The 'epidemic' of *indiki* spirit possession in Zululand, South Africa from the 1890s to 1914 is an interesting example (35).

More importantly, African cultures define two types of causes for illnesses (21,32). Firstly, a proximate cause, which accounts for how a disease is contracted (30). Infection and contagion from pollutants are examples of proximate causes (26). Secondly, an ultimate cause, which accounts for why a disease is contracted by a particular person. A simple explanation by Green (27) clarifies, 'a mother may recognize that her infant has diarrhea because flies settled on and contaminating its food (proximate cause), but she will also want to establish who sent the flies to harm her child (ultimate cause)'. The material types of treatment (i.e. herbal remedies) are often considered of secondary importance and only complementary. The primary concern of African traditional healing is to discover who, or what has caused the imbalance or illness (i.e. the ultimate cause).

Three main types of ultimate cause are often raised in explaining illness, contact with pollutants (literal and/or spiritual), sorcery and ancestral punishment. Pollutants are considered to often originate in other people's bodies, and include semen, menstrual discharge, vaginal secretions, and blood (26). Death is also believed to be a pollutant (23). Since contact with pollutants cannot always be avoided, people fortify themselves from contamination by maintaining strict moral codes and observing protective rituals (27). Illness is often suspected to be inflicted by people who have been offended by a victim's behavior. Failure to honor filial obligations, violence, or other forms of uncooperative behavior risk creating a level of offence which elicits kin or

neighbors to seek redress through ‘witchcraft’ (36). The ‘survival’ of ancestors in the spirit world depends on them being accorded regular attention from living offspring (e.g. respect paid to *mizimu* in Uganda and *amadlozi* in South Africa). This attention is manifest in rituals, sacrifices, avoidance of taboos, and high standards of social behavior. Where these requirements are not met, illnesses can be sent as a warning or punishment (23).

Both proximate and ultimate causes require treatment if a disease is to be cured. Diseases manifest themselves not only in physical symptoms, such as fever or pain, but in mystical disturbances of the blood commonly described in terms of ‘impurities’ or ‘heat’ (26). To treat proximate causes and physical symptoms, people may consult medical personnel and/or traditional healers for appropriate remedies. However, treatment for ultimate causes must also be sought, and since these lie within the mystical or spiritual domain, it is believed that only traditional healers or diviners are capable of useful insights and therapies (37).

These cultural beliefs make it difficult for outsiders to understand and determine the use, in western terms, of many African medicinal plants. Literature on African medicinal plants is replete with references to plants used to counteract curses and appease the ancestors. For example the plant *Asparagus virgatus* (= *Protasparagus*) is known by the Zulu people of South Africa as ‘*iphinganhloya*’ which means ‘what suppresses the ill-omen or curse’ (38). Many of these plants are not ingested but are usually carried about on the person in the form of a protective amulet or charm (referred to as ‘*imfingo*’ Zulu culture) or administered by sprinkling onto the person or around the homestead (referred to collectively as ‘*intelezi*’ Zulu culture). These are assumed to have no physiological effect on the patient, but perhaps provide some ‘psychosomatic’ protection to the person. Some remedies such as those referred to as ‘*amakhubalo*’ in isiZulu which are plant materials, that are ingested, for protection from ‘evil’ and thus, may have a physiological effect on the user. It is therefore not only important to know the symptoms, including the proximate and ultimate causes, but also the method of administration of the plant material, many of which are often neglected in ethnobotanical literature.

A closer look at plants used in African traditional medicine reveals a large number of plants with potential psychoactive properties. One study in South Africa list 306 plants from 94 families with reported psychoactive uses in southern Africa (39). Thirty plants belonging to 21 families and traditionally used in southern Nigeria by herbalists for the management of mental disorders (including amnesia, insomnia and senile dementia) have been reported (40). Neuwinger (41) cites more than 1 750 potential psychoactive traditional African medicinal applications (**Figure 2**); the majority of treatments were aphrodisiacs and sexual stimulants. What is also of interest is the large number of treatments with potential CNS suppressant effects, for example 300 plants for treating epilepsy and convulsions alone. In addition to these there are plants to calm the insane, to treat insomnia, hysteria and tranquilizers (41).

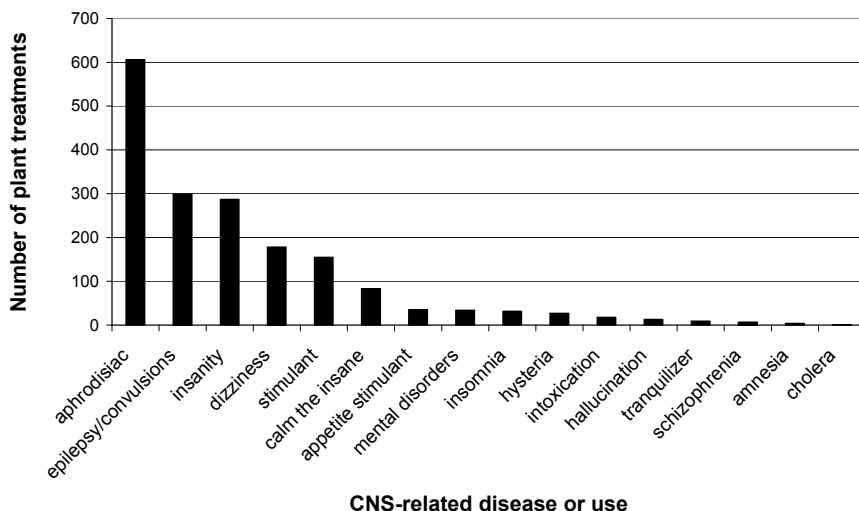


Figure 2. African traditional plant treatments with potential psychoactive properties based on their traditional uses reported in Newwinger (41).

There are surprisingly few traditional African treatments for memory loss (amnesia), to improve cognition or treat age-related neurodegenerative diseases like Alzheimer's and Parkinson's disease. Four species are reported to be used in southern Nigeria by herbalists for amnesia (40). These include *Boerhaavia diffusa* (Nyctaginaceae), the roots of which are reported to contain liriodendrin (lignan) a Ca^{2+} channel blocker (42). Decoctions of male inflorescences of *Carica papaya* (Caricaceae) with *Zingiber officinale* (Zingiberaceae) and *Pauridiantha viridiflora* (Rubiaceae) are also used to treat amnesia. *C. papaya* contains (*S*)-(-)-cotinine (pyridine pyrrolidinone) a major brain metabolite of nicotine and acetylcholine receptor agonist ($\text{IC}_{50} = 30 \mu\text{M}$) (42).

Plants and compounds with anticonvulsant, anxiolytic and sedative activity

Substances that slow or reduce brain activity, notably by a reduction in neuronal activity, are often referred to as CNS depressants. Central nervous system depressants are routinely used to treat anxiety, epilepsy and insomnia. They are also important in general anesthesia for major surgical operations.

Epilepsy is a symptom complex consisting of repeated unprovoked seizures. Seizures are classified according to the area of the brain in which the seizure originates; partial seizures and generalized seizures (43). During seizures some of the patients will experience convulsions. There are numerous causes for epilepsy (43). The link between abnormal electrical activity, as detected by electroencephalograph (EEG) and levels of chemical substance (such as neurotransmitters) is still relatively unexplored. However, abnormal activity of electrical impulses is often associated with epilepsy. Therapeutic use of antiepileptic drugs has focused on lowering Na^+ , K^+ , or Ca^{2+} flux in neurons,

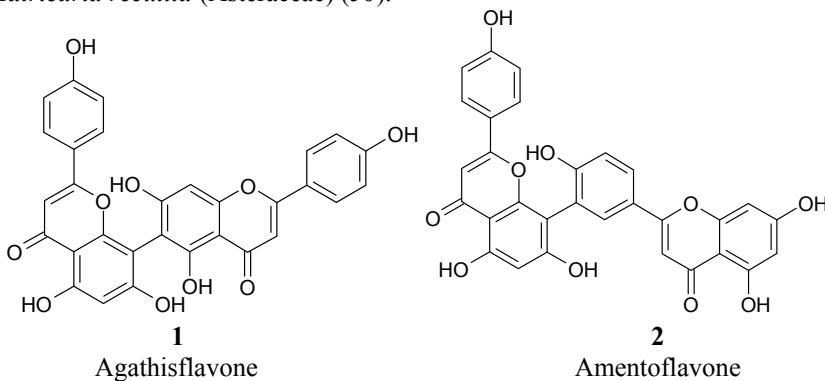
inhibiting glutamate neurotransmission, or promoting γ -aminobutyric acid (GABA) activity at Cl⁻ channels.

In many traditional Africa societies epilepsy in particular is thought to be due to possession by evil spirits (44), and is seen traditionally as a highly contagious (a pollutant) and shameful disease. It has severe social implications in African communities as it carries a stigma and sufferers are often shunned and discriminated against with respect to education, employment and marriage (45-50).

Overexcitement of the central nervous system, commonly results in mania, a collective term for symptoms that include frenzied or hyperactivity, lack of concentration, and irrational thoughts and behavior. Anxiety is also associated with overexcitement of the CNS, which greatly effects ones ability to function normally. The neurobiology and control of anxious states has been extensive reviewed (51).

A common group of anticonvulsant, anxiolytic and sedative agents are the benzodiazepines, which bind to the GABA_A-benzodiazepine receptor complex where they enhance the affinity for the inhibitory neurotransmitter GABA. A GABA stimulus on the GABA_A-receptor causes an influx of chloride ions into the cell. This influx causes hyperpolarization of the membrane, making it more difficult to generate action potential (52). As a result the neuronal impulse is inhibited, the CNS is suppressed and an anticonvulsant activity is achieved.

In an initial screening of southern African medicinal plants for compounds with an affinity for the GABA_A receptor benzodiazepine site several *Rhus* species showed interesting activity (53). It was later discovered that this activity was largely due to two biflavonoids (54). Agathisflavone [1] and amentoflavone [2] competitively inhibited the binding of ³H-Ro 15-1788 (flumazenil) with a Ki of 28 and 37 nM, respectively. Extracts of *Rhus dentata* and *R. petheri* were not as active as the extract from *R. pyroides*, this was found to be due the presence of amentoflavone only in *R. pyroides*, and the content of agathisflavone was higher in *R. pyroides* than in *R. dentata* and *R. petheri*. Amentoflavone [2] is found in a number of plants with CNS-related properties, including ginkgo (*Ginkgo biloba*) and St. Johns Wort (*Hypericum perforatum*) (55). The monomer apigenin, which was detected in *Rhus dentata*, only had a Ki value of 7.6 μ M. Thus, the biflavones are far more active than the monomer. Apigenin has also been detected in the flowers of chamomile, the popular herb *Matricaria recutita* (Asteraceae) (56).



The discovery of chrysin (5,7-dihydroxyflavone), and several other flavonoids shown to possess activity through interaction with the benzodiazepine receptor ($K_i = 4 \mu\text{M}$) (57), started the search for similar natural anxiolytics. Several flavonoids have been found to possess partial allosteric modulatory action at the GABA_A receptor complex, and can potentially play a role in the modulation of anxiety. These flavonoids therefore constitute a promising class of naturally occurring compounds for the treatment of anxiety. These naturally occurring flavonoids often bind to the benzodiazepine receptor with only moderate affinities. Through synthesis of chemical libraries and molecular modeling of the flavonoid binding to the benzodiazepine receptor pharmacophore, several research groups have been able to develop synthetic derivatives with higher affinities for the benzodiazepine receptor (58,59).

Several southern African plants have shown *in vivo* anticonvulsant activity against seizures produced in mice by pentylentetrazole, picrotoxin, bicuculline and *N*-methyl-DL-aspartic acid. However, the active constituents are yet to be identified. The plants include *Leonotis leonurus* (Lamiaceae) (60) which is reported to have narcotic effects and is used as a substitute for *Cannabis sativa* (61). The aqueous extracts of *L. leonurus* (400 mg/kg) protected against or delayed seizures induced by pentylentetrazole, picrotoxin and *N*-methyl-dl-aspartic acid, but did not protect against bicuculline-induced seizures. In a later study, the ethanol extracts of the three species of *Leonotis* had weak GABA_A-benzodiazepine receptor binding activity only at the highest concentration tested (10 mg/ml) (53) but the aqueous extracts were not active, suggesting that the anticonvulsant mechanism is not via GABA_A-benzodiazepine receptor.

Watt (62), one of the earlier researchers to recognize the potential of African plants in improving mental health, reported the use of *Cotyledon orbiculata* (Crassulaceae) leaves to treat epilepsy. Again, *in vivo* studies have demonstrated both aqueous and methanol extracts of *C. orbiculata* have anticonvulsant properties (moderate protection against pentylentetrazole, bicuculline, picrotoxin and *N*-methyl-dl-aspartic induced seizures in mice) (63). However, the ethanolic extract did not show *in vitro* GABA_A-benzodiazepine receptor binding activity (64).

Another study investigated a Northern Sotho remedy, *Sehlar sa Seebana*, for treatment of epilepsy. The recipe for this herbal remedy contains six plants, *Acrotome inflata*, *Aptosimum indivisum*, *Asparagus suaveolens*, *Barleria bolusii*, *Commiphora marlothii* and *Sesamum triphyllum*. Equal parts of the plants are placed in a red-hot clay pot and the patient inhales the smoke (65). Both aqueous and ethanol extracts of *Aptosimum indivisum* and *Asparagus suaveolens* and the aqueous extract of *Commiphora marlothii* showed good dose-dependent GABA_A-benzodiazepine receptor binding. Most of the plants have not been chemically investigated. Three metabolites: verbascoside, pinocembrin 7-neohesperidoside and shanzhiside methyl ester were isolated from *A. indivisum*. *B. bolusii* contains verbascoside, which is known to inhibit the GABA receptor, but did not show much activity (65,66).

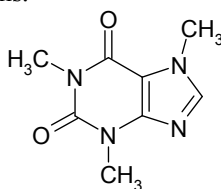
Although a large number of plants are used traditionally all over the world for the treatment of epilepsy and convulsions, there is still no substantial use of herbal products or constituents in western medicine that have been shown to be both efficacious and safe for this condition (10).

Plants and associated compounds which stimulate the CNS

The concept of using plants to help one stay focused and alert for long periods has a strong appeal. Stimulants are among the earliest psychoactive plants used by humans and are the most widely used psychoactive throughout the world today. There are strong cultural and social links with stimulant use and consequently, these plants have spread around the globe with the movement of people. Africa has adopted many customs of stimulant use from the numerous cultures that have settled on the continent. Betel chewing is one such practice, originating in southeast Asia where an estimated 200-400 million people are reported to use it (67). Betel is composed of the fruit or nut of the areca palm (*Areca catechu*, Arecaceae), the leaf of the betel pepper (*Piper betle*), and lime (calcium hydroxide). These plants can now be found through-out Africa. It is Africa that has made the largest contribution to stimulant use, coffee. The coffee plant, *Coffea arabica* originates from the highlands of south-west Ethiopia. The beverage consumed world-wide is produced from the seeds of mostly *C. arabica* but may contain other varieties (e.g. *C. canephora*, *C. liberica*).

Cola spp. (Sterculiaceae)

Caffeine [3] is possibly the most widely used psychoactive substance in the world. Caffeine is valued because of its stimulant-like behavioural activity on mood and performance. Caffeine is found in several plants which are widely known and employed throughout the world. Wherever these 'caffeine containing plants' were found, indigenous groups have recorded their mildly stimulant effects and have grown habituated to their use. The most well-known sources in contemporary western society are coffee, tea, caffeinated soft drinks, cocoa, chocolate and certain medications.



3

Caffeine

More traditional sources include *Ilex* and *Paullinia* species valued by the indigenous people of South American, whereas *Cola* spp. are an important social drug for West African peoples (68). Kola nuts have also played an important role in western Africa as a valuable commodity. The Yoruba farmers of western Nigeria recognize at least four kinds of kola nuts, which probably belong to three different *Cola* species (*C. acuminata*, *C. nitida* and *C. verticillata*) (3).

The main mechanism of action of caffeine is the antagonism of adenosine receptors (69-71). Adenosine decreases the firing rate of neurones and exerts an inhibitory effect on synaptic transmission and on the release of most

neurotransmitters, while caffeine increases the turnover of many neurotransmitters, including monoamines and acetylcholine (70).

Catha edulis (Celastraceae)

Khat, *Catha edulis*, is known throughout Africa by many names, e.g. qat in Yemen; in Kenya it is known as miraa; South Africa the bushman's tea, igqwaka (Xhosa) and inandinandi (Ndebele). The Shona of Zimbabwe call it mutsvahari. The spelling khat has been chosen here because it is the most widely used one in the literature but can also spelled qat, kat, cat, ghat or tchat.



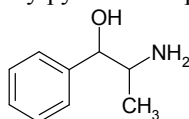
Figure 3. *Catha edulis* (khat), the two youngest leaves from fresh shoot tips are chewed in East Africa, the Middle East, including Ethiopia, Tanzania and North Yemen

The principal cultivation and production areas of *C. edulis* are in Ethiopia, particularly in Harar district, and in Yemen (72). It is also cultivated, to a lesser extent, on the slopes of Mount Kenya and grows wild in many mountainous parts of eastern Africa including South Africa, Uganda, Tanzania, Rwanda and Zimbabwe (72,73). The most debated historical issue about khat is whether it originated in Yemen and then spread to Ethiopia (74), or *vice versa* (75).

The psychotropic properties and use of *C. edulis* have been known for centuries in East Africa, the Middle East, including Ethiopia, Tanzania and North Yemen (8). The medicinal properties of khat for the treatment of depressive states, as an anorectic and stimulant are reported as early as 1237 by the Arabian physician Naguib Ad Din (76). However, khat use has largely lost its therapeutic aspects becoming a popular habit among several million people who consume khat daily because of its euphorogenic and pleasurable effects. Khat is reported to induce a clear anorectic effect (77), together with euphoria,

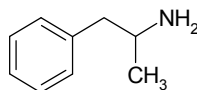
excitation and cheerful sensation (78,79). Among certain populations, particularly in Yemen, khat is mainly used in a social setting, and consumption of the drug has become part of the cultural tradition. The habit is socially sanctioned and even considered prestigious, with many houses having a special room devoted to khat chewing. The usual method of ingestion is chewing of the young leaves. Recently, use of this plant has reached other parts of the world (80). In southern Africa infusions of the leaves are used to treat coughs, asthma and other respiratory ailments (61).

These effects are produced mostly by phenylpropanolamines present in the leaves. These include cathinone [4] (*S*- α -aminopropiophenone), cathine [5] [(-)-1*S*, 2*S*-norpseudoephedrine] and (-)-1*R*, 2*S*-norephedrine (8). These substances have pharmacological properties similar to those of amphetamine [6] (81), as they induce the release and inhibit the uptake of dopamine and norepinephrine in CNS (82). In addition to the known phenylpropylamines, the presence of other amines such as merucathine, pseudomerucathine and merucathinone have been identified (83, 84). Cathinone, being a ketoamine base, is extremely unstable and, in particular, it can be transformed into (+)-norpseudoephedrine and (-)-norephedrine by an enzymatic reduction. It can also be oxidized to give 1-phenyl-1,2-propandione, while the cathinone dimers, such as 3,6-dimethyl-2,5-diphenylpyrazine are purely artifacts of the isolation (85).



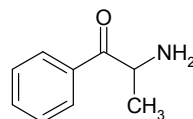
4

Cathinone



5

Cathine



6

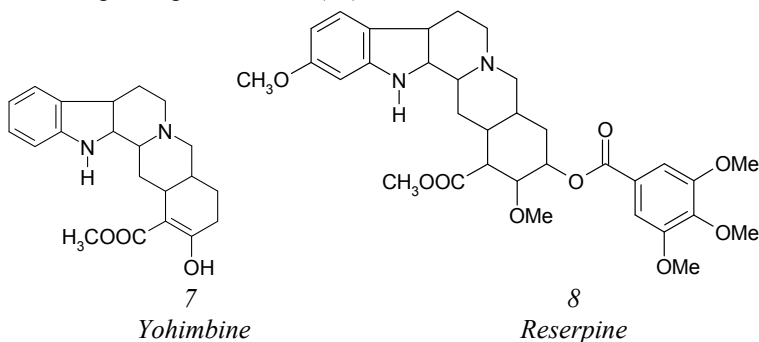
Amphetamine

Hartogiella schinoides (Celastraceae) leaves are reported to have similar stimulant activity to *C. edulis* when chewed (86). Leaves are chewed in southern Africa to relieve thirst, prevent fatigue and are reported to cause weight loss due to lack of appetite (61). Other members of the Celastraceae with psychoactive uses are *Maytenus senegalensis* and *M. heterophylla* which are used to treat epilepsy in Zimbabwe and East Africa respectively (87,88), however little is known about their chemistry.

Pausinystalia yohimbe (Rubiaceae)

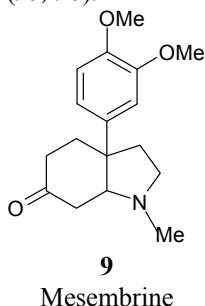
The stem bark of *P. yohimbe* (= *P. johimbe*; *Corynanthe yohimbe*), 'yohimbe' as it is known in Cameroon, Gabon and Congo is used traditionally as an aphrodisiac and stimulant to prevent sleep (41). The bark contains 1-6% of indole alkaloids, most of which are yohimbane-type alkaloids, the main one being yohimbine [7], which is structurally related to reserpine [8] (89). Yohimbine is a selective inhibitor of α -2- adrenergic receptors and, while at low dose it has hypertensive activity, at high dose it is hypotensive (vasodilation of peripheral vessels). It is the vasodilation of peripheral vessels, and especially vasodilation of the corpus cavernosum, which is the cause of the reputation of yohimbine as an aphrodisiac (90). Tests have shown, indeed, that increased

libido and easier ejaculation result from treatment with yohimbine. It is used with success in the treatment of erectile dysfunction (90). However, it is not sufficiently free from serious adverse effects (such as tremors, sleeplessness, high blood pressure and rapid heartbeat) and drug interactions, which warrant some form of prescription control (91).



Sceletium tortuosum. (Mesembryanthemaceae)

The mood elevating properties of *Sceletium tortuosum* have been attributed to mesembrine [9], an alkaloid with potent selective serotonin (5-HT) re-uptake inhibition activity (86). Other scelletium alkaloids as they are now referred to are mesembrenone, mesembrenol and tortuosamine. The use of mesembrine-type alkaloids was patented (92). Selective serotonin (5-HT) re-uptake inhibitors (SSRIs), like mesembrine, have become important treatments in the therapeutic management of depression (93). Several southern African medicinal plants, in particular Amaryllidaceae, have shown *in vitro* affinity for serotonin re-uptake transporters (94,95). Several active alkaloids have been identified from *Boophone* and *Crinum* species (95, 96).



Narcotic and hallucinogenic plants and their active constituents

Narcotic plants are used primarily to induce sleep and are used to treat insomnia and in anaesthesia (10). In South Africa the Zulu use the powder bark of *Tecomaria capensis* (Bignoniaceae) to make infusions that are said to induce sleep (39). There are many examples of plants used for similar purposes,

however, no research has been done to validate these. The pharmacology and chemistry of *Boophone disticha*, the bulb infusions of which are used to induce hallucinations for divinatory purposes, are well documented (16). Alkaloids isolated from *B. disticha* bulbs include buphanamine, buphanidine, buphanine, buphanisine, haemanthamine, nerbowdine, undulatine, lycorine, crinamidine, crinine, 3-*O*-acetylnerbowdine, ambelline, buphacetine and distchamine (95,97). Much of this research was stimulated by the toxic nature of this plant and the numerous poisonings and deaths that have resulted from its use.

Datura sp. (Solanaceae)

Datura stramonium, a naturalized toxic weed that is probably indigenous to tropical America but now widely distributed throughout the world including sub-Saharan Africa. The seeds are the most potent part of the plants, followed by the roots, stems, leaves, and flowers, and as few as ten seeds are sufficient for psychoactivity.

Atropine and scopolamine are found in jimson weed (*Datura stramonium*), nightshade (*Atropa belladonna*) and mandrake (*Mandragora officinarum*), and scopolamine alone is found in henbane (*Hyoscyamus niger*) (98), all of which are popularly grown as ornamental flowers. Dissociative rather than entirely hallucinogenic, both chemicals act as CNS depressants and competitively antagonize muscarinic cholinergic receptors. These two chemicals have considerable application in ophthalmology to dilate pupils (atropine), anaesthesia to decrease secretions and treat bradycardia, toxicology to treat organophosphate and nerve gas poisoning, and in emergency medicine for cardiac arrest (99).

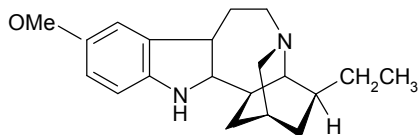
Scopolamine is also used as a treatment for motion sickness. In excess, these plants can cause a toxic delirium that may last hours to days, marked by amnesia, confusion, dissociation, hallucinations, delusions, euphoria, and sometimes episodes of bizarre self-injury (100).

The delirium caused by *Datura* is evident by the Afrikaans name, *malpitte* which translates as 'mad seeds' (86) and its Zulu name *iloyi* which is possibly derived from *-loya* which means to bewitch, or cast a spell on. In tropical West Africa, *Datura* spp. are used in native beer or in palm wine to add a stupefying or narcotic effect. A drink made from the seeds of *D. metel* is given as an intoxicant to Fulani youth to incite them in the 'Sharo contest' or ordeal of manhood (101).

Tabernaemontana iboga (Apocynaceae)

The Iboga people (Bwiti and Mbiri cults) living in Gabon and other nearby West African countries chew the roots of this plant in order to communicate with their ancestors (1,2). It is also used as a stimulant to keep hunters awake and motionless while stalking prey (2). European explorers in the 19th century, eating the roots had also stimulant and aphrodisiac effects and greatly increased endurance (102). Ibogaine [10] was isolated and identified in the beginning of

the 20th century (102) and at least 12 more related indole alkaloids have been isolated from the roots of *T. iboga* (Table 1) (42). Ibogaine, at low doses has primarily a stimulant effect, increasing alertness and reducing fatigue, hunger, and thirst (103). Pharmacological differences between Ibogaine and crude *T. iboga* extracts are slight, suggesting that the other indole alkaloids may play a role in the psychoactivity observed (for a review, see 102)



10

Ibogaine

Until the mid-1980s ibogaine had no apparent therapeutic value. Interest was stimulated with the filing of a patent for ibogaine treatment of opiate dependence. Four other patent filings followed in rapid succession for treatment of cocaine, amphetamine, alcohol and nicotine/ tobacco dependence syndromes (8,2). Current research has focused on 18-methoxycoronaridine, a synthetic derivative of ibogaine (104-106).

Ibogaine is only one representative of a class of complex indole alkaloids that are particularly abundant in the Apocynaceae family, and it is likely that other naturally occurring alkaloids with related structures will also exhibit interesting CNS activity. *Tabernaemontana* and *Voacanga* species are known to possess a similar chemistry to *Tabernaemontana* species (42). There is evidence that certain *Voacanga* species are used as stimulants in Africa (107). *V. africana* is used in Cote d'Ivoire to treat convulsions in children and madness (108). The stem bark of *V. bracteata* has a 2.46 % alkaloid content (109), although these alkaloids are chemically related to ibogaine, there is no evidence that they are psychoactive. Voacangine is also present in *Tabernaemontana coffeoides*, which is used as a stimulant in Madagascar (107).

Table 1. Biological activity of *Tabernanthe* alkaloids (adapted from Polya (42))

Compound	Biological activity
Tabernanthine (= 13-Methoxy- ibogamine)	Benzodiazepine receptor agonist (flunitrazepam displacement ($IC_{50} = 150 \mu M$), NMDA-Glutamate-receptor antagonist ($K_i = 11 \mu M$), $\sigma 1$ -receptor ligand ($IC_{50} = \sim 1 \mu M$) and $\sigma 2$ -receptor ligand ($IC_{50} = 0.2 \mu M$), Opiate (κO)-receptor ligand ($K_i = 0.2 \mu M$), μO -receptor ($IC_{50} > 100 \mu M$;), δO -receptor ($K_i = 3 \mu M$),
(\pm)-Coronaridine (= Carbomethoxy- ibogamine)	NMDA-Glutamate-receptor antagonist ($K_i = 6 \mu M$), Opiate (μO)-receptor ($K_i = 3 \mu M$), δO -receptor ligand ($K_i = 8 \mu M$), κO -receptor ligand ($K_i = 4 \mu M$), Voltage-gated Na^+ channel antagonist ($K_i = 16 \mu M$) cytotoxic, diuretic and oestrogenic
Ibogaine (=12-Methoxy- ibogamine)	5HT ₃ -receptor ligand ($IC_{50} = 4 \mu M$), Dopamine and 5HT re-uptake transporter inhibitor ($IC_{50} = 4$ and $0.6 \mu M$ respectively), Dopamine receptor (D1) ligand ($IC_{50} > 10 \mu M$), Dopamine receptor (D2) ligand ($IC_{50} > 10 \mu M$), NMDA-Glutamate-receptor antagonist ($K_i = 1 \mu M$), $\sigma 1$ -receptor ligand ($IC_{50} = 9 \mu M$) and $\sigma 2$ receptor ligand ($IC_{50} = 0.2 \mu M$), Opiate (κO)-receptor ligand ($IC_{50} = 25 \mu M$; $K_i = 2 \mu M$), μO -receptor ($K_i = 4 \mu M$), δO -receptor ($K_i > 100 \mu M$), Adenosine-receptor (subtype A_1) ligand, Muscarinic acetylcholine-receptor ligand, $\alpha 1$ -Adrenergic-receptor ($IC_{50} = 7 \mu M$), Voltage-gated Na^+ channel antagonist ($K_i = 9 \mu M$), Anticonvulsant, hallucinogen, inhibits morphine dependence, anti-addictive, increases synaptic 5HT
Noribogaine (=12-Hydroxy- ibogamine) Metabolite of Ibogaine	Dopamine receptor (D1) ligand ($IC_{50} > 10 \mu M$), Dopamine receptor (D2) ligand ($IC_{50} > 10 \mu M$), Opiate (κO)-receptor ligand ($K_i = 4 \mu M$), μO -receptor ($K_i = 0.2 \mu M$),
Ibogamine	NMDA-Glutamate-receptor antagonist ($K_i = 6 \mu M$), $\sigma 1$ -receptor ligand ($IC_{50} = \sim 1 \mu M$) and $\sigma 2$ receptor ligand ($IC_{50} = 0.1 \mu M$), Opiate (κO)-receptor ligand ($K_i = 3 \mu M$), μO -receptor ($K_i > 100 \mu M$), δO -receptor ($K_i > 100 \mu M$), Voltage-gated Na^+ channel antagonist ($K_i = 8 \mu M$), Brachycardiac activity, cytotoxic and hypotensive
Tubotaiwine	Adenosine-receptor (subtype A_1) ligand. Opiate-receptor ligand ($K_i = 2 \mu M$) Analgesic (mouse abdominal relaxant)

Members of the Apocynaceae worthy of further investigation are *Acokanthera oppositifolia* which is administered to treat convulsions and fits

(110). *Acokanthera* species are well documented as arrow poisons (cardiac glycosides), and are used extensively in Africa (111). Another source of poisonous cardiac glycosides for arrows are *Strophanthus* species. Some species (possibly *Strophanthus gerrardii* and *S. petersianus*) known to the Zulu of South Africa as 'ubuhlungubendlovu' or 'pain of the elephant' are reported to be used to treat hysteria (112), however no details are given as to how the plant is administered. Both *Acokanthera* and *Strophanthus* are also used as ordeal poisons in East Africa (113).

Conclusions

The African flora is indeed rich in psychoactive substances. There is clear evidence from ethnobotanical surveys and screening programs for CNS-related activity to support this. Africa's indigenous people have in the past and to a large extent continue to utilize these plants for cultural, medicinal and recreational purposes. As is the case in many areas concerning African traditional medicine, there is still much research required, to determine the active constituents, their mode of action and safety. Due to the supernatural properties attached to these plants by many African cultures it is often more difficult to identify psychoactive plants based on their traditional uses. The ethnobotany of many African cultural groups is poorly documented, and requires urgent detailed research before this valuable knowledge is lost. A better understanding of the indigenous or traditional interpretations of mental illness and treatments, especially on the use, preparation and administration of these plants, will help in deciphering the science behind their utilization and will potentially provide new candidates for the development of modern drugs

The chemistry and pharmacology of most African psychoactive plants are unknown. The safety and validation of these plants continues to be assessed with promising outcomes. There remains many opportunities where advances in this field could provide a better understanding of African traditional medicine and the mode of action of these phytochemicals.

Acknowledgements

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Chapter 19

Quality and Safety of African Medicinal Plants

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The trade in African medicinal plants is poorly regulated. The regulation includes three fundamental aspects: quality, safety and efficacy. The adverse effects of African traditional medicines are not well documented in the literature. Examples and case studies illustrate the current knowledge and future challenges relating to African traditional medicine. Pre- and post harvest factors which influence the safety and quality are discussed.

Approximately 70% of Africa's population relies on traditional herbal remedies to meet their primary health care needs (1,2). However, with little monitoring or few control systems in place, there is concern that certain aspects of health can be compromised. A general misconception is that medicinal plants are "pure" and "natural" which equates to "harmless". Based on their long history of use, users of traditional medicines deem them safe for human consumption. However, the absence of their regulation provides no such guarantee, and those plants known to contain such pharmacologically active substances to induce physiological changes in humans that lead to the cure and/or mitigation of a disease or illness may also have side effects that could lead to adverse responses as well (3). A number of African medicinal plant species, from a variety of plant families, have revealed genotoxic effects (3-8). Reliance on plants collected from the wild could cause not only a threat to medicinal plant biodiversity and population stability but it now also raises concerns relative to possible contamination from the environment due to anthropogenic activities such as industrial encroachment which has led to contamination of water tables and soil with pesticides, industrial chemicals, heavy metals and microbial contamination. Furthermore, potentially harmful

contaminants, either as a result of improper cultivation methods, harvesting or storage practices, result in degradation in quality. Assessing scientific validity of plant material is vital in the affirmation of plant efficacy.

The objective of this paper is to discuss factors which influence the safety and quality of medicinal plants and to provide insights on the current knowledge and future challenges of African medicinal plants.

Biological assays in validating the use of traditional medicines

To regulate the practice of traditional medicine, there is a need to establish whether there is scientific validation for the use of certain medicinal plants (9). *In vitro* tests, commonly known as biological assays, are used worldwide to demonstrate the pharmacological value of traditionally used plant parts. Examples of such assays include antibacterial, anti-inflammatory, antifungal, anthelmintic and anti-amoebic, antischistosomal, antimalarial, antioxidant and assays to test plants used for mental illnesses (10). However, there has been criticism regarding the lack of uniformity (single dose studies, no proper controls or repetition of simple bioassays for another extract or plant) for the various bioassays (11-13) and even the lack of defined or standardized plant materials and/or their respective extracts. “Too many papers are published which extrapolate results from *in vitro* tests to claim *in vivo* activity and efficacy without taking into account biopharmaceutical factors, traditional methods of making actives, treatment of the extract prior to administration, the effect of other added substances or the dose showing activity” (11). The Journal of Ethnopharmacology has issued clear guidelines regarding the criteria for high-quality ethnopharmacological research (13) and reviews on methodology are encouraged to advance the quality of research (12). Many external factors affect the reliability and legitimacy of the assay results which researchers should take into consideration namely seasonal impacts, plant age, plant part and location (9). The sections below discuss the influence of several pre- and post harvest factors on the biological activity of select African medicinal plant species.

Important pre-harvest aspects influencing safety and quality

The World Health Organization (14) has issued a set of guidelines for good agricultural and collection practices (GACP) for medicinal plants promoting sustainable cultivation, which conserves both the medicinal plant and the environment. Thus far, only the European Union and a few other countries, including China and Japan, have developed regional and national guidelines for good agricultural and collection practices for medicinal plants (14). Such guidelines are regulated and monitored to ensure that the proper plant material is identified for collection and/or cultivation and to ensure soil and irrigation water are within the limits or free from potentially harmful substances such as heavy metals, pesticides and herbicides. Such guidelines help put into place the documentation and traceability of the medicinal plants that enter into regional and international trade.

Unfortunately, environmental monitoring in Africa is limited, since restricted resources compel African governments to focus on immediate and urgent health concerns such as malnutrition and infectious diseases (15).

Wild harvesting vs. cultivation

Population increase has led to an increase in demand for a variety of medicinal plant species (16). Sustainable cultivation systems have been suggested to overcome the over-harvesting of certain wild species (2,17). In Europe, China and India, the cultivation of medicinal plants has been put into place to meet the demands of the people, but the most common practice in Africa is still to collect medicinal plants from the wild (18). Although cultivated medicinal plants are acceptable as an alternative to wild plants in some African countries, namely Ghana, South Africa and Swaziland, the more conservative traditional healers in South Africa and Botswana, for example, believe that cultivated medicinal plants will not have the same medicinal properties as those collected from wild populations (2). This is not scientifically unsound as harsh natural environmental conditions may influence secondary metabolite production which may not be expressed under mono-culture conditions (16). Many of these secondary metabolites are stress inducible so that abiotic and biotic stress conditions have often resulted in higher concentrations of such bioactives, though this also appears to be specific to the plant species, bioactive compound and actual environmental condition (19,20).

Researchers must bear in mind that a deviation in harvest sites can have a pronounced effect on biological activity (21) as secondary metabolites adapt to fluctuating environmental conditions such as temperature and light (e.g. antioxidants), stress (e.g. proline), infection (e.g. flavanoids) and herbivory (e.g. alkaloids) (22).

Effect of agricultural practices on phytochemical yield

According to WHO GACP (14), soil should contain suitable amounts of essential elements and organic matter for optimal plant growth and quality. Essential elements are involved in the structure of certain secondary metabolites yet can also have adverse effects on their regulation thus, optimizing nutrient content is a key factor in the quality of medicinal plants (23).

The use of fertilizers is often essential in order to obtain a large yield. Nitrogen supply to *Hypericum perforatum* L. (St. John's Wort) (Hypericaceae) plants had a profound impact on the levels of phytochemicals in the leaves. Decreasing the nitrogen levels resulted in an increase in secondary metabolite yield amounting to 2.4 to 3.3 fold (24). Likewise, a lower nitrogen concentration significantly increased hypoxide levels in *Hypoxis hemerocallidea* Fisch.Mey & Ave-Lall. (Hypoxidaceae) (25), a popular medicinal plant recommended to treat HIV/AIDS. Thus, elemental ratio in soils directly influences phytochemical yield therefore impacting on quality.

In addition, the sensitivity/tolerance of wild medicinal plants to essential and non-essential elements need to be established. A recent study indicated that the maximum permissible concentrations for trace elements and heavy metals in South African agricultural soils may be too high for the growing of wild medicinal plants (26).

The assessment of heavy metal tolerance would not only allow for maximum growth and survivability for the plants but would also indicate potentially harmful accumulatory species.

The use of agrochemicals to improve the growth of or to protect medicinal plants should be applied only when required and only approved plant protection products (such as pesticides and herbicides) should be used at the minimum effective concentration (14). The investigation into the biological activity of *Cyrtanthus suaveolens* Schönland (Amaryllidaceae), a medicinal plant widely used in Southern Africa, lead to the isolation of Captan, a commercial pesticide with known mutagenic, genotoxic and teratogenic activity (27).

Consequently, the use of greenhouse hydroponic cultivation has been suggested for the cultivation of medicinal plants (9). Controlled growth environments would ensure that the plants are grown under contaminant free conditions. This would result in not only better quality but also consistency (9).

Seasonal variation

Traditionally, wild ginger (*Siphonochilus aethiopicus* (Schweinf.) B.L. Burt (Zingiberaceae), is harvested during winter time as summer gathering is believed to cause storms and lightning (2). Investigations into the seasonal senescence of *S. aethiopicus* showed that the time of harvest had only a minimal influence on antibacterial and anti-inflammatory activity (28).

Seasonal variation in biological activity is an important aspect and work has been conducted in several popular South African medicinal plants (19, 28-30). A study on the effect of age, season and growth conditions on the anti-inflammatory activity of *Eucomis autumnalis* (Mill.) Chitt. (Hyacinthaceae) showed considerable differences between plants harvested before and after the growing season with the highest COX-1 inhibitory activity shown shortly before the onset of dormancy (19). The age of the plant affected COX-1 inhibition, with young plants having large amounts of COX-1 inhibitory activity, particularly in leaves. However, as the plants matured, more activity was associated with the underground plant parts (bulb and root) (19).

Collections of *Harpephyllum caffrum* Bernh. (Anacardiaceae) bark from the same female tree at different times of the year showed the highest antibacterial activity in summer, suggesting that the bark be collected during warmer months (29).

Such information is imperative in maximizing phytochemical yield.

Plant part substitution

Plant part substitution is an important conservation approach especially when it comes to slow growing endangered species. However, phytochemicals often differ between plant parts of the same species. A study on *Crinum macowanii* Bak. (Amaryllidaceae) showed significant organ-to-organ variation in the alkaloid distribution (31). Further work on four important medicinal plants of Southern Africa using TLC-analysis showed significant differences between plants (18): *Eucomis autumnalis* (Mill.) Chitt. subsp. *autumnalis* (Hyacinthaceae) revealed significant differences between plant parts, *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae) showed similarities in root and rhizome extracts as well as in leaf and stem extracts, and high similarity was seen in leaf and bark extracts for *Ocotea bullata* (Burch.) Baill. (Lauraceae) and *Warburgia salutaris* (Bertol.f.) Chiov. (Canellaceae).

Thus, although plant part substitution may be important for certain species, investigation into phytochemical distribution of individual plants needs to be conducted and quantified. Phytochemicals accumulate in specific cells or organs and it is therefore important to identify, characterize and protect these structures (23). For regional and international trade, and according to the GACP WHO, the specific plant part should be both identified, and authenticated in the study and/or trade of medicinal plants.

Traditional healers in KwaZulu-Natal, South Africa, utilize the bark of various *Cryptocarya* species (Lauraceae) as a substitute to the rare *O. bullata*. An investigation into the anti-inflammatory activity of bark extracts proved *Cryptocarya* species to be superior to *O. bullata* (32). As the COX-2:COX-1 inhibition profile of *C. woodii* and *C. wyliei* extracts was remarkably good, the isolation of the active compounds in both species is underway (32). Such studies are vital in the conservation and optimal utilization of scarce resources and endangered species.

Important post harvest aspects influencing safety and quality

Drying and Storage

Correct drying and storing of medicinal plants is imperative in preventing the deterioration of quality. By correctly reducing moisture content, metabolic activity can be slowed, and the medicinal plants less prone to depreciation (33). “While it is generally accepted that medicinal plants deteriorate after harvesting, very little is understood of the process and its effect on the chemistry of the plant” (34). Drying temperature is critical as volatile constituents (such as essential oils) will be depleted or lost at high temperatures (35). In addition, inadequate drying may result in mould and decay (35). In Southern Africa, plant material that is dried (bark and roots), or has an extensive shelf-life (bulbs, seeds and fruits) represents a large portion of the traditional medicinal markets (2). “It is speculated that leaf material generally has a shorter shelf-life than bark, roots and other underground storage organs. It is assumed that due to the fact that

bark, roots and other underground storage organs store assimilates and secondary metabolites that these compounds are relatively stable and these organs due to their structure (i.e. lower surface area to volume ratio) are better suited to protecting compounds from degradation than are leaves” (36). In Lesotho, traditional healers and herbalists generally air-dry their plants at room temperature and store them for up to five years (37). An investigation on storage effects of medicinal plants indigenous to Lesotho (38), revealed antibacterial activity of leaves and roots of *Malva parviflora* L. (Malvaceae) initially increased with storage of 3-6 months yet after 12 months no activity was detected. On the other hand, root extracts of *Asparagus microraphis* (Kunth) Baker. (Asparagaceae) retained their high anti-inflammatory (COX-1) activity throughout the 12 month experiment. The effect of storage (90 days, 1 year and accelerated aging with elevated temperature to 55 °C and 100% humidity) on biological activity was carried out on 9 frequently used medicinal plants of South Africa. On the whole, antibacterial activity was retained while COX-1 inhibition activity was lost rapidly (36). Thus, the shelf-life is species-specific and no assumptions can be made with regards to phytochemical stability (36). Furthermore, the determination of shelf-life is vital and will allow healers to make informed decisions when using stored plant material (38). The timeous disposal of medicinal plants with unstable compounds will contribute to the quality and efficacy of these plants. Contribution by researchers in sharing these types of data with the traditional healers can lead to an improved understanding by both groups and improve the overall quality and agreements as to acceptable practices.

Misidentification

Medicinal plants collected in the wild may be contaminated by other species or plant parts through misidentification which may have perilous consequences (14). Poisoning from traditional medicines is frequently a consequence of misidentification (39). The relocation of a Zambian tribe to a nearby re-settlement area led to an epidemic of poisoning and fatalities as commonly utilized edible plant species were misidentified and poisonous species were accidentally ingested (40). The users of traditional medicine in South Africa are reliant on sellers who are insufficiently equipped to supply the correct plant species, thus compromising the wellbeing of the patient (10). *Jatropha curcas* L. (Euphorbiaceae), a medicinal plant frequently associated with poisonings in South Africa (41), was the cause of 11% (50) of the total number of acute poisonings (42) admitted to Ga-Rankuwa Hospital (Pretoria, Gauteng) due to accidental ingestion of the seeds – all cases were children (42). Many traditional plants have various common or vernacular names which could lead to confusion and misidentification (43). In addition, plant-based products are rarely labeled but bulk stock may be identified by basic signage indicating a local vernacular name (10). Dispensed plant material is usually packaged in newspaper or plastic. Furthermore, taxonomically reliable characters may be lost during processing such as drying and/or grinding of plant material into powders thus rendering medicinal plant products particularly difficult to identify (10). Thus, an added

advantage of controlled cultivation is that it avoids the risk of incorrect identification.

Microbial contamination

Microbial contamination of agricultural produce is a major problem in the tropics and subtropics (44). Contamination of medicinal plant material is alarming and products need careful assessment before being sent to the drug and/or food industry (44). Compared to synthetic materials, materials of natural origin have a tendency to contain a much higher level of microbial contamination (43). A study on African herbal teas showed samples containing a high microbial count, coupled with a large variation in quality (color, aroma, flavor and taste) (45). "In general, hygiene quality, indicated by the absence of pathogens in at least 10 grams of product and a low level of microbial contamination (i.e. low total aerobic mesophilic, endospore and enterobacterial counts), should be the goal for each producer or trader in medicinal plants" (46). Furthermore, the consumer of medicinal plants is typically not in good health which means that higher risk levels and hazard classes have to be taken into account (46). The presence of yeasts and moulds in medicinal plants can cause opportunistic infections in humans which may be more significant in immunosuppressed HIV patients (47). A recent study on South African traditional remedies and plants recommended for the treatments of HIV/AIDS revealed that the high bacterial and fungal content indicated low environmental sanitation and low processing standard during the preparations of the herbal medicines (47).

Intentional adulteration and herbal-drug interactions

The deliberate addition of a biologically active substance to a plant preparation (known as adulteration) has become a major issue in the United States and elsewhere (48). Adulteration may be both purposeful and accidental. It is a common type of misconduct used to enhance the efficacy of the herbal products (43). Adulteration of herbal remedies by synthetic therapeutic substances is well documented (49-51). Until recently there had been no reports of deliberate adulteration of traditional African herbal remedies (52). The first such study reports two separate incidences in which South African traditional remedies were adulterated with western pharmaceuticals causing severe toxicity (52).

Such adulteration is perilous owing to the unanticipated drug-herb interactions that may arise (43). Recently, two African medicinal plants, *Hypoxis hemerocallidea* (African potato) and *Sutherlandia frutescens* (L.) R.Br. (Fabaceae) often recommended for treatment of HIV/AIDS, showed a negative interaction with antiretroviral medication, thus patients may be at risk from treatment failure, viral resistance or drug toxicity (53). Consequently, even when these interferences do not actively cause morbidity and mortality, they can weaken the therapeutic value of concomitantly administered conventional drugs

(54). Unfortunately, the overall impact on public health is an aspect of ethnopharmacology which is seldom considered (48).

Heavy metal contamination

Heavy metals are a known contaminant or adulterant of many traditional plant-based remedies (50,51,55,56) with clinical manifestations widely documented (57,58).

The maximum permissible levels in raw materials used in traditional medicines as set by the World Health Organization (59) are as follows: cadmium and lead; 0.3 and 10 mg/kg, respectively. However, as few African countries have formal monitoring systems, quality control is non-existent and the testing and screening of medicinal plants for heavy metals is costly. Several attempts have been made to determine the heavy metal content in African medicinal plants (55, 60-63). In Nigeria, a study revealed that certain herbal remedies contained excessive levels of iron, nickel, cadmium, copper, lead, selenium and zinc, sufficient to cause adverse health conditions when taken as recommended (55). In South Africa, traditional herbal remedies which give rise to severe renal pathology may be related to heavy metal toxicity (63). The metals most frequently implicated in South Africa are arsenic, chromium and magnesium (58).

Several possibilities exist to explain the presence of heavy metals in medicinal plants and plant-based remedies thus strict monitoring through plant growth stages to the finished product is vital.

Trading sites - traditional markets and medicine shops

In spite of rapid urbanization in Africa, traditional medicines are still sought after and extensively used in cities (64). Informal markets are commonly situated in the hub of the city centre allowing easy public access to commuters but also placing plant material in contact with various kinds of urban pollutants. In addition, plant material may be exposed to microbial and insect attacks as well as fluctuating light and temperature (36) (Figure 1). The lack of storage facilities and trading infrastructure results in the spoiling of raw plant material (65). A survey on rural clinic patients (n=100) in South Africa revealed that 83.7% would favor more hygienically packaged traditional medicines. As the market conditions are poor, most consumers indicated the need for more modernized and hygienic trading sites (65).

Fumigation against pests is a common practice in traditional medicine shops. However, shop owners do not appear to be concerned about the effect of potentially toxic residues which could contaminate the plant material (34).



Figure 1. Informal street market, Pietermaritzburg, South Africa

Poisonings caused by Traditional African remedies

Significant risks are associated with the improper use of medicines in all healthcare systems (10), since poisoning from African traditional medicines is common (10,66). The occurrence of liver lesions in children of East Africa has been traced to widespread use of *Crotalaria* in treating measles (1). The extensive use of *Crotalaria*, *Cynoglossum* and *Scenecio*, have been implicated in the occurrence of liver and kidney diseases in certain parts of Ethiopia (1). Acute renal failure is one of the most severe consequences resulting from the use of traditional herbal remedies (67,68). Although the Southern African plant *Urginea sanguinea* Shinz (Hyacinthaceae) is well-known for its toxic effects on livestock, it is widely administered by traditional healers (69). *Urginea sanguinea* contains potentially harmful cardiac glycosides (69). Autopsies (41) from different areas in South Africa were carried out where death was presumed to have been caused by traditional medicine (70). The presence of cardiac glycosides was found in 44% of the patients.

Another important group of plant toxins are pyrrolizidine alkaloids (PAs) (71) of which even a single administration can be fatal (72). Poisonings by PAs are prevalent in Southern Africa (73), with a number of cases recorded for children (74).

Recognizing toxic symptoms is imperative for immediate medical action to be taken. However, toxic medicinal plants are sold by herbalists with only a general knowledge of toxic effects and symptoms based on patient observation (75). In South Africa, eight patients were admitted to the intensive care unit after admitting to consulting a traditional healer 48 hours before. Six out of the eight patients died (64).

In Mali, traditional practitioners view diarrhea and vomiting as common signs of medicinal plant intoxication (75). A South African based study on patients (n=103) admitted to hospital with a confirmed history of traditional remedy use showed the most frequent clinical features to be dehydration (51%), vomiting (46%), jaundice (40%), diarrhea (39%), altered mental state (37%) and oligoanuria (30%). Renal dysfunction was present in a large number of patients (76%) and liver dysfunction in 48%. The overall mortality was 34% (76). Thus prevention and treatment of poisoning by traditional plant-based remedies should be given high priority with regards to the regulation of African traditional medicines.

Case studies of poisonings - hospital records:

Case study 1 – South Africa

Over a 12 month period (1981 – 1982), 18% (50) of the 277 Black patients admitted to Ga-Rankuwa Hospital (Pretoria, Gauteng) with acute poisoning was caused by traditional medicines. A large number (32%) of the patients stayed in hospital for longer than 6 days. A total of 15 patients died, 13 of them from acute poisoning from traditional medicines (77).

A more comprehensive study at the same hospital between 1981 – 1985, (78) revealed that poisoning with traditional medicines resulted in 16% of the total number of patients (n=1306) being admitted for acute poisonings. In 15% death was due to traditional medicine. All of these were accidental. The most frequently encountered symptoms included vomiting, diarrhea and abdominal pains. The lungs, liver and central nervous system were commonly affected. Most of the traditional medicines (83%) were bought from a traditional healer while 12% was bought from traditional medicine shops. The patients (83%) took the traditional medicines orally, while in 11% of cases it was administered as an enema.

Enemas are more commonly used for administration of traditional medicine than emetics in the case of children. However, medical staff consider enemas to be the cause of some poisoning cases and liver damage seen in hospitals (79).

A study comparing the number of patients admitted to Ga-Rankuwa Hospital (Pretoria, Gauteng) for acute poisoning based on five categories of poisoning (paraffin, pesticides, drugs, plants and traditional medicines) over three time periods (66) revealed that the number of deaths from traditional medicine poisoning decreased from an average of six per year (1981-1985) to one per year (1996-2000) (Table 1).

Table 1. Numbers (and percentages) of admissions due to acute poisonings from Traditional medicines at Ga-Rankuwa Hospital, Gauteng, South Africa (Modified from 66).

Year	Total no. of poisonings	Poisonings from Traditional medicines	Deaths due to Traditional medicine poisonings
1981-1985	1164	204 (17.5)	31
1987-1992	3394	313 (9.2)	42
1996-2000	2067	98 (4.7)	5

Case study 2 -Zimbabwe

A retrospective study of poisoning admissions to the six major referral hospitals in Zimbabwe over a 10-year period (1980-1989) revealed 6018 cases. The main cause (23%) was from acute poisoning by traditional medicines. Total mortality was 15%, with traditional medicines being the second highest cause of fatalities (80).

A later study on eight referral hospitals over a 2-year period (1998-1999) showed the pattern had changed over the last decade and increased pesticide and pharmaceutical poisonings were higher when compared with traditional medicine poisonings (81).

Future prospects and limitations

The number of plant extracts assayed and compounds identified from the screening of African medicinal plants emphasizes the prospective areas for future work (10). It will be a long time before a functioning system will be put into place. Routine screening for toxicity, efficacy and stability of medicinal plant compounds considered for drug development, should also support screening programmes to provide a conclusive understanding of the effect of these plants on human health (10). An aspect of ethnobotanical research in Africa which draws criticism is the insufficient transfer of relevant findings to the community of medicinal plant users (34). Unfortunately, researchers are not conscious of the importance of relaying their findings to the public (72). Scientific findings need to filter through to the consumers of traditional medicines, and just as importantly, consumer reports on adverse reactions should be accepted as a serious source of information, highlighting current and relative problems. The above mentioned case study on poisonings by *Jatropha*

curcus concluded that based on their findings, an educational poster campaign was underway (42). This type of positive action needs to be encouraged.

Information regarding adverse reactions to traditional medicines need be accessible to the public - consumers, manufacturers, scientists and medical practitioners. A comprehensive database of toxic plants and adverse reactions could speed up investigations ensuring that well-informed decisions are made to treat the first signs of poisoning. Correct analysis of data is imperative and an accurate representation of data needs to be put into proper context (66). Such data should help to identify trends in poisonings, such as seasonal poisonings when certain plant parts are more readily available.

WHO (82) has a set of guidelines for safety monitoring of herbal medicines which urges the development of national and regional guidelines/policies. Berger (54) states that the Traditional Health Practitioners Act of 2004 sends a “confusing and unscientific message” from the South Africa’s Department of Health. “While the document is long on rules and procedures it is extremely short on substantive statements that set out standards of skill, knowledge and training, or that address the safety and efficacy specifications required of materials and methods used in the practice of traditional medicine”.

In order to advance the various aspects of the current extensive African medicinal plant trade much work needs to be done. This would not only ensure a product of quality and safety for the consumer, but also ensure that medicinal plant species are utilized in a sustainable manner.

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Chapter 20

Voacanga africana: Chemistry, Quality and Pharmacological Activity

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This review examines the natural products in *Voacanga africana*, a small tree, whose seeds are the most economically important plant part though in traditional medicines the bark and root are also widely used. *Voacanga* is used to treat or cure a wide range of diseases in Africa. This paper also discusses the botany, chemistry and pharmacological properties and presents current and potential applications of this species. The alkaloids are the principal bioactive compounds responsible for its use in traditional medicine. The major alkaloids include voacamine, voacangine, vobasine, and ibogaime. Others include voacristine, voacamidine, voacarine, voaphylline, vobtusine, voalfolidine and tabersonine. With over 1,600 tons of seeds exported annually from Ghana and Cote D'Ivoire, *voacanga* is an important source of additional income for harvesters and exporters in West Africa.

Introduction

Voacanga africana is a small tree or shrub, reaching up to 6 meters in height with a low widely spreading crown. The leaves are opposite, obovate and acuminate. They are dark green and glossy above greenish-green below, usually

stalkless. The flowers are white borne in axillary or terminal loosely branched glabrous inflorescences. The fruits occur mainly in pairs, spherical, mottled green with seeds wrapped in yellow pulp (1) (Figure 1). The seeds are the most economically important part of the plant though the bark and roots are also widely used in traditional medicines. The seeds are numerous, dark brown and ellipsoid in shape and embedded in pulp. The seeds are important annual source of additional income for harvesters and exporters in West Africa. Voacanga is sometimes planted in newly established cocoa plantations to provide shade in the first three years of establishment and finds ready use as stakes for yams. Field observation revealed that a fully grown plant yields between 2,000 - 3,000 pods. About 110-120 pods yield a kilogram of the dry seeds which gives an estimated yield of 10-23 kg/tree of seeds per plant or approximately 800-1,200kg/acre.

Traditional Uses

In Cote d' Ivore voacanga is traditionally used against leprosy, diarrhea, generalized edema, convulsions in children, madness, diuretic and infant tonic (1-3). Bark or root bark decoctions is drunk for cardiac spasms, stomach, hernia and post partum pain, kidney troubles (3).

A decoction of the stem bark and root is used in the treatment of mental disorders. The latex is applied to carious teeth. A decoction of the bark is considered an analgesic and is added to embrocation mixtures used as pastes during fracture repair. In southeastern Nigeria the plant is featured in many healing rituals (1). Also it used to induce hallucinations and trances in religious rituals.

Also, in Congolese traditional medicine macerations of root bark of *V. africana* are used as anti- amoebial. Intestinal amoebiasis is one of the current diseases in tropical regions causing diarrhea (4).



Figure 1. Voacanga fruits, characteristically spherical, mottled green with seeds wrapped in yellow pulp.

Habitat and Harvesting

Voacanga africana is distributed mainly in West Africa though reaching as far as the Congo and east to Tanzania. The plant is widely distributed over secondary forests and transitional zones. It grows in the wild and is therefore subject to degradation by land clearance for farming, bushfires and unsustainable harvesting methods.

The maturity of the pods and harvesting periods vary with ecological zones and rainfall patterns. However, voacanga generally flowers in May and matures in July. The harvesting season extends from July to September. A fully-matured pod will show signs of cracking along the cleavage line.

Harvesting is done by climbing the trees to pluck the pods or pollarding the fruit-bearing branches. Pickers sometimes cut down the tree to harvest the fruits – a practice which depletes stocks in the wild and is unsustainable. A few people pluck the fruits with long sticks.

At present, there are no national and international standards nor are there any grades and norms in handling and processing the seeds, the product of commerce. This work aims to review the chemistry and pharmacological activity and develop quality control standards (QC) for *V. africana* seeds that can be used in the development of standards and norms and also to identify potential pharmacological uses of the seeds. The development of QC will help to determine the best harvesting conditions and post-harvest handling methods which will provide the optimum levels of the important chemical composition for the buyer as well as high yields for the supplier. Seed moisture content, density, clustering levels, and ash levels were investigated.

Materials and Methods

A total of 26 samples of dry seeds of *Voacanga* from six endemic regions in Ghana were included in this study. All procedures were run in quadruplicate.

For moisture content, seeds were dried in an oven at 85°C for 5 days to a constant dry. The loss of weight after drying was expressed as moisture percent. A sieve test was conducted to determine the relative amounts of seed clusters, whole seeds and broken seeds in the samples of *V. africana*. Two US standard sieves (size 4, opening 4.75 mm and size 10, opening 2 mm) were used to separate the samples in three groups; particles higher than 4.75 mm (seed clusters), particles between 4.75 and 2 mm (whole seeds, broken seeds), particles less than 2.0 mm (fine particles and other foreign matter). All seeds were inspected and foreign material separated. The densities of the seeds were calculated according to the weight and volume of each sample.

Total ashes and acid insoluble ashes were calculated according to the Food Chemicals Codex (5). Ground voacanga seeds (2 g) were placed in ceramic crucibles and then ignited at 600°C, the ashes were then dissolved in a hydrochloric acid solution, filtered and the crucible ignited at 650°C to obtain the acid insoluble ashes that represent the contamination with sand and earths. Total seed alkaloids concentration was measured according to Sreevidya and Mehrotra (6).

Results and Discussion

Most of Voacanga seeds have a brown color; the moisture content was below the 12%, the maximum percentage according to international standards for botanical products. Only 5 samples exceeded that amount (Table I), indicating that these seeds were improperly dried. High moisture content can decrease shelf life due to microbial growth and enzymatic reaction, leading to seed deterioration.

Regarding to the total ashes and insoluble ashes we observed that 38% of the samples showed high levels of acid insoluble ashes (Table I).

The amounts of seeds cluster is an important quality parameter, since seeds harvested from immature trees form tight clusters; very difficult to separate. When seeds are harvested immature, they are literally piled-up and allowed to ferment to facilitate their separation (Figure 2). This is not a recommended practice, since the fermentation may degrade the active principles. The amount of seed clusters among the samples was highly heterogeneous ranging from 3 (sample 4) to 89 % (sample 7) (Table II). Almost 46 % of the samples showed levels higher than 30% of clusters, suggesting that these seeds were collected from immature fruits. Only 6 samples exhibited the presence of high levels of broken seeds, indicating that these seeds were broken when collectors were trying to disaggregate the clusters.



Figure 2. Immature seeds are collected, piled up and covered to allow fermentation. After this fermentation, the seed clusters are disaggregated with the help of a tool.

Significant amount of seeds (27%) showed low densities (less than 0.4 g/ml). Our results, suggest that seed density could be a useful parameter to

evaluate the quality of whole seeds since low density seeds seem to correlate with high amounts of clusters and thus immature seeds (Table II).

Table I. Color, Moisture, Total Ashes and Acid Insoluble Ashes of *Voacanga africana* from Ghana

Spl ¹	Location and Region	Color	Moisture (%)	Total ashes (%)	Acid insoluble ashes (%)
1	Tema (GAR)	dark brown-gray	10.6 ± 0.7 ²	3.6 ± 0.1	0.5 ± 0.0
2	Tema (GAR)	dark brown	13.9 ± 0.1	2.7 ± 0.0	0.5 ± 0.3
3	Tema (GAR)	light brown	9.4 ± 0.1	3.2 ± 0.1	0.7 ± 0.3
4	Nsuhia (BAR)	light gray	10.6 ± 0.1	3.9 ± 0.0	0.8 ± 0.1
5	Nwonmaso (CR)	dark brown	10.6 ± 0.1	3.2 ± 0.3	1.0 ± 0.4
6	Agogo (AR)	dark brown	9.9 ± 0.2	5.0 ± 0.3	1.4 ± 0.0
7	Agogo (AR)	gray-brown	10.1 ± 0.4	6.2 ± 1.8	1.5 ± 0.4
8	Nkrankwanta (BAR)	black	9.9 ± 0.05	9.0 ± 0.5	1.3 ± 0.2
9	Nkrankwanta (BAR)	very light gray	8.4 ± 0.1	3.2 ± 0.1	0.8 ± 0.2
10	Nkrankwanta (BAR)	brown	8.6 ± 0.4	6.8 ± 2.0	2.3 ± 0.7
11	Nkrankwanta (BAR)	light and dark gray	9.1 ± 0.03	4.9 ± 0.4	1.8 ± 0.7
12	Bepong (ER)	light gray-brown	8.4 ± 0.4	3.7 ± 0.1	0.6 ± 0.1
13	Bepong (ER)	dark brown	10.5 ± 0.2	2.5 ± 1.7	0.3 ± 0.5
14	Anyan-Denyira (CR)	dark brown	10.5 ± 1.02	3.0 ± 0.1	0.5 ± 0.1
15	Obo (ER)	dark brown	8.3 ± 0.2	2.9 ± 0.1	0.3 ± 0.0
16	Obo (ER)	light gray	8.2 ± 0.1	3.8 ± 0.4	0.9 ± 0.1
17	Obo (ER)	dark brown-black	9.1 ± 0.4	4.1 ± 0.0	0.7 ± 0.1
18	Kasapi (BAR)	gray-brown	9.7 ± 0.2	4.4 ± 0.4	1.6 ± 0.2
19	Kasapi (BAR)	very dark brown-black	10.8 ± 0.3	5.7 ± 0.2	1.7 ± 1.1
20	Ampaha (AR)	light-dark brown	19.0 ± 6.0	2.8 ± 0.0	0.6 ± 0.2
21	Ampaha (AR)	dark brown-black	11.6 ± 0.5	4.5 ± 0.1	1.7 ± 0.1
22	Sunyani (BA)	dark brown	9.3 ± 0.6	4.7 ± 0.4	1.3 ± 0.3
23	Ayiem (WR)	dark brown	15.5 ± 0.9	2.6 ± 0.0	0.6 ± 0.2
24	Hohoe (VR)	dark brown	14.0 ± 0.4	2.5 ± 0.3	0.6 ± 0.0
25	Togo	brown-black	12.4 ± 0.1	3.6 ± 0.0	0.9 ± 0.1
26	Togo	light brown-gray	11.3 ± 0.3	3.8 ± 0.0	1.9 ± 0.4

NOTE: ¹. Sample number. ². Average Value ± Standard Error. GA, Greater Accra Region, BAR, Brong Ahafo, CR, Central, AR, Ashanti, ER, Eastern, WR, Western Region, VR, Volta.

Table II – Percentage of Seed Clusters, Whole, Broken Seeds, Density and Alkaloid Content from *Voacanga africana*

Spl ¹	Average Seed Clusters % ²	Average Whole Seeds %	Average Broken Seeds %	Average Density (g/ml)	Total alkaloids (%)
1	16 ± 1.3 ³	82.5 ± 1.3	1.2 ± 0.2	0.51 ± 0.14	0.9 ± 0.0
2	54.3 ± 0.4	44.9 ± 0.1	-	0.45 ± 0.01	0.5 ± 0.1
3	13.7 ± 4.1	85.9 ± 3.6	-	0.47 ± 0.02	0.6 ± 0.0
4	3.1 ± 2.4	96.6 ± 2.1	0.2 ± 0.06	0.47 ± 0.01	1.2 ± 0.1
5	15.1 ± 4.6	84.6 ± 4.4	0.7 ± 0.2	0.49 ± 0.01	0.4 ± 0.0
6	39.8 ± 18.1	59.2 ± 19.1	0.4 ± 0.09	0.5 ± 0.01	0.2 ± 0.0
7	89 ± 3	10.5 ± 3.1	0.5 ± 0.06	0.33 ± 0.00	1.2 ± 0.2
8	53.4 ± 3.4	46.1 ± 3.4	1.1 ± 0.6	0.41 ± 0.06	1.5 ± 0.2
9	8.3 ± 4.5	90.4 ± 4.7	1.8 ± 1.2	0.42 ± 0.01	0.8 ± 0.3
10	24.2 ± 7.1	74.9 ± 8.1	0.9 ± 0.03	0.47 ± 0.01	0.5 ± 0.2
11	28.9 ± 14.6	70.6 ± 14.4	0.4 ± 0.2	0.39 ± 0.01	0.9 ± 0.3
12	69.6 ± 4.7	29.6 ± 5.2	0.4 ± 0.2	0.41 ± 0.01	0.9 ± 0.1
13	37.7 ± 0.9	61.7 ± 1.1	0.4 ± 0.3	0.47 ± 0.01	0.5 ± 0.1
14	52.4 ± 0.6	46.8 ± 1.1	0.1 ± 0.04	0.39 ± 0.03	0.7 ± 0.1
15	65.1 ± 13.2	34.8 ± 13.1	0.1 ± 0.04	0.46 ± 0.01	0.3 ± 0.1
16	20.2 ± 5.8	79.5 ± 5.8	0.2 ± 0.01	0.53 ± 0.00	0.4 ± 0.2
17	38.8 ± 1.5	60.5 ± 1.8	1 ± 0.1	0.3 ± 0.01	1.0 ± 0.3
18	16.6 ± 8.2	81.5 ± 5.1	0.8 ± 0.4	0.37 ± 0.05	0.2 ± 0.1
19	27.6 ± 5.2	69.1 ± 3.5	2.5 ± 0.7	0.21 ± 0.08	0.3 ± 0.1
20	53.3 ± 2.2	46.5 ± 2.1	0.3 ± 0.2	0.47 ± 0.00	0.3 ± 0.0
21	10.6 ± 0.1	84 ± 3.5	2.2 ± 0.09	0.27 ± 0.01	0.9 ± 0.1
22	12.8 ± 7.3	87.1 ± 7.3	-	0.48 ± 0.02	0.3 ± 0.1
23	84.6 ± 3.4	15 ± 3	-	0.56 ± 0.04	0.2 ± 0.1
24	33.5 ± 1.2	66.2 ± 1.2	0.02 ± 0.01	0.56 ± 0.01	0.4 ± 0.1
25	15.6 ± 3.9	84 ± 4.1	0.2 ± 0.04	0.48 ± 0.00	0.6 ± 0.3
26	22.1 ± 4.1	77.5 ± 4.2	0.1 ± 0.07	0.49 ± 0.00	0.3 ± 0.1

NOTE:¹Sample number. ²% is of total weight. ³Average values ± Standard Error.

According to the number of whole seeds, samples 4 and 9 appear to be the best quality samples (Table II). The seeds were light gray in color. Sample 9 also showed high levels of broken seeds (1.8%), as well as samples 19 (2.5%) and 21 (2.2%). An acceptable value of broken seeds will be 1% or less.

Another important, classic and simple quality control parameter is the total ashes and insoluble ashes (Table I). We observed that 38% of the samples

showed high levels of acid insoluble ashes indicating that they are contaminated with earth and/or sand. The results suggest that these samples were dried on the bare ground. Interventions easy to overcome the presence of sand and other soil contaminants would be to ensure all seeds are dried on materials off the ground.

Total insoluble ashes, or sand content, should be less than 1% of the ground seeds for economic reasons. Botanical products are purchased on a weight value; thus, if a large amount of sand is present in the product then the buyer is paying for a percentage of sand and not the product. As an example, one-ton sample of the seeds having a value of insoluble ashes of 3% will contain 30 kg of sand.

The total alkaloids in Voacanga seeds ranged from 0.2% to 1.5% (Table II). Highest content of alkaloids were found in samples 7 (1.2%) and 8 (1.5%).

In this initial study, there was no clear relationship between the selected quality control parameter and the alkaloid content and thus the total amount of alkaloid was not found to be correlated with the harvesting and processing of the seeds. The observed differences could be related with high genetic variation in the alkaloid content in the selected populations.

Our results suggest that the best postharvest handling method for Voacanga seeds is simply to use the seeds from mature pods, to avoid the formation of seed clusters, and subsequent fermentation that lead to seed deterioration, and which later involve additional labor. The mature seeds tend to be of higher density; being heavier than the immature seeds. Thus, the seed clusters and seed density are important parameters to assess the quality of voacanga seeds. To avoid excessive contamination with sand, the voacanga seeds need to be dried in raised platforms to avoid contact of the seeds with the bare ground. Field research has shown that by harvesting immature seeds, collectors/growers lose 40-50% of the potential yields from same population/plant

In summary, to better assess the quality of *V. africana* seeds, we propose additional standards, such as percent of clusters, density, ashes and total alkaloids (Table III). In Ghana, the seeds are purchased according to their weight. Thus, the seed clusters and seed density are important parameters to assess the quality of voacanga seeds.

Table III. Proposed Quality Control Standards for *Voacanga africana* Seeds

<i>Characteristic</i>	<i>Voacanga seeds</i>
Organoleptic evaluation	
Color	Dark brown or brown
Macroscopic and sieve analysis	
Extraneous/foreign matter content, % (m/m) max.	0.5
Damaged seeds, % (m/m) max.	0.5
Percent of clusters (particles > 4.75mm), % (m/m)	30
Physical-Chemical parameters	
Seed density (g/mL), min.	0.4
Moisture (%), (m/m) max.	12
Total ashes, %(m/m) max.	4
Total insoluble ashes, %(m/m) max.	1
Total alkaloids, %(m/m) min.	0.5

Chemical Composition of *Voacanga* Species

Different chemical components have been isolated from different organs of *Voacanga* species. The presence of flavonoids, tannins, phenols, steroids and terpenes, alkaloids has been reported in the leaves, seeds, stem bark and roots (4, 7, 8).

In the past decades, many studies were conducted and reported that focused on the identification of the wide array of *V. africana* alkaloids and the main ergot alkaloids have been isolated. With the improvement of the isolation procedures many minor components have been identified (Table IV). *Voacanga* alkaloids have been isolated from leaves, seeds (mature and immature), trunk and root bark (Table IV).

Analysis of root and bark extracts of *V. africana* showed the presence of the alkaloids voacamine, voacangine, and vobasine as the principal ones (9). Other compounds found in the plant include voacristine, voacamidine, and voacarine. Voaphylline, vobtusine, voalfolidine occur in the leaves and tabersonine is a constituent of the seeds (1, 10). Also the presence of ibogaine has been obtained from bark (11) and seed (12). Many papers have been published with the structural determination of minor ergot alkaloids occurring in *Voacanga* species (Table IV). Alkaloids found in *Voacanga* have also been found in other species of the family Apocynaceae (Table V).

Table IV. Isolated Alkaloids in *Voacanga* spp. and the Main Organ of Accumulation and Distribution

<i>Alkaloid</i>	<i>Organ distribution</i>	<i>Reference</i>	<i>Species/Sources</i>
amataine	Root bark	13	<i>V. chalogina</i>
-(-) tabersonine	Cell culture	14	<i>V. africana</i>
folicangine	Leaves	10,15	<i>V. africana</i>
isovoafoline		10	<i>V. africana</i>
O methyl 16 epi vincanol	seed	16	<i>V. africana</i>
subsessiline		10	
subsessiline lactone		10	
vincamol	seed	16	<i>V. africana</i>
vincamone	seed	16	<i>V. africana</i>
voacafricine	bark	17	<i>V. africana</i>
voacafrine	bark	17	<i>V. africana</i>
voacamine	Stem, bark	18, 19	<i>V. africana</i>
	Bark	11	<i>V. thousarsii</i>
		9, 20	<i>V. africana</i>
		21	<i>V. braceata</i>
voacangine	Trunk bark	22	<i>V. africana</i>
	Bark	11	<i>V. thousarsii</i>
		9, 20	<i>V. africana</i>
		21	<i>V. braceata</i>
voacangine	Tree bark	20, 23	<i>V. africana</i>
hydroxyindolenine			
vacorine		19	<i>V. africana</i>
		10	<i>V. africana</i>
		1	
		20	<i>V. africana</i>
		19	<i>V. africana</i>
voacristine		23-25	<i>V. africana</i>
	Bark	11	<i>V. thousarsii</i>
	Bark and bark root	1, 10, 20, 24, 26	<i>V. africana</i>
voacamidine	Bark and bark root	1, 10, 24, 26, 27,	<i>V. africana</i>
voacryptine		26	<i>V. africana</i>

Table IV. Continued.

<i>Alkaloid</i>	<i>Organ distribution</i>	<i>Reference</i>	<i>Sources</i>
voafolidine	leaf	10	<i>V. africana</i>
voafoline	leaf	10	<i>V. africana</i>
vobasine		9, 20, 23	<i>V. africana</i>
vobtusine		10	<i>V. thousarsii</i>
	Bark	11	<i>V. thousarsii</i>
	leaves	10, 28	<i>V. africana</i>
	Bark	13	<i>V. chalogina</i>
		1, 20	<i>V. africana</i>
vobtusine-lactone	leaves	28	<i>V. africana</i>
deoxyvobtusine	leaves	28	<i>V. africana</i>
vobtusine 3-lactam Nb ² - oxide		10	<i>V. africana</i>
vobtusine 3-lactam, ibogaine	Bark	11	<i>V. thousarsii</i>
	seeds	12	<i>V. africana</i>
iboluteine	Bark	11	<i>V. thousarsii</i>
18 ² - decarbonethoxyvoacamine	Bark	11	<i>V. thousarsii</i>
voaluteine	Bark	11	<i>V. thousarsii</i>
20 ² hydroxyl-voacamine	bark	29	<i>V. africana</i>

Table V. Voacanga Alkaloids Reported in Other Species of the Apocyanaceae Family

<i>Alkaloid</i>	<i>Organ</i>	<i>Reference</i>	<i>Source specie</i>
amataine	roots	30	<i>Hedranthera barteri</i>
voacamine	Bark, stem	31	<i>Ervatamia coronaria</i> ¹
		32	<i>Peschiera laeta</i> ²
		33	<i>P. fuchsiaefolia</i>
voacangine	Stem, bark	22, 34	<i>Tabernaemontana</i>
	Bark, stem		<i>australis</i>
	Leaves, immature fruits	35	<i>T. hilariana</i>
		36	<i>T. calcarea</i>
		37	<i>P. fuchsiaefolia</i>
vobasine		9, 20	<i>Voacanga africana</i>
vobasine	leaves	38	<i>T. corymbosa</i>
voacristine	Stem, bark	39	<i>Pechiera buchtiene</i>
	Leaves, fruits	36	<i>T. calcarea</i>
	stem	31	<i>E. coronaria</i>
voacamidine	Stem, bark	39	<i>P. buchtiene</i>
voacorine		21	<i>T. braceata</i>
voaphylline	Stem, bark	34	<i>P. buchtiene</i>
	leaves	40	<i>T. divaricata</i>
vobatricine	Stem, bark	38	<i>T. corymbosa</i>
vobasonidine	leaves	38	<i>T. corymbosa</i>
ibogaine		22, 41	<i>T. iboga</i>
		34	<i>T. australis</i>
ibogamine		34	<i>T. australis</i>
		22	<i>T. iboga</i>
		39	<i>P. buchtiene</i>
		35	<i>T. hilariana</i>
		36	<i>T. calcarea</i>
		37	<i>T. fuchsiaefolia</i>
ibogaline		34	<i>T. australis</i>
		22	<i>T. iboga</i>

SOURCE: ¹*Ervatamia coronaria* (Jacq) Stapf (syn. *Tabernaemontana divaricata* R.Br. ex Roem. et Schult.) ²*Peschiera* syn. *Tabernaemontana*

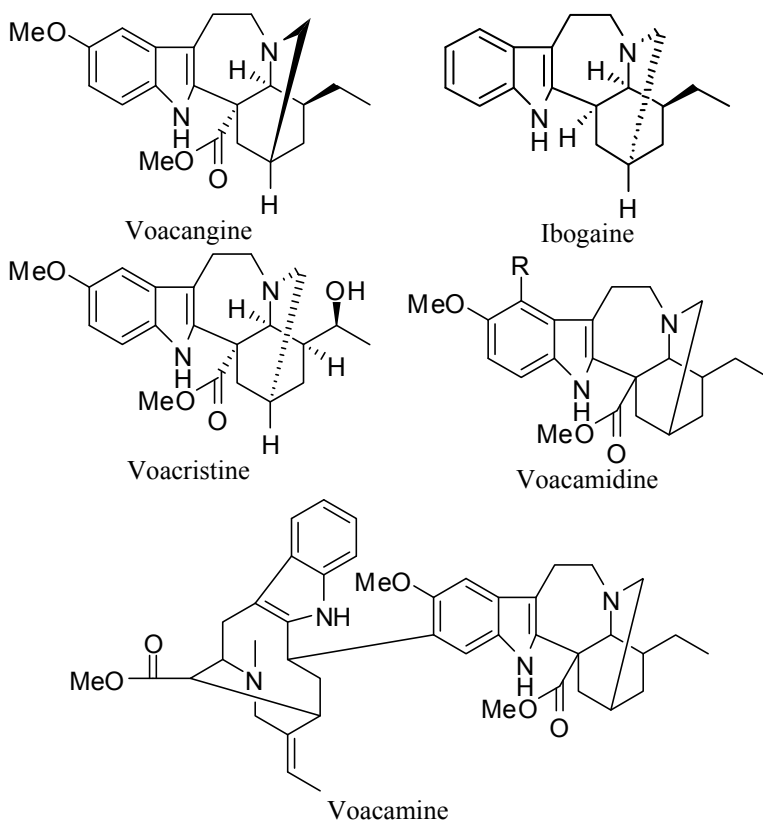


Figure 3. Chemical structures of some alkaloids isolated from *Voacanga africana*.

Pharmacological Activity of Voacanga Alkaloids

Traditional medicines based upon single or a mixture of plants is still commonly used across Africa, playing a significant role in the primary health care of the population. Traditional medicine is deeply rooted in a specific socio-cultural context, and thus is different among the communities yet an integral part of the family, community, village, and nation (3, 42, 43, 70).

The majority of plants used in traditional medicine are directly collected from the wild with few cultivated. The most common plant remedies are used as infusions, powders, decoctions (boiled for few minutes or hours), macerations (steeped in cold water for a period of time), added in foods or drinks (3).

Among the natural products that can be isolated from *Voacanga* species, it is the alkaloids which account for the wide range of pharmacological activities and thus medicinal uses. For instance, the bisindole alkaloid amataine isolated from *Voacanga* exhibited cytotoxicity effects *in vitro* and could be used in chemotherapy as cytotoxic group (44).

Voacamine, extracted from *Peschiera fuchsiaefolia*, has shown anticancer activity by enhancing the cytotoxic effect of DOX on Multi drug resistant cells by favouring a lethal autophagic process (33). Voacamine extracted from *V. africana* or *P. fuchsiaefolia* has shown immunostimulant activity, by modulating and reinforcing the immune system, thus with anti- HIV properties. The mechanism of actions was associated with an increase of TH1 cell levels relative to TH2 cells (45).

A mixture of alkaloids from *V. africana* and *V. obtuse* have been shown to have cardiostimulant, sympatholytic and hypotensive properties (9, 46, 47).

In Nigeria, fruits of *V. zenkeri* have been successfully used to treat lumbago and arthritis associated with different type of cancers and inflammatory diseases (48). In Cameroon, *V. africana* seeds extracts are used in traditional medicine to treat orchitis, tooth decay and gonorrhoea, and these uses were related to the antimicrobial and antifungal activity of the extracts (7). An infusion based on the bark from *V. africana* has been used for the relief stomach complaints and root decoctions for hernia pain and kidney troubles (3).

The antiulcerogenic effect of the bark of *V. africana* was also reported using different experimental models. Tan and collaborators (2, 49) showed the cytoprotection of aqueous extracts for the gastric mucosa against erosive action of corrosive agents (HCl/ EtOH). Thus, *V. africana* possess anti-ulcer properties and these properties were not related with an increase on mucus production, or reduced pepsin activity, however the cytoprotection was related to a mechanism involving the physico-chemical re-enforcement of the gastric mucous layer.

Ibogaine is a psychoactive indole alkaloid and powerful hallucinogens (50). Ibogaine appears to be the main alkaloid in the root of *Tabernanthe iboga*, a naturally occurring shrub from Africa. The root bark of *T. iboga* has been used for many centuries in West African ceremonies. Of interest, is that ibogaine induces several peripheral and central nervous system (CNS) effects, one of which is effective in the treatment of withdrawal symptoms and to reduce the cravings in drug addicts (51). Modern studies showed that ibogaine was able to interrupt addiction to heroin (52, 53), cocaine (54, 55), amphetamine (54), nicotine (56), alcohol (57) in animals and humans (50, 58, 59).

V. braceata has been identified as an African botanical with hallucinogenic potential. Stem barks of *V. braceata* contain 2.46% of alkaloids mainly voacamine/voacamine N-oxide, 20- epi voacamine and voacangine, although these alkaloids are related with ibogaine, it was concluded that there is no evidence of hallucinogenic properties reported De Smet (21).

Ibogaine and voacangine (one of the major alkaloid in voacanga) are closely related. Voacangine differs from ibogaine in that voacangine contains an additional carboxy function (60). Recently, it was reported the semisynthesis of ibogaine from voacangine, obtained from bark trunk of *V. africana*, as the most promising alternative source of ibogaine (22). Also, it was reported the presence

of the alkaloid ibogaine, in seeds and bark of voacanga (11, 12), supporting thus its use in ritual in traditional medicine.

Malaria is an important parasitic disease and extensive attention has been gained in the last years (61, 62). Many drugs are suitable to treat malaria disease in modern therapy. Because of the rapid development of resistance toward current drugs, traditional remedies have been an important source of antimalarial drugs and provide novel and effective treatments (61). The bisindole alkaloid voacamine exhibit *in vitro* antiplasmodial activity against chloroquine sensitive D₆ and chloroquine resistant W₂ strains (63, 64). *In vivo* antiplasmodial activity of voacamine was less pronounced than that of the reference antimalarial chloroquine. This result was associated with the potential effect on nuclear division of parasite, possibly with the DNA and or protein synthesis (65). Moreover, voacamine, and voacamine isomers has been reported as effective to prevent or treat malaria and are non-toxic. These compounds can be combined with other effective drugs such as chloroquine, arthemisin and qinghaosu (66). Such studies strongly suggest additional research is warranted as the search for new antimalarial agents continues worldwide. Voacamine and its derivatives isolated from *Peschiera* or *Voacanga* may also have application and used directly as antimicrobial, antiparasitic or antiviral agents (67).

Extracts of the root bark of *V. africana* has demonstrated *in vitro* active antiamebic activity (MIC =62.5 µg/ml) against *Entamoeba histolytica* supporting its' traditional use as antidiarrhoeic medicine (4). Recently, Tan and collaborators (68) reported that methanolic extracts of *V. africana* bark exhibited antimicrobial activity against *Helibacter pylori* (human pathogen associated with gastro duodenal ulcers (69) and *Campylobacter jejuni*, thus providing a rationale for the wide traditional use of stem bark in traditional management of peptic ulcer disease. Moreover, these same researchers reported voacanga extracts were active against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Alcaligenes spp*, suggesting the possible presence of broad spectrum of antimicrobial principles.

Extracts of *V. africana* are used to treat AIDS. Phytochemical screening revealed the presence of important antioxidant bioactive molecules such as tannins, flavonoids and phenols in bark extracts (8). They concluded that the antioxidant activity in this medicinal plant may be a contributing factor to its therapeutic applications. Additional medicinal applications of Voacanga have been reviewed in relation to other African medicinal plants (3, 70).

Conclusions

Voacanga is a well known and respected African medicinal plant used in many West African countries for a wide variety of applications. The biological activity is due largely to the presence of alkaloids found in the seeds, bark and roots. There is a quantitative and qualitative difference in alkaloid content and composition by plant tissue and species. Sustainable harvest and preservation of this key African medicinal strongly suggests that Good Agricultural and Collection Practices as recommended by the WHO should be followed (71). The original research presented here as well as the review of the botany, chemistry and Pharmacognosy were to assist in the development of grades and standards for the raw Voacanga seed. High quality seed well defined and characterized is just one step in the larger quality control of the final extractable alkaloids or in the standardization of the seeds and the seed extracts.

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Chapter 21

Quality Control and 5-HTP (5-Hydroxy-L-tryptophan) Analysis of Griffonia (*Griffonia simplicifolia* (DC.) Baill.) Seed Accessions Collected in Ghana

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Griffonia, *Griffonia simplicifolia* DC. Baill., is an important West African medicinal plant because the seeds contain high concentrations of 5-hydroxy-L-tryptophan (5-HTP) which are extracted and then purified for use in the dietary supplemental market as an antidepressant, for anxiety, treating fibromyalgia, insomnia and weight loss. *Griffonia* seeds were collected from 16 key *Griffonia* collection and trading centers around Ghana and investigated for their quality characteristics. The collection of different populations provided preliminary information on the natural variation that could be expected as expressed either by genetics and environment in order to develop quality control recommendations and product specification ranges for the raw seed. The seeds were physicochemically characterized and the content of 5-HTP in seeds from each population and collection site were analyzed using high performance liquid chromatography suggesting seed characteristics and 5-HTP content that should be considered as the standard methods and levels for the quality control of *Griffonia* seeds.

Griffonia (*Griffonia simplicifolia* (DC.) Baill.) is an important African medicinal plant of interest because the seeds are a rich source of 5-hydroxy-L-tryptophan (Figure 1) also known as 5-HTP, the bioactive compound used as an antidepressant, in the treatment of headaches, panic attacks and anxiety, insomnia, fibromyalgia, and weight loss (1). As a serotonin precursor, the purified 5-HTP is on the US marketplace as a dietary supplement used also to treat serotonin deficiency. Griffonia seeds (Figure 2) which can reach as high as 20% 5-HTP in fresh material also contain a number of additionally known biologically active compounds such as β -sitosterol, stigmasterol and campesterol (1,2). The 5-HTP is also found in the pods and in lower concentration in the leaves of mature plants (Figures 2 and 3). The seed, the marketable product of commerce as well as the extractable 5-HTP are both of increasing interest in the global medicinal plant market.

Traditional African medicinal uses of *G. simplicifolia* include wound healing, pain, kidney problems, diarrhea, vomiting, purgative, aphrodisiac and as an enema (3). The related species *G. tessmannii* is also used for some of the same applications such as vomiting and as an aphrodisiac but also in contrast to Griffonia is used in the treatment of gonorrhoea, stomach disorders, fever reduction in children and internal injuries (3).

Griffonia seed is harvested by hand, and in general dried on the ground, mats, rooftops or areas kept clean for the spreading out of the seed (Figures 2 and 3). Griffonia seeds can later be further dried using artificial forced air heat. The seeds are generally sold domestically, collected by brokers and traders and aggregated for larger volume sale and export; where the seeds are then further processed. Processing to extract the 5-HTP generally takes place in China and afterwards the purified 5-HTP enters into the global marketplace.

Ghana is the principal world supplier of Griffonia and to enable greater trade and export, the establishment of basic standards and grades are needed. The Ghana Standards Board (4, 5) is working towards this end, and in support of that work, other groups are also contributing (6) and this study was conducted. Developing a quality control program and standards including a quantitation method for 5-HTP in Ghana, would empower the collectors, traders and export council to better understand the Ghanaian product and its economic value. Currently, quality and payment is often based only on the cleanliness, color and moisture content of the seed. Routine quantitation of 5-HTP is normally not performed in-country in advance of sales. To better protect both the collector and trader as well as the processor and to explore in-country processing to yield further value-addition in Africa, defined seed characteristics including minimum and maximum ranges of 5-HTP are needed.

Apart from the chemical standardization that is needed; this species now faces serious challenges in its continued sustainable production due to unsustainable harvest and post-harvest practices. As Griffonia is not cultivated but grows wild, the climbing shrubs are subject to destruction by bushfires and land clearance for farming. Additionally, unsustainable harvesting methods are sometimes used to gather the pods (6). We recommend that all collection of Griffonia seed should be conducted in compliance with the WHO Good Agricultural and Collection Practices of Medicinal Plants (7). Such trainings for sustainable collection and quality control have begun and there is now an

illustrated pictorial guide to all the parameters of quality and acceptance at the collectors' level (8). There is also interest to begin to cultivate this plant as a way to both ensure the preservation of this species and the availability of product. Long term prospects for cultivation are unclear due to the high price fluctuations for the seeds.

The Ghana Standards Board (GSB) and participating Ghanaian botanical industry have long recognized the importance of seed quality in the definition and quality of Griffonia seed. The recognition that the product comes from wild, not cultivated trees focuses attention on the actual physical character and cleanliness of the seed. High quality and even acceptable seed is considered to be from intact mature seeds of normal color and seed coat (vs. broken, split, cracked, shaven, immature) and free from mold, mite and insect infestation and of characteristic color (both seed coat and endosperm). The presence of non-Griffonia materials in association with the seed (e.g. weeds, dirt, extraneous materials of plant, animal, rodent, or manufacturing origin) is undesirable and upper allowable ranges for each have been set (4, 5). Seeds should have the characteristic odor both on the seed coat and endosperm. The Ghana Standards Board has documents now describing Griffonia as well as an Inspection Manual for Griffonia Seeds which specifies the minimum quality of Griffonia for export and outlines the maximum allowable defects in seeds and aflatoxins as well as other chemical parameters (4, 5).

This study was conducted to evaluate the chemistry and quality of Griffonia seed 'crop' in Ghana, as a first step in characterizing the range of seed characters and the content of its main bioactive component, 5-HTP.

Material and Methods

Sun dried seed samples were collected across the key Griffonia collection and trading centers around Ghana by the coordinating efforts of ASNAPP-Ghana. These key regions included the Volta, Ashanti, Eastern, Central, Western and the Brong Ahafo regions.

The physical analysis of the seeds was conducted by measuring the diameters of 10-15 seeds from each sample at the widest point and weighing each seed. Seed coat and endosperm color were visually evaluated. The moisture, total ashes, and total insoluble ashes were assessed using methods described by the Food Chemical Codex (9).

Griffonia seeds from 16 accession samples were analyzed using HPLC/UV method outlined below, developed at the New Use Agriculture and Natural Plant Products Program, Rutgers University. Three replicates per sample were tested with more than five seeds per replicate. 5-hydroxy-L-tryptophan (Sigma Chemical Co.) was purchased and used for compound identification using an Alliance HPLC equipped with Waters 2695 Separation Module and Waters 2996 Photodiode Array Detector, and Waters Millennium Software. The column was a Prodigy 5 μ ODS3 150 \times 3.2 mm (Phenomenex Co.).

About 300 mg of each powdered sample after drying for 24 hrs at 60°C was extracted with 40 mL of 50 % methanol/water by shaking at 150 rpm for 10 hrs, and then diluted two times. Extract solution was centrifuged and transferred to 2

mL autosampler vial for HPLC injection. The water was of HPLC grade (Fisher Scientific Co.). HPLC separation was performed with mobile phase of 1.0 % acetonitrile in water in isocratic condition. The flow rate was 0.6 mL/min. UV chromatogram was detected at 275 nm. 5-HTP elution time was approximately 7.0 min.

Results and Discussion

Of the 16 samples, seed color ranged from brown to black and endosperm color ranged from bright yellow to green/yellow (Figure 2). Seed weight ranged widely from 0.38 g up to 0.61 g/seed; while seed diameter ranged from 1.50 to 1.84 mm (Table I). While seed moisture in general was well below 10%, as commonly called for relative to many botanical products traded internationally, only 9 of the 16 samples would meet the maximum allowable seed moisture content as recommended by the Ghana Standards Board of 8% (4). Moisture content of seven samples exceeded the 8% maximum seed moisture levels with sample collected in Bayive, VR having an exceptionally high seed moisture of 19%. Of these samples, only two did not meet the 10% minimum requirement as described for inspectors of Griffonia seed (5).

Ash content and insoluble ash contents were low, though a low variation was observed (2.9 to 4.1%, 0.27 to 0.68%, for total and acid insoluble ashes, respectively (Table I). The acid insoluble ashes are a classic determination of cleanliness in botanical products; usually a maximum of 1% is accepted for food products. All Griffonia samples were well below that threshold. In both the Inspection Manuals, there are defined degrees of tolerances allowed (i.e. % by number or weight of defective fruit) (4). Ash content and insoluble ash contents are generally part of ISO specifications and should be considered to include in future standards of Griffonia seeds.

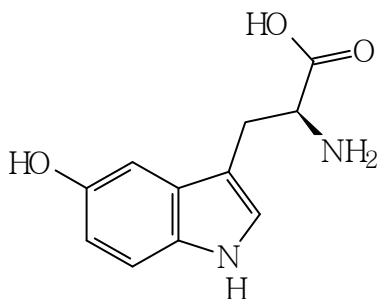


Figure 1. Chemical structure of 5-hydroxy-L-tryptophan (5-HTP)

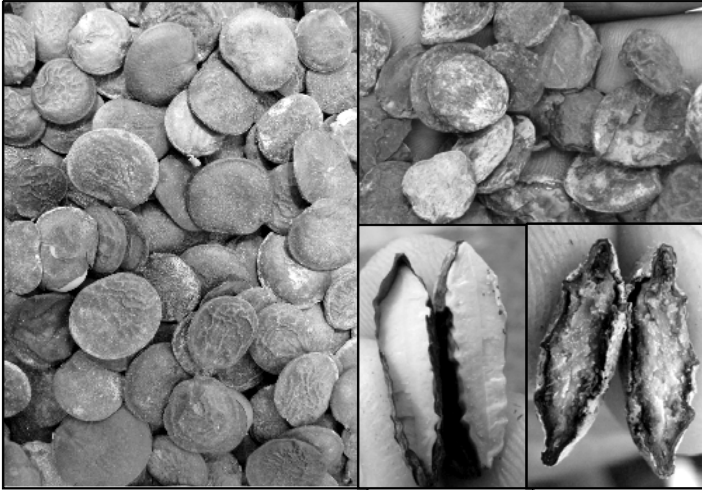


Figure 2. Dried and acceptable quality Griffonia seeds (left), poorly dried seeds (upper right), typical endosperm (yellow) of good quality well-dried seed (lower right), endosperm of an improperly dried seed (lower left).



Figure 3. Collector of Griffonia drying seeds on the roof of his house (left). Griffonia leaves (upper right) and pods (lower right).

Table I. Griffonia Seed Characteristics, Ghana.

<i>Collection Sites¹</i>	<i>Seed Color²</i>	<i>Endosperm Color</i>	<i>Weight³</i>	<i>Diameter⁴</i>	<i>Moisture⁵</i>	<i>Ash⁵</i>	<i>AIA⁵</i>
Xavi, VR	Blk /Brn	Light	0.381	1.50	7.82	2.86	0.51
Bayive, VR	Blk /Brn	Green Bright Yellow	0.453	1.66	18.88	3.16	0.44
Race Course- K'si, AR	Blk	Yellow	0.547	1.84	8.13	3.09	0.68
Kwahu Bepong, ER	Blk	Green /Yellow	0.543	1.70	10.38	3.38	0.48
Ampaha, AR	Blk	Yellow	0.495	1.69	7.75	3.81	0.50
Gomoa	Dark	Green	0.617	1.77	7.48	3.24	0.36
Fawomanye, CR	Brn	/Yellow					
Akurabadze, CR	Dark Brn	Yellow /Green	0.612	1.80	7.61	3.10	0.37
Gomoa	Blk	Yellow	0.592	1.66	7.35	3.19	0.59
Kyiren, CR							
Abreshia, CR	Blk	Yellow	0.500	1.71	8.28	3.38	0.50
Essakyir, CR	Blk	Yellow	0.495	1.75	7.72	3.47	0.42
Mankessim, CR	Blk	Yellow	0.546	1.72	8.82	3.41	0.35
Wassa, WR	Blk	Yellow	0.654	1.79	7.52	3.55	0.33
Aowin	Blk	Yellow	0.533	1.71	7.75	3.70	0.36
Suaman, WR							
Japekrom, BAR	Blk	Bright Yellow	0.462	1.69	8.28	4.11	0.28
Nkrankwant a, BAR	Blk	Green /Yellow	0.582	1.70	8.02	3.36	0.27
Kasapin, BAR	Blk /Brn	Yellow	0.617	1.81	7.51	3.44	0.33

¹VR, Volta region; AR, Ashanti region; ER, Eastern region; CR, Central region; WR, Western region; BAR, Brong Ahafo region; ²Blk, black; Brn, brown; ³g/seed; ⁴Seed diameter (mm); ⁵o m/m, AIA=acid insoluble ashes.

5-HTP Content of Griffonia

The content of 5-hydroxy-L-tryptophan (HTP) in 16 samples of Griffonia seeds determined using the HPLC method as described was found to be on average $15.7 \pm 0.63\%$ of HTP, ranging from 14.3 to 17.1% (Table II). These results suggest little variation in 5-HTP content across a wide range of seeds, and conditions for a single season, including the one with the exceptionally high seed moisture content.

Table II. Content of 5-hydroxy-L-tryptophan in Seeds of Griffonia Accessions.

<i>Collection Regions in Ghana¹</i>	<i>5-Hydroxy-L-tryptophan (%)</i>
Xavi, VR	14.3 ± 1.4
Bayive, VR	15.6 ± 1.9
Race Course-K'si, AR	16.3 ± 0.7
Kwahu Bepong, ER	15.4 ± 1.7
Ampaha, AR	15.9 ± 0.3
Gomoa Fawomanye, CR	15.4 ± 1.5
Akurabadze, CR	15.7 ± 0.8
Gomoa Kyiren, CR	16.0 ± 0.3
Abreshia, CR	16.0 ± 0.53
Essakyir, CR	16.0 ± 1.0
Mankessim, CR	15.6 ± 1.5
Wassa, WR	15.6 ± 1.3
Aowin Suaman, WR	15.7 ± 1.6
Japekrom, BAR	17.1 ± 1.2
Nkrankwanta, BAR	14.7 ± 1.0
Kasapin, BAR	16.1 ± 0.9
Mean	15.7 ± 0.6

¹VR, Volta region; AR, Ashanti region; ER, Eastern region; CR, Central region; WR, Western region; BAR, Brong Ahafo region.

This study evaluated the quality of Griffonia seed across the main Griffonia collection and trading centers around Ghana in a single year. In addition, average content of 5-HTP was 15.7 ± 0.63 % across the 16 samples of Griffonia seeds, ranging from 14.3 % (Xavi, VR) to 17.1 % (Japekrom, BAR). Variation of 5-HTP content was low in this collection of Griffonia accessions.

There appears to be a correlation between endosperm color and 5-HTP content. The highest values of 5-HTP were found in seeds with yellow endosperm, while the sample from Xavi, showing a light green endosperm, showed the lowest values (14.3%). The endosperm of the sample from Nkrankwanta was green/yellow and showed a slightly higher 5-HTP value (14.7%) (Tables I and II). There was no correlation between the 5-HTP content (as % of seed wt) and seed weight or seed moisture, while there was a weak correlation with the seed diameter ($r^2=0.24$) and total ashes ($r^2=0.35$).

These relationships are important to recognize as many buyers prefer to purchase Griffonia seed with high concentrations of 5-HTP and the linking of grading and final purchase price with 5-HTP content in the seeds is a major issue facing Ghanaian traders. The Ghana Standards Board is considering the minimum/maximum range of 5-HTP to be acceptable for exported Griffonia seeds and have recommended values of 10-12% while using agreed upon chemical methods of analysis. Our results suggest that the ranges now being considered for acceptable 5-HTP should be reviewed to allow for the range that one would find in Ghana over several years. These results also suggest that cross

lab validation and a comparison of the analytical techniques now employed and being considered as acceptable for routine analysis and screening be undertaken. The values we report here were not compared to other analytical methods.

While, this paper purposefully focused on 5-HTP, other compounds are of interest and natural Griffonia seeds are also known to 4-indole-3-acetyl-aspartic acid (IAAA) and 5-hydroxyindole-3-acetic acid (5-HIAA). In addition, the GSB recognizes the importance of the fatty acid profile of the seeds, and that changes in the fatty acids will reflect seed and oil age, quality and purity. As such, routine measurements of both free fatty acids and the peroxide value of the Griffonia seed oil are recommended to be tested and included in grades and standards.

In 1999, a major unknown compound was found in the chromatograms of Griffonia seed for the quantitation of 5-HTP. This caused a major industry concern as the unknown peak was thought to possibly be an industrial contaminant. Later, Klarskov et al. (10) reported that this unknown peak, named 'peak X' was actually a breakdown product of 5-HTP and not a contaminant. Now recognized as an oxidative form of 5-HTP, called peak X1 or 4,5-tryptophane-dione, the toxicity remains unknown but the compound can be removed during processing by crystallization. Peak X is not a contaminant of Griffonia but rather an artifact from the processing and analysis of the samples. Peak X has a main absorption band at 350 nm, and in this study we did not find any peak at this wavelength that relates to Peak X or Peak X1.

Since the lack of information on product chemistry and quality can affect its market access and limit prices received in country of origin, we propose the initial and selected standards for Griffonia seeds as presented in Table III, to strengthen the relationship between the collectors, processors and exporters and to provide clarity and product definition to the international buyers and users.

Table III. Proposed and selected QC Standards for *Griffonia simplicifolia* seeds.

<i>Characteristic</i>	<i>Griffonia seeds</i>
Organoleptic evaluation	
Seed color	Black, brown
Endosperm color	Yellow, Yellow/green
Macroscopic and sieve analysis	
Extraneous/foreign matter content (% m/m) max.	0.5
Damaged seeds (% m/m) max.	0.5
Physical-Chemical parameters	
Seed weight (g), min.	0.38
Seed diameter (cm), min.	1.5
Moisture (% m/m) max.	10
Total ashes (% m/m) max.	5
Total insoluble ashes (% m/m) max.	1
5-HTP content (% m/m) min.	12

Conclusions

Griffonia is a well known and valued West African medicinal plant used in Traditional African Medicine for a variety of conditions. The global market and interest in this plant is because the seeds contain high concentrations of the serotonin precursor, 5-HTP used in dietary supplements in the treatment of depression, anxiety, fibromyalgia and weight loss. Results of this research which examined and characterized the seed characteristics and quality from 16 growing regions illustrated the expected natural variation that may be expected across region and handling techniques. Data procured provided the foundation for developing quality control recommendations for the Griffonia seed which includes both the physical and chemical characteristics of the seed as well as the actual 5-HTP concentration.

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Chapter 22

Characterization of Essential Oils from *Cinnamomum camphora* T. Nees & Eberm. and *Ravensara aromatica* Sonnerat from Madagascar

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The commercial and identical Malagasy denominations, of ravintsara and ravensara essential oils have generated confusion in the marketplace. The essential oil of ravintsara is obtained from the leaves of *Cinnamomum camphora* which was introduced from Taiwan and now is growing widely in Madagascar. This essential oil has been misreported and traded as ravensara botanically classified as *Ravensara aromatica*. The true ravensara essential oil is extracted from the leaves of an endemic tree locally known in the Malagasy language as Havozo. The objectives of this study were, a) to provide collectors and the international community with morphological characters to differentiate the two species; and b) to characterize the chemistry and quality of both essential oils by profiling a large number of samples and to examine the genetic and chemical diversity which could lead to the identification of new chemotypes. This work has shown that the trees in the wild can be easily identified, thus avoiding misidentification during the collection and storage of the leaves. The organoleptic, physicochemical and chemical profiles of the essential oils of ravintsara (*C. camphora*) and

ravensara (*R. aromatica*) oils showed that both essential oils can also easily be differentiated using chemical fingerprinting. We propose new standards for their botanical and essential oil authentication and species identification.

Indigenous essential oils from Madagascar are important for income generation. Madagascar has been a significant exporter of essential oils, thus the country has tremendous potential to further expand the essential oil industry. However, the misinformation of their essential oils and the lack of standards to define product identity and quality are among some of the problems and obstacles that affect the industry (1-2). Typical examples of this lack of information are the ravintsara and ravensara essential oils that have been traditionally misidentified in the marketplace (3-6).

The essential oil of ravintsara is obtained from the leaves of *Cinnamomum camphora* which was introduced from Taiwan and now is growing widely in Madagascar. This essential oil has been misreported and traded as ravensara or *Ravensara aromatica*. The true ravensara essential oil is extracted from the leaves of an endemic tree locally known as Havoza (6-10).

Previous work have reported the initial standards for these two oils and compared with commercial samples (8). The essential oil of ravintsara (*C. camphora*) is dominated by high levels of 1,8-cineole, and is characterized by a fresh and eucalyptus-type aroma. The essential oil of ravensara (*R. aromatica*) is characterized by varying levels of limonene, methylchavicol and methyleugenol, with licorice and spicy notes.

Since *R. aromatica* is an endemic tree, the presence of chemical variation in the essential oils within the species is anticipated (7-8). The objectives of this study were, a) to provide collectors and the international community with morphological characters to differentiate both trees; and b) to characterize the chemistry and quality of both essential oils by sampling a large number of samples to better characterize each essential oil and identify new chemotypes.

This was a collaborative effort between the Agribusiness in Sustainable Natural African Plant Products (ASNAPP) program at Rutgers University, Landscape Development Interventions (LDI – USAID/Madagascar) and PRONABIO (Association of Malagasy Exporters of Natural Products), to develop grades and standards for Madagascar to ensure export product quality, consistency and traceability.

Material and Methods

The essential oil of *C. camphora* (199 individual samples) were obtained from leaves of cultivated trees harvested from two geographical areas, the central highlands and the eastern coast. The essential oil of *R. aromatica* (130 individual samples: 115 sabinene - limonene types, 10 methyleugenol types, and 5 methylchavicol types) were manually collected from leaves of wild indigenous

plants of the Malagasy natural rainforest located in the central highlands and the Eastern coast.

The essential oils were extracted by hydrodistillation and the organoleptic and physicochemical (density, refractive index, optical rotation and flash point) properties were assessed for each essential oil, according to the Food Chemical Codex protocols (11) (Table II).

The analysis of essential oil components was performed by GC/MS analysis. Individual identifications were made by matching their spectra with those from mass spectral libraries (Wiley 275.L) and the identity of each component was confirmed by comparison of its Kovat's index (12) with those from literature(13) (Table III). Individual oil constituents were reported as relative percent of total essential oil.

Results and Discussion

One of the factors that has contributed to the botanical misidentification of both species, is that collectors are usually not trained to differentiate the trees (9,10). The morphological characters of the leaves, flowers and fruits clearly showed that both trees can be easily differentiated, thus providing a guide for collectors to avoid the misidentification in the field (14, 15).

The leaves of the *C. camphora* tree were elliptic, with a cuneate base and a characteristic domatia, while the leaves of *R. aromatica* were usually asymmetrical, obovate lacking the domatia (Table I).

As previously reported, the ravintsara essential oil showed fresh notes, recalling that of the pure 1,8 cineole. However, the oil was also characterized by low spicy notes. The oil was colorless or slightly yellow with refractive indexes ranging from 1.4608 to 1.4688, with densities from 0.900 to 0.916 and optical rotations -15 to -20°, with a flashpoint of 43°C (Table II). The essential oil composition showed that the ravintsara essential oil was rich in 1,8 cineole (49-69%), with minor and varying amounts of sabinene (8-17%) (Table III).

In contrast, the essential oil of ravintsara was highly homogeneous, and the essential oils of ravintsara showed variation in the organoleptic and physicochemical profiles (Table II-III).

Table I. Morphological Differences between *C. camphora* and *R. aromatica* (14,15,16,17)

Plant part	Characteristics	<i>C. camphora</i> E.	<i>R. aromatica</i> S.
		Introduced to Madagascar, native to China and Japan	Endemic to Madagascar
Leaf	Blade shape	Usually elliptic, rarely ovate	Usually obovate, rarely elliptic
	Blade base	Cuneate	Asymmetrical
	Venation	With domatia	Without domatia
	Petiole	Reaching 3 cm	Maximum 1.5 cm
Flowers	Tepal color	Yellow	Green
	Shape	Ovoid	Sub-globular
Fruit	Type	Berry	Drupe
	Locule	1 septum	6 septa
	Seed	1	6
	Odor	Odorless	Characteristic of aroma of cloves

Three distinct chemotypes were observed. The first chemotype, which is the most common in the marketplace, is characterized by a slightly yellow/greenish color with spicy and liquorish notes. The other two chemotypes yielded light yellow or colorless essential oils with eugenol and anise-like notes, respectively (Table II). Each of these essential oils showed a characteristic physicochemical profile, the first chemotype showed the lowest refractive index 1.4858, while eugenol and anise-like essential oils showed higher levels (1.4920 and 1.5170, respectively). The first chemotype showed the lowest density, suggesting low amounts of phenols, while the second chemotype was 1.008 and the third slightly lower (0.973). The first chemotype included the most optically active essential oils (-47), whereas the essential oils from the second chemotype exhibited lower values (-22), and the oils of the third was the least active (-4.15). The flash point of 47°C of the first chemotype suggested the presence of more volatile components as opposed to the second and third chemotype (>100°C and 70°C, respectively) (Table II).

Table II. Comparative Organoleptic and Physicochemical Properties of *C. camphora* and *R. aromatica* Essential Oils from Madagascar

Name	<i>C. camphora</i>		<i>R. aromatica</i>	
Plant part	Leaves		Leaves	
Chemotype	1,8-cineole	Sabinene-limonene	Methyleugenol	Methylchavicol
Number of samples	199	115	10	05
Organoleptic evaluation				
Aroma	Fresh, cineole type, low spicy note	Spicy, liquorish	Eugenol-like	Anise-like
Color	Colorless to slightly yellow	Slightly yellow green reflection	Slightly yellow	Colorless
Physicochemical properties				
Refractive Index	1.4608 to 1.4688	1.4658 to 1.5058	Average 1.4920	Average 1.5170
Density	0.900 to 0.916	0.867 to 0.907	Average 1.008	Average 0.973
Optical Rotation	-15 to -20°	-40° to -34°	Average -22°	Average -4.15°
Flashpoint	43°C	47°C	> 100°C	70°C

The essential oil composition as determined following the chemical analysis of the ravensara essential oils was as expected and in accordance with the described organoleptic and physicochemical profile. The first chemotype was dominated by varying amounts of sabinene, limonene and methylchavicol. The second chemotype was dominated by high levels of methyleugenol and the third type was characterized by high levels of methylchavicol.

Table III. Chemical Composition of Ravintsara (*C. camphora*) and Ravensara (*R. aromatica*) Essential Oils from Madagascar.

Botanical name	<i>C. camphora</i>		<i>R. aromatica</i>	
Plant part	Leaves		Leaves	
Chemotype	1,8-cineole	Sabinene-limonene	Methyeugenol	Methyl chavicol
α -pinene	2.5 - 7.5 ¹	3 - 10	0.22	0.52
β -pinene	2 - 4	0 - 6	-	0.28
sabinene	7.5 - 17	3 - 26.5	0.30	1.42
δ -3 carene	-	1 - 6	tr	0.12
myrcene	0.5 - 2.5	0 - 7	tr	0.24
α -phellandrene	-	0 - 5	-	0.1
α -terpinene	0 - 2	-	-	0.1
limonene	0 - 2	4 - 31	0.87	2.95
1,8-cineole	49 - 69	0 - 4	0.81	0.21
γ -terpinene	0 - 2.5	0 - 5	-	-
linalool	-	1.5 - 8	0.92	1.21
β -caryophyllene	-	0 - 7	tr	1.35
terpinene 4-ol	0.5 - 4.5	0.4 - 5	0.99	0.42
α -terpineol	3 - 13	-	Tr ²	0.13
methylchavicol	-	0 - 19.5	1 - 5	up to 76
germacrene-D	-	0 - 9.5		0.75
methyleugenol	-	0 - 17	Up to 77	7 - 10

NOTE: 1. GC average of total area %, 2. tr: traces amount (less than 0.1%)

Three chemotypes are attributed to *R. aromatica*: (1) the sabinene-limonene chemotype which is the common and the one typically found and traded in the Malagasy and international marketplace (8,18); (2) a methyleugenol chemotype, already described in previous work and confirmed in this study (19,10); and finally (3) a methylchavicol chemotype (Table III). This is the first description of this chemical variation in this species and was likely found only due to the high number of plants sampled to comparatively identify botanical and chemical markers that would facilitate botanical authentication for both species. Of all those samples only five were of this newly described chemotype (Table II).

The Principal Components Analysis (PCA) and Factorial Discriminating Analysis (FDA) (20,21,22,23) were used to discriminate the different ravensara chemotypes. One major cluster dominated the variability of the ravensara essential oil, showing a higher complexity in the essential oil profile, with different components found in high amounts (>5%) (e.g. α -pinene, sabinene, limonene, methylchavicol, methyleugenol, β -caryophyllene and germacrene D). The other two minor clusters were characterized by methylchavicol and methyleugenol (Figure 1).

PCA and FDA methods were previously used to solve the Ylang-Ylang oils classification problem (24) and identification of niaouli essential oil chemotypes (25), showing the usefulness of these methods in the characterization of different essential oils.

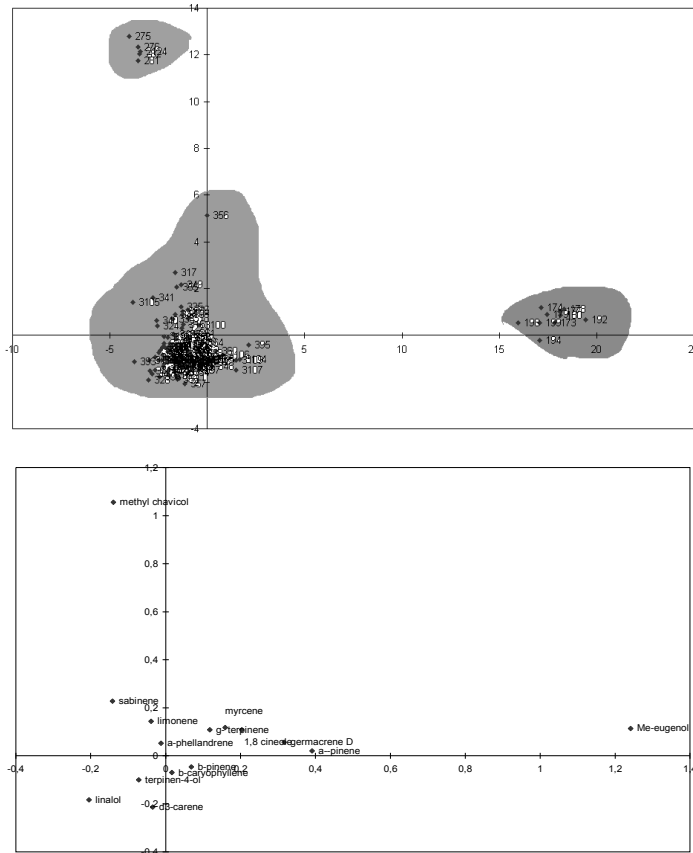


Figure 1. Principal Components Analysis (PCA) and Factorial Discriminating Analysis (FDA) of *Ravensara aromatica* Essential oils (26).

Table IV. Quality Standards¹ for the Essential Oil of Ravintsara (*C. camphora*) and Ravensara (*R. aromatica*)

Name	<i>Cinnamomum. Camphora</i> (L.) J. Presl	<i>Ravensara aromatica</i> Sonn.
Plant part	Leaves ²	Leaves ²
Chemotype	1,8-cineole	Sabinene-limonene
Aroma	Fresh, cineole type, low spicy note	Spicy, liquorish
Color	Colorless to slightly yellow	Slightly yellow to green
Refractive Index	1.4608 - 1.4688	1.4658 - 1.5058
Density	0.900 - 0.920	0.867 - 0.907
Optical Rotation	-11 - -22°	-40° - -34°
Flashpoint	43°C	47°C
Chemical composition		
α -pinene	2 – 8	3 - 10
β -pinene	2 – 5	0 – 6
sabinene	7 – 18	3 – 27
δ -3 carene	–	1 – 6
myrcene	0.5 – 3	0 – 7
α -phellandrene	-	0 – 5
α -terpinene	0 – 2	-
limonene	0 – 2	4 - 31
1,8-cineole	49 – 69	0 - 4
γ -terpinene	0 – 3	0 - 5
linalool	-	1 - 9
(E)-caryophyllene	-	0 - 7
terpinene 4-ol	0.5 – 5	0.4 - 5
α -terpineol	3 – 13	-
methylchavicol	-	0 – 20
germacrene-D	-	0 – 10
methyleugenol	-	0 – 17

NOTE: ¹These standards were developed from the chemical screens conducted during this study and which built upon prior work conducted in Madagascar (8); ²Leaf shape as described in Table I would be used in species authentication.

Conclusions

The results from this work has shown that the trees in the wild can be easily identified using both botanical as well as chemical markers, thereby avoiding misidentification while collection the leaves. The organoleptic, physicochemical and chemical profiles of the essential oils from both ravintsara (*C. camphora*) and ravensara (*R. aromatica*) oils also showed that the essential oils can also be used to differentiate the species. Based upon our data set of 199 individual plants and samples, we offer revise quality control standard specifications (Table IV), to describe the essential oil of ravintsara and ravensara

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Chapter 23

Quality and Consumer Studies in the USA of African Herbal Teas for the Natural Product Industry Development in Sub-Sahara Africa

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In Africa, many plant products have been used over the centuries as herbal teas, and as popular local drinks used as refreshing beverages and for their perceived health and medicinal attributes. With the regionalization and globalization of foods, beverages and natural products, the introduction of new drinks such as African teas into other countries and even regions in Africa can provide new herbal teas to a wide range of new consumers. African herbal teas can also be exported outside the continent into Europe, North America and Asia and in doing so have potential to contribute to the economic development of those involved in the collection or production, processing, packaging and distribution of such products. Information on the quality, chemical analysis and health attributes, and consumer response to new products contribute to the commercialization and marketing success of any product. The aim of this study was to assess the quality parameters, proximate analysis of a few selected African herbal teas (Honeybush, Kinkeliba, Lemongrass, Lippia, Rooibos) during three consecutive years (2004 through 2006). This study also sought to conduct a consumer study to evaluate the intensity of color and flavor

and liking ratings for four different African teas to an American college-based audience in the USA.

In Africa, many valuable natural plant products and botanicals have been used for centuries as traditional foods and medicines for health and well being and for the prevention and treatment of diseases (1), and are becoming increasingly important as income generating activities for rural communities (2). African herbal teas are becoming increasingly popular not only because of their aroma and flavor but also because of their functional properties and consumer interest in the health promoting properties of such beverages. Herbal teas are important sources of polyphenolic antioxidants (1), but they can also provide modest amounts of minerals to the diet (3). The improvement of locally produced herbal teas, as well as the development of new and improved products can generate new commercial opportunities for African communities at the local and regional levels. Studies that can identify new applications and uses of exotic products can also potentially lead to new bioactivities that improve health and ultimately assist growers and rural communities by increasing interest in their products (4). African herbal teas have been used for centuries in Africa either as refreshing beverages or as medicines, and they are beginning to be more widely available both across sub-Saharan Africa, as well as of increasing interest to international consumers.

Numerous factors play a role in the behavioral response of consumers to novel foods and beverages. Of particular importance are their sensory and overall quality, and attitudinal or personality variables of potential consumers (5). Another significant factor that can affect consumer response is the available information on the products and the “story” behind a product. Thus, the lack of familiarity and access as well as the lack of scientific information on the chemical composition, quality parameters and health benefits of herbal teas from Africa, may certainly limit the access to western oriented and Asian new food and beverage markets (6).

This study evaluates leading national or regional ‘bush teas’ which refer to the local indigenous herbal teas, popular in each of the African countries yet all but one are not well known to the North American, European and Asian markets. Kinkeliba is a popular infusion in Senegal, that is prepared from the dried leaves from a tree (*Combretum micranthum*). The leaves showed diuretic and collagogue properties and several components have been identified including flavonoids, catechins, and organic acids (7).

Lippia multiflora is known as Gambian bush tea. In Ghana the infusion of sun-dried leaves is consumed as a tea, often with sugar and appreciated by children as well as adults for its taste and for medicinal purposes (digestive complaints) (8) and as a carminative and stimulant depending upon the time of day when it is consumed. In Ghana, this herbal tea is traditionally used to relieve stress, relax, induce a good strong sleep and as a mild laxative.

Honeybush (*Cyclopia spp.*) is a traditional African tea native to the Western Cape Province in South Africa. The leaves and the flowers showed a characteristic honey scent from which the name Honeybush is derived (9). The

tea is produced from the aerial parts (leaves, flowers and stems) of the shrubs through a process of fermentation and drying. Honeybush tea is a rich source of bioactive polyphenolic compounds with the major ones being mangiferin and hesperidin (1). Traditionally, honeybush is used also as a carminative, to treat colic in infants, digestive aid and to overcome sleeplessness. Rooibos, *Aspalathus linearis* (Fabaceae) which among all the African herbal teas is the one whose market has significantly expanded into the international trade comes from the leaves of a shrub indigenous to the to the Cedarburg and neighboring mountains of the Western Cape Province. Rooibos, also known as red tea, is another very popular fermented and dried tea indigenous to South Africa (10).

Lemongrass tea, made from the dried leaves of the grass, *Cymbopogon citratus*, is a well know infusion in international markets, widely used in traditional medicine. Lemongrass is native to southeast Asia but long naturalized in many regions across sub-Sahara Africa, This is the one product of our study that is familiar to many in the North American, European and Asian market. The leaves make a refreshing lemon-like infusion. This grass has been used as antispasmodic, hypotensive, and analgesic, among other uses (11).

The aim of this study was to assess the quality parameters and proximate analysis of the African herbal teas Honeybush (*Cyclopia* sp., South Africa), Kinkeliba (*Combretum micranthum*, Senegal), Lemongrass (*Cymbopogon citratus*, Zambia), Lippia (*L. multiflora*, Ghana), and Rooibos (*Aspalathus linearis*, South Africa). These teas, available through a number of retailers and packagers in each of these African countries as well as under the Mpuntu brand of African teas (www.asnapp.org), were studied during three consecutive years (2004 through 2006).

Understanding the response of targeted consumers to any new product is a key strategy that can contribute to the commercialization and marketing success of a product. As such, this study also sought to conduct a consumer study to evaluate the intensity of color and flavor and liking ratings for four different African teas (Honeybush, Kinkeliba, Lippia, and a blend of Honeybush and Lippia). The Lemongrass and Rooibos Mpuntu teas were not included in this consumer assessment since both herbal teas are well-known and already sold in the USA as found in a multitude of commercial products both alone and in a wide variety of blended formulations. This study was limited in that we drew heavily upon a college audience and did not reach out to specific ethnic communities living in the USA which would either be more familiar with the specific herbal tea from their or their family's country of origin or to those simply preferring teas over coffee and unique 'herbal infusions' or teas over traditional green or black teas.

Material and Methods

Plant Material

Five commercial teas from the Mpuntu Brand of African Teas (ASNAPP South Africa, Welgevallen Farm, Stellenbosch) produced in the years 2004, 2005 and 2006 were used in this study. This included Honeybush, Kinkeliba, Lemongrass, Lippia, and Rooibos.

Ten boxes of teas, each box containing 20 tea bags (2.5 g of dried tea, 1 g for Kinkeliba) were randomly selected to make two composite samples of 100 grams each, thus all the quality and chemistry procedures were run in duplicate.

Quality and Chemistry Procedures

The color of the dried teas was determined visually. Each subsample was weighed (2 g) and then gently placed in an oven (85°C) until constant weight for the determination of moisture percent was reached. The dried teas were then ground (mesh 20) and total ashes, and acid insoluble ashes were determined for each sample using methods described by the Food Chemical Codex (12). A sieve (250 μm) was used to separate the fine particles and then weighed to calculate their percentages in relation to the total mass of dried teas (10 g). For total phenols and antioxidant activity the ground teas were extracted in 60% methanol (in water) following the Folin Ciocalteu's and ABTS procedures (13). The dried teas were submitted to the Agricultural Analytical Services Lab (Penn State University Soil and Plant Testing Laboratory) to determine the concentration 11 elements including: phosphorus, potassium, calcium, magnesium, manganese, iron, copper, boron, aluminum, zinc and sodium.

All the samples were tested for total caffeine using an HPLC-ESI-MSD analysis compared against a prepared caffeine standard and a commercial coffee sample (Maxwell House Original Roast, Northfield, IL, USA). The caffeine standard was prepared by dissolving 5 mg of caffeine in 25 mL of 70% methanol in water and the coffee and tea samples were prepared by sonicating 200 mg of material in 25 mL of 70% methanol for 30 minutes. The extracts were filtered through a 0.45 μm filter before injection into the HPLC. The LC method utilized ODS3 column, 5 μm , 150 x 3.2 mm, and a solvent system consisting of 5% to 30% acetonitrile in 0.1% formic acid in water over 40 minutes. This method allowed the caffeine peak to elute at approximately 15.5 minutes.

Consumer Study

Panelists were recruited through E-Mail notification from the School of Environmental and Biological Sciences, at Rutgers University. The panelists were healthy men and women ages 18 and over. A total of 74 panelists participated in the study; however four were not included in the data analysis as

they consumed tea less than once per month. Two similar studies with these herbal teas were conducted two months apart and as the results were similar, only the results of this second study are presented.

Four herbal teas were evaluated: Honeybush tea; Kinkeliba tea; Lippia tea; and a blended tea (10:1 Honeybush:Lippia). These teas belonged to the 2005 production. One liter of each sample was brewed with 14.4 g of tea (six tea bags) every hour. Each liter of tea was sweetened with 20 grams of sugar and kept warm in an eight-cup coffee server. At the end of each hour, the unused tea was discarded and the servers were rinsed and filled with the freshly brewed tea. Each tea was poured just before the panelist rated it to ensure all the teas were served at the same temperature.

Panel Procedures: A single session was conducted in the Sensory Evaluation Lab in the Food Science Building on the Cook College Campus. The session ran from 10:00 am–2:00 pm. Panelists were given general instructions on how the taste panel would run before the samples were distributed.

Forty-five mL of each sample was poured into Styrofoam cups labeled with a three-digit code number and covered with a lid. The four samples were presented to panelists one at a time in a random order. Panelists were provided with water, a spit cup, and a napkin, and instructed to rinse between samples. After completing the test, the panelists were compensated for their participation.

Ballot: Nine-point end-anchored category scales were used to measure both the intensity and liking of the following attributes: color darkness, tea aroma, sweetness, bitterness, tea flavor, mouth drying, and aftertaste. The intensity scales were anchored with “very weak” on the far left and “very strong” on the far right. The liking scale was anchored with “dislike extremely” on the far left, “nl/nd” (neither like no dislike) in the center, and “like extremely” on the far right. After rating the intensity and liking of the attributes, the panelists were instructed to check boxes next to the terms “woody,” “green” (i.e., vegetative), “spicy,” “earthy,” and “dried herbs,” if they felt any of the terms applied to the tea being tasted. The panelists were also given the opportunity to write, in their own words, other terms that they felt described the tea. Finally, overall liking and how likely panelists would be to buy the tea were rated on nine-point end-anchored scales for each tea.

Demographic information collected included gender, age, and ethnicity. Data was also collected on frequency of hot tea consumption in the past month, the type of hot tea usually drunk (black, green, herbal, decaffeinated, or more than one kind) and the type of condiments that were used (milk/dairy, sweetener, lemon, or nothing). Panelists also checked a box to indicate whether or not they had participated in the previous tea study.

A short description of the teas was placed at the beginning of the ballot as follows:

“The herbal teas you are tasting today are organic and caffeine-free. These distinctive teas come from wild plants that are native to South Africa, Ghana and Senegal. We searched the countryside to find plants that give these teas the best flavor and aroma. The tea leaves are grown or collected by local communities and part of the profits from these products is returned to these communities.”

Data Analysis: Mean intensity and liking ratings for all attributes and products were calculated and the data were analyzed using SAS 9.1 (SAS Institute, Inc. Cary, NC USA). If the ANOVA results were significant, a Duncan's multiple range test was performed to determine which samples were different from one another. Two and three-factor ANOVA models were also calculated to determine the influence of the demographic variables and tea drinking habits on the results. Frequency distributions of responses for overall liking and purchase intent were also constructed to probe for trends in the data. These data were analyzed using Chi-square analysis. The frequency of selection of the check-box terms was also tabulated and analyzed by Chi-square analysis. The statistical cut-off criterion used was $p < 0.05$ for all tests.

Frequency responses for overall liking and purchase intent were analyzed using Chi-square analysis. For ease of interpretation, the nine response categories were condensed into three categories. For overall liking, response categories 1-3 were condensed into a single category labeled "dislike", response categories 4-6 were condensed into one category labeled "neutral", and response categories 7-9 were combined into one category labeled "like". The likelihood to buy ratings was also condensed into three categories labeled "unlikely", "neutral" and "likely" to buy.

Results and Discussion

Quality

The raw dried material of each the tea exhibited the characteristic and distinctive colors expected of the botanical (Table I). Honeybush in each of the three years was characterized by light red/orange color; while Kinkeliba dried leaves were dark green. Lemongrass dried leaves were light green, with the samples from 2005 and 2006 exhibiting a yellow/green to green color. Lippia tea from 2004 was dark brown while the 2005 and 2006 samples showed lighter colors. The dark brown color could suggest excessive heat during drying. Rooibos was characteristically red brown (Table I). These results were expected as each tea had been quality control tested prior to being permitted to be used in the Mpuntu brand.

All packaged finished herbal teas exhibited low values of moisture ($<10\%$), while Lippia was higher at moisture contents ranging from 11 to 12% (Table I). Rooibos and Honeybush were characterized by low levels of fine particles (less than 0.3%), while all the other herbal teas contained higher values ($>1\%$), and the Kinkeliba teas processed during 2005 and 2006 with the highest levels (8%).

The amount of total ashes found showed that the African teas exhibited varying levels of total minerals (Table I). Honeybush and Rooibos exhibited low levels of total minerals for each of the three years (1.4-4.5 and 1.8-2.2%, respectively). Kinkeliba was characterized by intermediate values (4.6-4.8%), Lemongrass contained higher levels (6.8-8.7%), while Lippia had consistently the highest levels (12-13%) (Table I).

Total insoluble ashes, a classical determination of the cleanliness of botanical products, were low (less than 1%) in Honeybush, Kinkeliba and Rooibos, showing a low contamination with sand and earth. Lippia and Lemongrass, exhibited higher levels while a visual examination indicated no content of sand and earth. Our previous results (4), suggest maximum values of 4%. For Lemongrass, commercial standards suggest maximum values of 9%. The results obtained in this evaluation suggest that the high levels of insoluble minerals were due to the an endogenous content of insoluble minerals rather than to contamination with sand and earth (Table I).

Table I. Color, moisture, fine particle and ash contents of African Herbal teas produced from 2004 through 2006

<i>Herbal Tea and Year</i>	<i>Color</i>	<i>Moisture¹</i>	<i>Fine Particles¹</i>	<i>Total Ash¹</i>	<i>Total Insoluble Ash¹</i>
Honeybush '04	Light red	9.0 ± 0.4	0.1 ^a	1.5 ^a	0.2 ^a
Honeybush '05	Light red	8.3 ± 0.4	0.3 ^a	1.4 ^a	0.2 ^a
Honeybush '06	Light red	8.1 ± 0.2	0.1 ^a	1.5 ^a	0.2 ^a
Kinkeliba '04	Dark green	9.4 ± 0.1	1.2 ± 0.4	4.8 ^a	0.5 ^a
Kinkeliba '05	Dark green	9.1 ± 0.4	8 ± 0.6	4.6 ^a	0.7 ^a
Kinkeliba '06	Dark green	9.0 ± 0.0	7.5 ± 1.3	4.6 ^a	0.4 ^a
Lemongrass '04	Light green	9.0 ± 0.5	0.2 ^a	6.8 ^a	2.9 ^a
Lemongrass '05	Light yellow green	8.4 ± 0.2	3 ^a	7.6 ^a	2.7 ^a
Lemongrass '06	Light yellow green	9.5 ± 0.4	2 ^a	8.7 ^a	2.0 ^a
Lippia '04	Dark brown	11.3 ± 0.2	8 ^a	11.6 ^a	3.3 ^a
Lippia '05	Lighter dk. brown	12.4 ± 0.3	5 ^a	13.2 ^a	4.5 ^a
Lippia '06	Lighter dk. Brown	12.3 ± 0.4	3 ± 1.5	12.5 ^a	3.5 ^a
Rooibos '04	Red brown	9.7 ± 0.5	0.1 ^a	2.19 ^a	0.3 ^a
Rooibos '05	Red brown	9.6 ^a	0.1 ^a	1.8 ^a	0.3 ^a
Rooibos '06	Red brown	10.0 ± 0.2	0.2 ^a	2.1 ^a	0.2 ^a

NOTE: ¹percentages (g/100 g dry tea), ^a standard error less than 0.1.

High levels of fine particles are usually not desired in a tea since it can produce cloudiness in the infusion. The results we observed suggest the fine particles were originated from the dried material itself and not from the contamination from sand and earth.

The raw materials of each tea exhibited varying amounts of macro- and micro- nutrients (Table II). In general, Honeybush and Rooibos contain low levels of each element, as compared with the others teas (Table II). Both teas were low in calcium (<0.22%), potassium (less than 0.32%), magnesium (<0.22%) and iron (<10 mg/100 g dry tea, DT) (Table II). Lemongrass was high in potassium (2.3-2.4%), while Lippia was high in calcium (2.3-2.6%), and magnesium (0.6-0.8%), with lower levels of potassium (1-1.6%). Lippia also contained the highest levels of iron (15-27 mg/DT).

Malik et al. (3) reported for both Rooibos and Honeybush slightly higher levels of potassium (0.36% and 0.52%, respectively), similar levels of calcium (0.19% and 0.21%) and iron (12 mg and 6 mg/DT). These results support the fact that the Lemongrass and Lippia were rich in mineral teas.

Table II. Macro (P, K, Ca, Mg) and micro (Mn and Fe) elemental composition of African Herbal teas (2004-2006 production)

<i>Herbal tea</i>	<i>P</i> ¹	<i>K</i> ¹	<i>Ca</i> ¹	<i>Mg</i> ¹	<i>Mn</i> ²	<i>Fe</i> ²
Honeybush '04	0.03 ^a	0.26 ^a	0.22a	0.09 ^a	6.2 ± 0.1	6.6 ± 0.3
Honeybush '05	0.02 ^a	0.32 ^a	0.16a	0.09 ^a	2.6 ± 0.1	5.6 ± 0.3
Honeybush '06	0.03 ^a	0.30 ^a	0.19a	0.08 ^a	6.2 ± 0.2	9.9 ± 0.2
Kinkeliba '04	0.16 ^a	0.87 ^a	0.97b	0.28 ^a	85 ± 22	17.8 ± 6
Kinkeliba '05	0.14 ^a	0.74 ^b	0.94b	0.29 ^a	104 ± 5	21 ± 0.2
Kinkeliba '06	0.14 ^a	0.76 ^b	0.94a	0.29 ^a	101 ± 4	15 ± 1
Lemongrass '04	0.09 ^a	2.29 ^a	0.34a	0.16 ^a	8.0 ± 0.1	15 ± 0.8
Lemongrass '05	0.24 ^a	2.32 ^a	0.46a	0.20 ^a	5.6 ± 0.0	9 ± 0.2
Lemongrass '06	0.23 ^a	2.37 ^a	0.50a	0.16 ^a	6.6 ± 0.1	7.9 ± 0.0
Lippia '04	0.17 ^a	1.58 ^c	2.25a	0.55 ^a	5.7 ± 0.0	26 ± 0.5
Lippia '05	0.13 ^a	1.35 ^b	2.63b	0.69 ^a	7.8 ± 0.4	27 ± 12
Lippia '06	0.11 ^a	1.08 ^a	2.52a	0.78 ^a	7.4 ± 0.2	15 ± 0.1
Rooibos '04	0.05 ^a	0.29b	0.16a	0.15 ^a	4.3 ± 0.2	8.4 ± 0.4
Rooibos '05	0.02 ^a	0.24 ^a	0.17a	0.21 ^a	6.6 ± 0.2	8 ± 0.5
Rooibos '06	0.06 ^a	0.31 ^a	0.20a	0.20 ^a	4.8 ± 0.2	8.6 ± 0.7

NOTE: ¹percent (g/100 g dry tea), ²mg/100 g dry tea, ^a standard error equal or less than 0.01, ^b0.02, ^c0.03

This study also suggests that some of the African herbal teas can provide low but still significant amount of elements. Kinkeliba was significantly higher in manganese (~0.1%). These findings suggest that one tea bag of Kinkeliba can provide up to 50% (1 mg) of the USA recommended dietary allowances (RDA) of manganese (2 mg/day) for adults (14). A cup of Lemongrass tea (2.5 g of dry tea) could provide up to 1.6% (57 mg out of 3500 mg) of the RDA of potassium, with similar levels of potassium found in most sport drinks (36 mg).

The African herbal teas also contained varied levels of aluminum, boron, copper, sodium and zinc (Table III). Each of the teas was found to have low and safe levels of copper and aluminum. Kinkeliba and Lippia were found to contain higher levels of aluminum (18-28 mg/DT), though these values were much lower than the lowest-observed-effect level for aluminum according to animal studies (130 mg/kg/day) (15). All teas were also found to be low in sodium, though it is interesting to highlight that Honeybush and Rooibos contained the highest levels of sodium (76-109 and 242-243 mg/DT, respectively), while Lemongrass and Lippia showed very low levels (<8 mg/DT). All the teas were

also low in zinc, not providing significant amounts of this element (14) (Table III).

Table III. Micro (Cu, B, Al, Zn, Na) elemental composition of African Herbal teas (2004-2006 production)

<i>Herbal tea</i>	Cu^2	B^2	Al^2	Zn^2	Na^2
Honeybush '04	0.4 ^a	3.3 ^d	4.9 ± 0.4	0.8a	109 ± 2
Honeybush '05	0.2 ^a	2.8 ^d	6.1 ^d	0.5 ^d	76 ± 1
Honeybush '06	0.3 ^a	2.7 ^d	5.9 ^d	1.3 ^d	88 ± 1
Kinkeliba '04	0.6 ^d	3.2 ± 0.4	19.6 ± 12	2 ± 0.2	13.4 ± 6
Kinkeliba '05	0.6 ^d	3.1 ^a	26.5 ± 0.4	1.8 ^d	20 ± 0.7
Kinkeliba '06	0.6 ^a	3.1 ^a	22.5 ± 1.4	1.8 ^a	20 ± 1
Lemongrass '04	0.3 ^a	0.3 ^a	15 ± 0.4	1.5 ^d	2 ± 0.2
Lemongrass '05	0.4 ^a	0.6 ^a	5 ± 0.3	2.1 ^a	8 ± 0.2
Lemongrass '06	0.3 ^a	0.5 ^a	4.2 ± 0.7	1.9d	5 ± 1
Lippia '04	0.8 ^a	7.2 ^d	27.8 ± 2	1.6 ^a	7.5 d
Lippia '05	0.7 ^d	9.9 ± 0.3	17.4 ± 0.3	1.1 ^a	5.1 ± 1
Lippia '06	0.7 ^a	10 ± 1	18.2 ± 2	1.4 ^a	3.1 ^d
Rooibos '04	0.8 ± 0.5	3.9 ^b	7.6 ± 0.2	1.3 ± 0.3	343 ± 9
Rooibos '05	0.4 ^a	1.4 ^d	6.5 ± 0.4	1.0 ^d	242 ± 6
Rooibos '06	0.3 ^a	2.9 ^b	8.3 ± 0.3	0.9 ^a	330 ± 10

NOTE: ¹percent (g/100 g dry tea), ²mg/100 g dry tea, ^a standard error equal or less than 0.01, ^b0.02, ^c0.03, ^d equal or less than 0.1

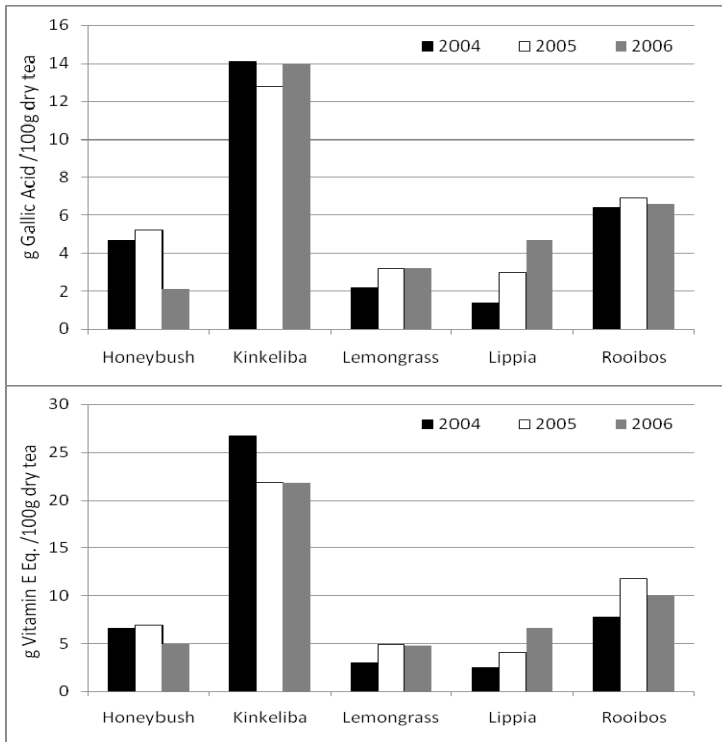


Figure 1. Total phenolics (top) and antioxidant activity (bottom) from five African herbal teas (2004-2006 production years)

The content of total phenolics and antioxidants also showed variation among the different herbal teas (Figure 1). Kinkeliba exhibited the highest amount of total phenols (13-14%), followed by Rooibos (6-7%). For the years 2004 and 2005, Honeybush showed slightly lower levels (4-5%), though for the year 2006, the polyphenols content were much lower (Figure 1 top). This could suggest the use of a lower grade material (e.g. one higher in stems to leaves) decreased the levels of polyphenols for 2006.

This same trend was also observed in the total antioxidant activity (Figure 1 bottom). The antioxidant activity also showed a similar trend, with Kinkeliba as the leading antioxidant tea (20-26%, expressed as g of vitamins E equivalent in 100 g of dried product (Figure 1 bottom).

The amounts of polyphenols increased during the years of study for both Lemongrass, from 3 to 5%, and Lippia, which increased from 3 to 6%. Germplasm selection and processing techniques (e.g. improved drying) produced an increment of antioxidant polyphenols in Lippia for 2006 (4).

For Rooibos and Kinkeliba, the consistent amount of phenols during the production years 2004 through 2006 (Figure 1), suggest a shelf life of at least two years, and a longer one if stored under good conditions.

Each of the African herbal teas including Honeybush, Kinkeliba, Lemongrass, Lippia and Rooibos were found to be caffeine free. Analysis by

HPLC confirmed that all the teas were devoid of caffeine when compared to commercial coffee and pure caffeine which was used as the standard (Figure 2).

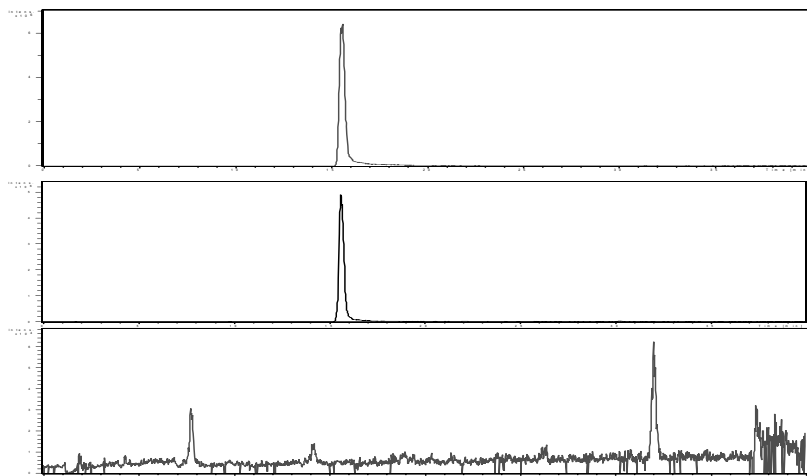


Figure 2. HPLC chromatogram of caffeine standard (top), coffee (middle) and Honeybush (bottom). The chromatograms for all of the African herbal teas also were devoid of the caffeine peak.

Consumer Reactions to the African Herbal Teas

Demographic and Tea Habits

Of the panelists participating, 69% were women and 31% were men. The largest group of respondents (46%) were between 26-35 years of age, with the other age groups distributed as follows: 27% 18-25 yr, 11% 36-45 yr, 11% 46-55 yr, and 4% older than 55 years of age. The majority of the panelists were either white (53%) or Asian (37%), with the remainder being, Pacific Islander (3%), and other (6%).

With regards to how often the panelists drank tea, 34% said they drank tea “everyday,” 39% responded “a few times per week,” 23% said “a few times per month,” and 4% responded “once per month.” The panelists usually drank more than one kind of tea (36%), while 27% drank only black tea, 16% drank only green tea, 20% drank herbal tea, and 1% drank decaffeinated tea. Of the 70 panelists participating in the test, 57% added at least one condiment (sweetener, milk, or lemon) to their tea, while 43% did not. Of the panelists participating in this taste-test, 50% had participated in the previous test as well.

Intensity and Liking

The intensity and liking ratings were analyzed using ANOVA and a Duncan's Multiple Range Test, where applicable. The results showed the perceived intensity of the darkness of color ($p < 0.0001$), tea aroma ($p < 0.0105$), sweetness ($p < 0.0001$), and tea flavor ($p = 0.0203$) were significantly different according to tea type (Figure 3). Duncan's multiple range test ($\alpha = 0.05$), showed, for darkness of color, that Honeybush, Lippia, and Kinkeliba were rated significantly different from one another, such that Honeybush was rated the highest and Kinkeliba was rated the lowest. The blend was not significantly different from either Honeybush or Lippia, but was rated significantly higher than Kinkeliba for darkness of color. For tea aroma intensity, Honeybush, Lippia, and the blend were rated significantly higher than Kinkeliba. For sweetness intensity, Honeybush, Lippia, and the blend were rated significantly different from one another, such that Honeybush was rated the highest and the blend was rated the lowest in sweetness. Kinkeliba was not significantly different from Honeybush or Lippia. Finally, for tea flavor intensity, Honeybush, Lippia, and the blend were all rated significantly higher than Kinkeliba, but were not different from each other. There were no differences for perceived intensity of bitterness, mouth drying, and aftertaste.

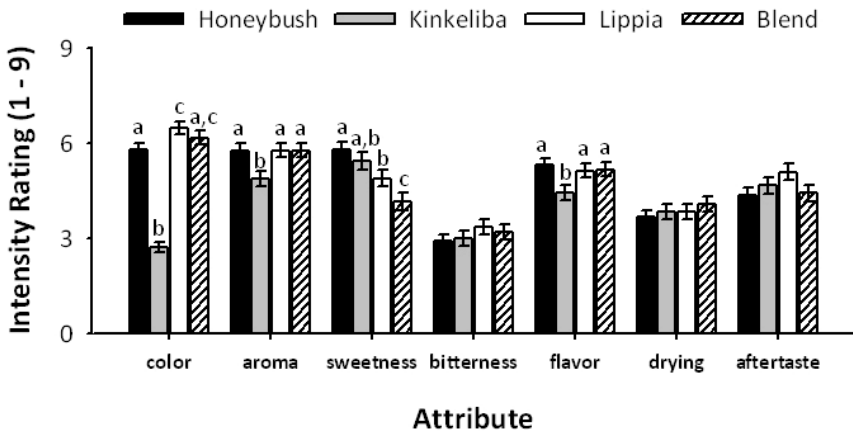


Figure 3. Intensity attribute ratings for African herbal teas (Honeybush, Kinkeliba, Lippia, and the blend).

Liking ratings for intensity of color ($p < 0.0001$), tea aroma ($p = 0.0004$), tea flavor ($p = 0.0005$), and overall liking ($p = 0.0017$) were significantly different according to tea type (Figure 4). Duncan's post-hoc test ($\alpha = 0.05$) showed that Honeybush and the blend were rated similar to each other and significantly higher than either Kinkeliba or Lippia for liking of darkness of color. Honeybush, Lippia, and the blend were rated similar to each other in liking of tea aroma and flavor, and all three teas were significantly higher than Kinkeliba for liking of these attributes. As stated above, there were differences in sweetness intensity among the teas (Figure 3), but no differences in sweetness

liking were observed by tea type. There were no differences among teas for sweetness, bitterness, mouth drying, or aftertaste liking. Overall liking ratings showed that Honeybush and the blend were liked the most and both teas were liked significantly more than Kinkeliba. Liking ratings for Lippia were in the intermediate range and not significantly different from any of the other teas.

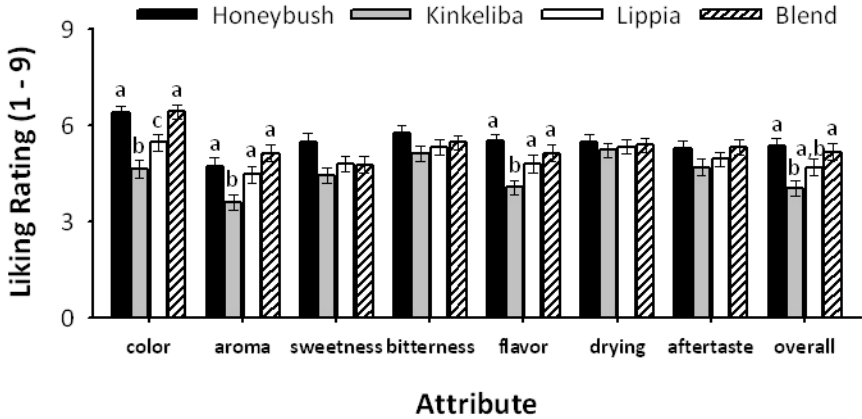


Figure 4. Liking attribute ratings for African herbal teas (Honeybush, Kinkeliba, Lippia, and the blend).

The check box attributes were analyzed using a Chi-square analysis (Figure 5), but none of the attributes were significantly different by tea type (at $\alpha=0.05$).

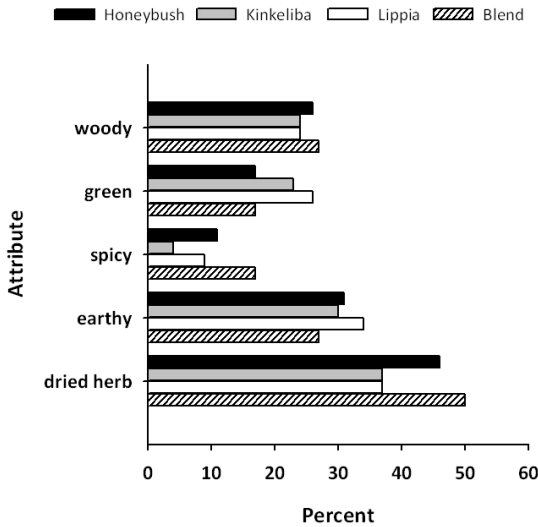


Figure 5. Percent of panelists selecting each attribute by each African herbal tea (Honeybush, Kinkeliba, Lippia, and the blend).

Overall Liking and Likelihood of Buying

Regarding the distribution of overall liking of the individual teas by frequency of tea drinking (categorized as “everyday” or “less than everyday”), no differences were observed in the pattern of overall liking responses of panelists who drank tea everyday as compared to those who drank tea less often for any of the teas (Figure 6). However, liking ratings for Kinkeliba were skewed toward the negative end of the scale for the panel as a whole.

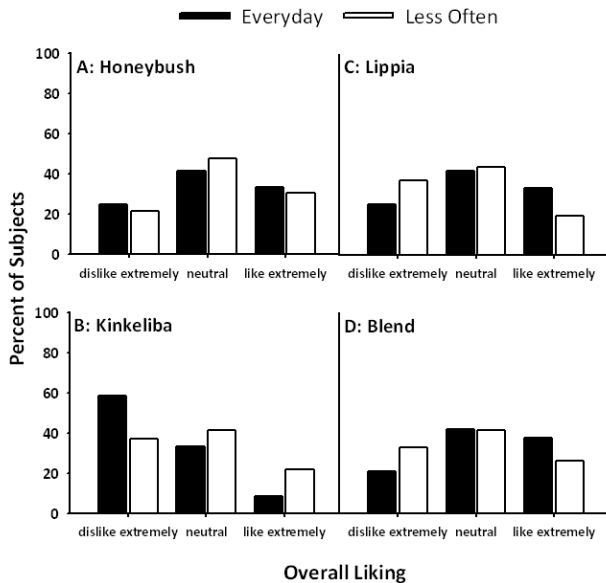


Figure 6. Overall liking ratings by frequency of tea drinking for African herbal teas (Honeybush, Kinkeliba, Lippia, and the blend).

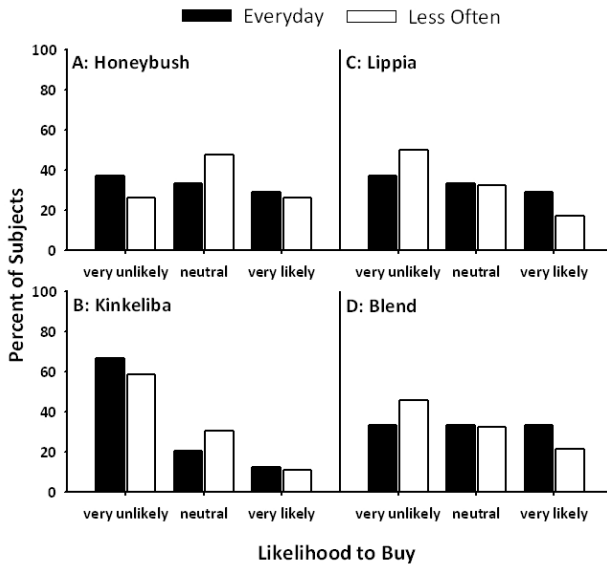


Figure 7. Overall likelihood of buying by frequency of tea drinking for African herbal teas (Honeybush, Kinkeliba, Lippia, and the blend).

The distributions of likelihood of buying the teas by frequency of tea drinking (categorized as “everyday” or “less than everyday”), was negatively skewed for Kinkeliba and Lippia but no differences were observed between everyday drinkers and those who drank tea less often (Figure 7).

The results of this study showed that intensity of color, aroma, sweetness and flavor distinguished the four teas to this group of panelists. Honeybush, Lippia, and the blend were rated similar in color, aroma, and flavor intensity, and all three teas were rated higher in intensity for these attributes than Kinkeliba. The teas showed a range of sweetness intensities. Honeybush received the highest sweetness rating, Lippia and Kinkeliba received intermediate sweetness ratings, and the blend received the lowest sweetness rating. It is unclear why the blend received lower sweetness ratings than Honeybush since the blend contained mostly Honeybush tea (10:1 Honeybush:Lippia). However, these differences in sweetness intensity did not affect liking of the sweetness of any of the teas. Rather, differences in color, aroma and flavor intensity contributed to differences in liking among the samples. Honeybush and the blend were liked the most for these attributes as well as liked most overall. Overall, Kinkeliba was preferred the least and Lippia was liked moderately well. Although the blend was liked as well as Honeybush, this formulation did not optimize consumer acceptance. Honeybush and the blend were the most preferred teas for this audience, and received positive ratings between 5 and 6 on the 9-point scale. Our findings are in line with overall acceptability ratings for Rooibos teas by Turkish consumers (16) and Oolong tea drinks by Taiwanese consumers (17).

Conclusions

Each of these African herbal teas showed distinctive sensory and chemical profiles. In general, all the teas showed low and acceptable levels of moisture. Lippia showed levels of moisture close the maximum accepted 12% for international markets, and as a consequence longer term storage and accelerated storage studies are needed to evaluate the effect of this level of moisture on the quality and shelf life. Rooibos and Honeybush herbal teas were also characterized by low levels of fine particles. Future efforts in all African herbal teas should be made to reduce botanical dust in the teas particularly for Kinkeliba. Fine particles can also be problematic depending upon the actual mesh of the tea bag itself leading to a cloudier tea after infusion. Honeybush and Rooibos contained low levels of mineral, while Lippia and Lemongrass can be considered as teas rich in minerals. Kinkeliba tea showed the highest amounts of antioxidant polyphenols.

Overall, Honeybush was 'liked the most' while Kinkeliba was 'liked the least' and Lippia was liked moderately well. These findings do not suggest one herbal tea will be more popular and successful than another but rather suggest that U.S. consumers are generally receptive to African teas although the marketing of each tea needs to be carefully planned and targeted to ensure that each product reaches the right consumers who would prefer that product. This study suggests that the market could be expanded as more consumers get to know these herbal teas and become familiar with their names, taste and associated story. The new information on the quality and chemistry of these African herbal teas will also contribute toward their commercialization, though in the end we conclude that color, taste and aroma will have a far greater impact on the consumer decision than nutritional benefit alone. Further studies should target consumer segments that have an interest in organic, green and fair traded products. Herbal African teas with exciting stories linking the products back to their communities may provide added value and purchasing incentives in the US marketplace.

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Chapter 24

Moringa (*Moringa oleifera*): a Source of Food and Nutrition, Medicine and Industrial products

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Moringa is the sole genus of Moringaceae family. There are 14 known species of the genus *Moringa*, which grow in the tropics. *Moringa oleifera* is the most widely distributed, the best known and the most utilized of the *Moringa* species. *Moringa oleifera* has a lot of potential for production of food products, medicinal products, water purification processes, renewable polymer products, animal and aquaculture feeds and even potential to be utilized in production of other crops. This chapter outlines the extent of research on *Moringa* towards utilisation as a food medicine and industrial product.

The Botany of Moringa

Family, Genus and Its Distribution

Moringa is the sole genus of Moringaceae family. It remains one of the most investigated and widely dispensed species among the monogeneric family Moringaceae. It is for its size, one of the most phenotypically varied groups of angiosperms (1). Almost all *Moringa* species are native to the sub-Himalayan

regions (India, Pakistan, Bangladesh and Afghanistan), from where they have been introduced to many warm countries (2, 3). There are 14 known species of the genus *Moringa*, now grown in the tropics including Africa, Arabia, Southeast Asia, the Pacific and Caribbean islands, and South America (4, 5, 6, 7).

Classification of Moringaceae

The classification of the Moringaceae has drawn a lot of controversy. Moringaceae, although very similar to the Capparidaceae, is said to form a family of their own (8). The monogeneric family Moringaceae has been traditionally placed in or near the Capparales because of the shared presence of glucosinolates and mustard-oils, along with several other characters, such as zygomorphic flowers, the presence of a gynophore, parietal placentation, and lack of endosperm. Especially in the light of recent cladistic analyses using macromolecular and morphological data, *Moringa* appears as part of an extended Capparales (5,9). A sister-family relationship of the Moringaceae with the Caricaceae is strongly supported by molecular phylogenetic studies (1,10).

The relationship of the Moringaceae with the Caricaceae contrasts with previous systematizations, none of which explicitly recognized a close relationship between the Moringaceae and Caricaceae (1). The comfortable position of *Moringa* in the Capparales has been challenged. Unusual characters are the regular pentamerous calyx and corolla (though occasionally present in a few Capparales such as Pentadiplandraceae), obhapplostemony with five antesepalous staminodes, hairy filaments with monotheical anthers, a trimerous gynoecium with deviating placentation, an aberrant pollen morphology, absence of a chlorophyllous embryo, and seed coat characteristics (121). In one instance, there is no evident serological relationship between *Moringa* and the other Capparales. Although *Moringa* has been placed in the Capparales, there is an admission that 'the resemblance (of *Moringa*) to some Sapindaceae may be of phylogenetic significance since *Moringa* is strongly reminiscent of some members of this family (122). The unusual laminal position of the placentation (in the middle of the carpels) in the tricarpellate gynoecium of *Moringa*, not parietal (alternating with the carpels) as in most Capparales is a significant differences that is noted (5,121).

Proposals have been made to shift Moringaceae to Violales near Violaceae, because of superficial similarities in the pentamerous zygomorphic flowers and embryological characters (33). A notable observation is the sufficient morphological evidence to raise Moringaceae to an order Moringales due to similarities with Malvales (123). On the basis of vegetative characteristics, there appears to be no evidence for a relationship of *Moringa* with either Violaceae or Capparaceae (84). *Moringa* has also been placed together with Bretschneidera within Leguminosae (Caesalpinaceae). Certain similarities of *Moringa* and Bretschneidera with Leguminosae are indeed impressive, such as the stipules reduced to glands ('Stipularnarben'), compound leaves, pentamery, hairy filaments with versatile anthers, a hypanthium ('Becherkelch'), and the curved 'Leguminosae'-style associated with zygomorphy. Furthermore, Moringaceae

has been removed from the Brassicales and transferred to Sapindineae of Rutales (5, 124, 125). However, the investigation of the floral development and anatomy of *Moringa* in the context of the disputed view of a capparalean affinity further concluded that, although *Moringa* shares important morphological features with certain members of the Sapindales and Capparales, differences in ontogeny make a close relationship with either Capparales or certain Sapindales appear uncertain (5). Another view is that, wood anatomy provides characters that are of potential phylogenetic utility at a variety of levels of relationship and this adds another moringaceae sister relationship with Caricaceae; concluding that based on wood anatomy and geography, the most likely sister taxon to *Moringa* is Cylicomorpha (Caricaceae) (1). Despite these several association and resemblance to several families, *Moringa* remains a moringaceae family member and is classified as such.

Moringa Species

At present, 14 species of *Moringa* have so far been identified (8,11,12, 13,14,15). However, just 13 species have been identified in the dry tropics of the Old World, spanning a vast range of life form (habit), from massive ‘bottle trees’ in Madagascar and Africa, to slender trees in Arabia and India, to shrublets with ephemeral shoots and underground tubers in Northeast Africa. *Moringa* trees show marked differences in their morphology. Based on gross appearance in the field, *Moringa* is divided into four habit or form classes: bottle trees, sarcorrhizal trees, slender trees, and tuberous shrubs. The bottle trees, slender trees, and tuberous shrubs display considerable within-class anatomical homogeneity. In contrast, the two species of sarcorrhizal trees show marked anatomical differences despite habitat similarity (1).

Bottle Trees (*Moringa drouhardii*, *Moringa hildebrandtii*, *Moringa ovalifolia*)

The bottle trees which include *M. drouhardii*, *M. hildebrandtii*, *M. ovalifolia* and *M. stenopetala* are characterized by massive trunks and swollen roots. The form of the trunk and roots are both similar, as is the bark colour and pattern of fissuring. Such bloated trees are also called ‘tank trees’ in reference to the large amounts of water stored in their swollen trunks. *M. hildebrandtii* is the largest member of the genus, commonly reaching 15m and can reach 25m. Trunk diameter of these species is commonly 60–100 cm but can exceed 2m (1).

Sarcorrhizal Trees (*Moringa arborea*, *Moringa ruspoliana*)

The Sarcorrhizal trees are characterized by a more slender trunk than the bottle trees with tough, smooth bark and thick, soft, fleshy, somewhat contorted roots that differ markedly in appearance from the trunk, having paler bark that is much softer and with different patterns of fissuring from that of the stem. They include *M. arborea* and *M. Ruspoliana* (1).

Slender Trees (*Moringa concanensis*, *Moringa oleifera*, *Moringa peregrina*)

The three principally Asian *Moringa* species, *M. concanensis*, *M. oleifera* and *M. peregrina*, have slender trunks at maturity and tough, fibrous roots with bark that is smoother, spongier, and more fragile than that of the stem. The root bark of all three species is less fissured than that of the very base of the stem (1)

Tuberous Shrubs (*Moringa borziana*, *Moringa longituba*, *Moringa pygmaea*, *Moringa rivae*)

Tuberous shrubs include *M. borziana*, *M. longituba*, *M. pygmaea* and *M. rivae*. Stems of the first three species are short-lived and persist only through favorable years, dying back to the large underground tuber during periods of extended drought. Only *M. rivae* forms permanent shoots with age but nevertheless also has a very large, very soft tuber underground. The root is much greater in diameter than the stem, is much softer, and has softer, paler bark that is more prone to forming polygonal plates rather than longitudinal fissures (1)

Moringa Oleifera - Importance

M.oleifera is the most widespread or widely distributed *Moringa* species (2,11). It is now found throughout the tropics and the best known and most utilized of all the *Moringa* species (2,15,16,17,110). A measure of the importance of the *M. oleifera* tree to communities across the globe is the multitude of indigenous names ascribed to it in individual countries and regions (36). *M. oleifera* is a multi purpose tree with most of its parts being useful for a number of applications (16). It is a tree of significant economic importance because of the several industrial and medicinal applications and various products used as food and feed which can be derived from the entire plant (6,7,27,72). The tree is now generally known in the developing world as a vegetable, a medicinal plant, coagulant and a source of oil (24).

Moringa Oleifera – Morphology And Physical Characteristics

M. oleifera is known under different names in different countries (20). In some parts of the world *M. oleifera* is referred to as the ‘drumstick tree’ or the ‘horse radish tree’ (Fig. 1) (15,18,19,21,80), whereas in others it is known as the kelor tree (21,22,80). In the Nile valley, the name of the tree is ‘Shagara al Rauwaq’, which means ‘tree for purifying’ (126). In Pakistan, *M. oleifera* is locally known as ‘Sohanjna’ (21,127). It is also known as benzolive tree, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree (22).



Figure 1. *M. oleifera* tree

M. oleifera has thick grey bark, fragrant white flowers (Fig. 2) and long green pods (Fig. 3) (7). The tree ranges in height from about 2–12 m (7,15, 23). It has a wide-open, umbrella-shaped crown and single stemmed; with white or cream coloured flowers . It has pale green, compound, tripinnate leaves; 3-6 cm in length, with 3-5 small leaflets that are 1-2cm long. It has characteristic elliptical lateral leaflets and obovate terminal leaflets, slightly larger than the lateral ones (Fig. 4). The fruits range usually 20–50 cm long (15,24). When matured, the fruit becomes brown and has 10–50 seeds inside (23,24,41). Fully mature, dry seeds are round, globular or triangular in shape, the kernel surrounded by a light wooded shell with three papery wings (15, 24). On average the seeds weigh approximately 3.0–4.0 g, 1.0–1.4 cm long and 1.0–1.7 cm wide (24). Dry matter (DM) yield of the tree is as high as 15 tons/ha/year (25).



Figure 2. *M. oleifera* flower



Figure 3. *M. oleifera* pod



Figure 4. *M. oleifera* leaves

***Moringa Oleifera* - Natural Habitat**

M. oleifera is native particularly to North India and sub-Himalayan regions (India, Pakistan, Bangladesh and Afghanistan) but now distributed in many locations in the tropic and subtropics countries of the world (15,16,17,26,27,110). *M. oleifera* now grows in Africa, Asia and South America regions (17,26,28,110). It is reported to exist in the Pacific region (7), West Africa (94), as well as Central America and the Caribbean islands

(6,7,17,27,110). It is also distributed in the Philippines, Cambodia, Central America, North and South America and the Caribbean Islands (21).

Moringa Oleifera - Cultivation

M.oleifera is a quick-growing ornamental tree (26,29,30,31). Large scale cultivation of *M. oleifera* has been reported in specific countries such as Malawi, Kenya and India (6) and until recently in Zambia. It can be propagated from seeds or cuttings. Seed propagation is first done in a nursery and the seedlings transplanted to the main field after 2 to 3 months. In stem propagation, mature stem cuttings of about 1.0 - 1.5 m long are planted direct into the main field. The *M. oleifera* plant is fast growing and depending on cultivar, produces its first fruits between the sixth and twelfth month after planting (96). Trees can reach 4 m high, flower and fruit in one year and multiple seed harvests are possible in many parts of the world (32). *M. oleifera* grows in all types of soil, from acid to alkaline, and at altitudes from sea level to 1800 m (25). These attributes make *M. oleifera* to be widely cultivated in tropical regions of Africa, South and Central America and in parts of Asia (96). It has been reported that the cultivation cost for producing 1 kg (3400 seeds) of *M. oleifera* is approximately US\$2 (24).

Moringa Oleifera – Drought Resistant Tree

M. oleifera is characterized as a drought resistant species and can tolerate a wide range of soil and rainfall conditions (4). It can grow well in the humid tropics or hot dry lands, can survive destitute soils, and is little affected by drought and is abundantly available throughout the year (12). *M.oleifera* flourishes well in humid and hot, semi-arid regions and wet conditions with annual rainfall between 250–3000 mm and temperature ranges from 26 to 40°C (12,20,34). It grows even during the 6 months of the dry season as it adapts under water stress (25). It is well adaptable to a variety of soil conditions, with well-drained sandy or loamy soils and a pH of 5.0–9.0 as the optimum (21,35).

Moringa Oleifera - Food

History of Food Use

As a traditionally important food commodity, *M. oleifera* has received attention as “natural nutrition of the tropics” and is consumed by humans in various forms and ways (17, 80). Almost every part of the *M. oleifera* tree is of value for food (15). The leaves, flowers, fruits and roots of this multipurpose tree are esteemed as a vegetable (17, 80). Leaves and seeds of *M. oleifera* represent an important source of nutrients for rural populations in certain areas of India and West Africa (27,100). *M. oleifera* leaves are currently the focus of

several development projects promoting their use as a valuable nutritional source for human consumption (36, 111).

Moringa Oleifera – Food Use

Different parts of *M. oleifera*, i.e., leaves, flowers, roots and fruits, have traditionally been used for dietary purposes (110). In India and Philippine, village people use the fresh leaves to prepare fat foods (38,110). Fresh leaves have been used by Indian inhabitants for the preparation of cow and buffalo ghee from butterfat (107). The leaves mixed with chicken soup are used by Philippino women to enhance breast milk production (4). The leaves are also eaten commonly as a food by infants and children in South India (28). The tree leaves are eaten as greens in salads and in vegetable curries in Malaysia and the Indian sub-continent (15,39). In Nigeria, *M. oleifera* leaves are eaten as cooked vegetables (28).

M. oleifera seed oil is pleasant tasting, highly edible and resembles olive oil in its fatty acid composition (15). The seeds are extracted for oil or used in curry powders (6). In Haiti and elsewhere, the oil has been used as general culinary and salad oil (15). In India, the oil obtained from *M. oleifera* seeds are used for cooking or frying (27).

M. oleifera kernels taste from sweet to very bitter (6,38). Seeds are consumed after frying and taste like peanuts. The fresh beans after roasting also make a palatable dish in many populations (38,48, 80,113).

Nutritional Composition

The *M. oleifera* plant has been consumed by humans for a long time in diverse culinary variety depending on the part of the plant. Almost all parts of the plant are consumed in one way or another and have been termed the “cow plant” in some parts of Zambia. Its unique composition in both macro and micro nutrients has been recognized and is the reason for it being advocated as part of diet in communities with food insecurity. The macro- and micro-nutrients of *M. oleifera* are differently distributed along its anatomical parts and this forms the basis for the preparation and processing of the plant parts for consumption. It's proximate composition is as shown in Table I.

Carbohydrates And Energy

Carbohydrate contents in seeds of *M. oleifera* ranges between 16.5-17.8%. The crude fibre contents ranges between 3.5-4.8% (15). The gross energy by bomb calorimetry approximates 18.7 MJ kg⁻¹ while the metabolizable energy is 9.5 MJ kg⁻¹ in *Moringa* leaves (6).

Proteins

The *M. oleifera* oil seeds have been found to contain high crude protein contents falling between 36.7 – 38.4% (15). The crude protein content of the leaves has been found to be 25.0% (6, 40). The true protein content of these leaves averages 81.3% of the total crude protein, with non protein nitrogen contents of 4.7%. Solubility of the proteins can reach 24% of the crude protein for the leaves. All essential amino acids including sulfur-containing amino acids in *M. oleifera* are higher when compared with recommended amino acid pattern of FAO/WHO/UNO reference protein for a 2 to 5-year-old child (6). The composition of amino acid analysis of *M. oleifera* leaves are given in Table II.

Crude Oil

M. oleifera oil has been characterized for different properties (15,47,127). *M. oleifera* has high contents of oil which makes it a potential raw material for the vegetable oil industry. Oil contents ranging between 25.1 and 41.4% have been reported depending on the extraction medium (2,15). The oil contents in the leaves ranges between 5.4 and 10.6% (6,40). However, the oil contents depend on a number of factors with significant variations in oil content as a function of season and agroclimatic locations (4,127).

Table I. Proximate composition of different anatomical parts of moringa plant

Sample and Reference ¹	M.(%)	Prot. (%)	Carb. (%)	C. oil (%)	Ash (%)	Energy
Seed(79)	6.8-7.1	32.1-34.4	21.1	39-43.4	4.3-4.5	588.4
Leaf Powder(95)	7.5	27.1	38.2	2.3	-	205
Leaves (Fresh) (95)	75.0	6.7	13.4	1.7	-	92
Pods(95)	86.9	2.5	3.7	0.1	-	26
Seeds(15)	-	36.7 – 38.4	16.5-17.8	-	3.2-6.5	-
Leaves (Fresh) (82)	-	6.7	-	-	-	95
Leaves (dried) (76)	-	26.4	-	ND	8.9	19.4 (MJ/kg)
Seeds(76)	-	36.7	-	41.7	3.8	26.7 (MJ/kg)
Fat free seed meal(76)	-	61.4	-	ND	5.6	19.4 (MJ/kg)
Leaves (dried, Whole) (6)	-	25.1	-	5.4	11.5	18.7 (MJ/kg)
Leaves dried ethanol extracted(6)	-	43.5	-	14.0	10.0	17.7 (MJ/kg)
Leaves dried(78)	8.6-9.0	30.5-31.8	-	-	-	-
Leaf meal(78)	-	23.3	-	-	-	-
Leaf meal(40)	-	25	-	10.6	8.4	-
Seed(142)	5.9–7.0	28.5–34.0	6.5–7.5 (Fiber)	-	6.5–7.5	-

NOTE: ¹ M.: Moisture, Prot.: Protein, Carb.: Carbohydrate (Fiber), C. oil: Crude Oil, Energy (kcal/100g)

Table II. Amino acids composition of *Moringa oleifera* leaves

Amino acid	g/kg DM (6)	g/kg DM (40)
Alanine	18.37	-
Arginine	15.64	12
Aspartic acid	22.16	-
Cystine	3.39	-
Glutamic acid	25.65	-
Glycine	13.73	-
Histidine	7.50	6
Isoleucine	11.30	9
Leucine	21.84	18
Lysine	14.06	11
Methionine	4.97	-
Phenylalanine	15.51	-
Proline	13.63	-
Serine	10.34	-
Threonine	11.70	8
Tryptophen	5.27	7
Tyrosine	9.71	-
Valine	14.26	11
Methionine + Cystine	-	6
Phenylalanine + tyrosine	-	19

Physical Properties of the Oil

Table III gives the physical and chemical properties of the *M. oleifera* oil. Some of the properties of the oil depend on the extraction medium. The *M. oleifera* oil is liquid at room temperature and pale-yellow in colour. Electronic nose analysis shows that it has a flavor similar to that of peanut oil. The melting point estimated by differential scanning calorimetry is 19°C (15). The chemical properties of the oil depicted in Table III below are amongst the most important properties that determines the present condition of the oil. Free fatty acid content is a valuable measure of oil quality. The iodine value is the measure of the degree of unsaturation of the oil. The unsaponifiable matter represents other lipid- associated substances like, sterols, fat soluble vitamins, hydrocarbons and pigments. The density, iodine value, viscosity, smoke point and the colour of *Moringa* oil depends on the method of extraction, while the refractive index does not. Varietal differences are significant in all physical characteristics apart from refractive index and density (2). The heating profile of the *M. oleifera* seed oil using the differential scanning calorimetry (DSC) conventional scan rate shows that there is one major peak B and, two small shoulder peaks A and C

(Table IV) (15). The onset (T_o) and offset (T_E) temperatures in Table IV indicate the start and the end of the melting process, while T_p represents the peak melting temperature. The shoulder peak A represents the melting temperature of unstable crystals of the low melting triglycerides (TAG) that melt prematurely. The more stable low melting unsaturated TAG crystals melts at a higher temperature peak B. The higher melting, more saturated TAG peak (C) appears at higher temperatures. The appearance of shoulder peak A is attributed to incomplete resolution of the melting peaks at conventional scanning rate. The incomplete resolution of peaks at 5°C/min (conventional) scan rate is due to re-crystallizing of the unstable low melting TAG crystals as slow scan rates allow the melting of unstable crystals that re-crystallize into more stable crystal forms when the temperature is raised. This causes the formation of shoulder peaks that would have otherwise formed separate peaks. Using HyperDSC™ (100°C/min) scan rate, the shoulder peak A is completely eliminated and the two peaks (B and C) representing the low and high melting TAG of the oil are completely separated, which makes characterization of the TAG species according to their melting behavior more accurate (15).

The cooling profile of the *M. oleifera* oil samples shows that the two sets of TAG crystallize at different temperatures. The higher melting TAG crystallizes at -5.1°C while the lower melting TAG crystallizes at -37.5°C. The complete melting and crystallization points are determined as the offset temperature values of the last melting and crystallization peaks, respectively. The peak temperature in the cooling profiles is considered as the temperature at which most of the oil crystallizes (15).

Fatty Acids Profile of the Oil

Extraction methods slightly influence the relative amounts of the fatty acids in *M. oleifera* oil (15). On the contrary, the extraction method does not significantly influence the fatty acid composition of the oils. Variety is also found to influence in the case of C16:0, C18:0, C18:1, C20:0 and C26:0 (2). However, the *M. oleifera* oil is highly unsaturated because of the high percentage of oleic acid (Table V). Oleic acid content ranges between 67.3 and 77.6%. The high percentage of oleic acid in the oil makes it desirable in terms of nutrition, high stability cooking and frying oil. Prominent saturated fatty acids in *M. oleifera* seed oil include palmitic (3.5 to 7.8%), stearic (4.0 to 8.3%), arachidic (2.5 to 4.7%) and behenic (4.6 to 6.7%) acids.

Table III. Physical and chemical properties of *M. oleifera* seed oil

Parameter	Cold pressure ^s		n-Hexane ^s		Chloroform:Methanol [†]		Light petroleum ether ^s
	(2)	(2)	(114)	(2)	(2)	(114)	
Oil content (g Oil/100g Seed)	25.1	38.1	35.7	41.4	31.2	30.8	
Density at 24°C (mg/mL)	0.899	0.909	0.8809	0.911	0.9182	-	
Refractive index (n_D 40°C)	1.460	1.457	1.4549	1.459	1.4581	-	
Colour – Red Index (Lovibond)	1.90	0.80	0	2.00	3.3	0.7	
Colour – Yellow Index (Lovibond)	30.00	35.00	40	35.00	72	5.9	
Smoke point (°C)	203	200	198	206	202	-	
Viscosity (mPa s)	80	45.05	57	56.10	66	-	
Free fatty acids (% as oleic acid)	1.94	1.12	0.85	1.39	0.91	2.48	
Saponification value (mg KOH/g)	199.32	188.36	178.11	186.32	176.23	164	
Iodine value (g I/100g or Wijs)	65.73	65.58	66.83	65.46	66.66	65.4	
Melting point (°C)	-	-	-	-	-	19.0	
SFC (%) ^a	-	-	-	-	-	11.1	
Unsataponifiable matter (%)	-	-	-	-	-	0.74	

NOTE: ^a SFC: Solid Fat Content values obtained at 0°C; ^s Extraction solvent used to extract oil

Triglycerides of the Oil

The *M. oleifera* seed oil contains up to 36.7% triolein as the main triacylglycerol. Other triglycerides include POO + SOL (12.4%), SOO (11.4%), OOA (7.7%), POL (5.0%) and OOGa (4.1%) with small amounts of OOLn (0.8%), OLA (0.6%) and OOL (0.5%) (Table VI). The types and percentages of the triglycerides are not affected by the extraction method.

The composition of acyl chains and their positions in the triacylglycerols of the oil extracted from seeds of *M.oleifera* have been studied using ^{13}C NMR spectroscopy. The unsaturated chains of *M. oleifera* seed oil comprises of mono-unsaturated fatty acids only and, in particular, two ω -9 mono-unsaturated acids, (*cis*-9-octadecenoic (oleic acid) and *cis*-11-eicosenoic acids) and one ω -7 mono-unsaturated acid [*cis*-11-octadecenoic acid (vaccenic acid)]. The mono-unsaturated fatty acids are detected as separated resonances in the spectral regions where the carbonyl and olefinic carbons resonate according to the 1,3- and 2-positions on the glycerol backbone. The unambiguous detection of vaccenic acid is also achieved through the resonance of the ω -3 carbon (41).

Table IV. Melting behavior of *Moringa oleifera* seed oil by DSC at two different scan rates

Parameter	Light petroleum ether extraction		
	T _O	T _P	T _E
5°C/min scan			
(a) Low melting unstable TAG crystals	-24.5	-21.6	-19
(b) Low melting stable TAG crystals	-12.6	-4.4	3.4
(c) High melting saturated TAG crystals	14.7	16.1	19.0
100°C/min scan			
(a) Low melting unstable TAG crystals	-	-	-
(b) Low melting stable TAG crystals	-21.42	-12.09	-6.05
(c) High melting saturated TAG crystals	1.46	13.10	18.93

SOURCE: (15)

Table V. Relative percent fatty acid composition of *M. oleifera* seed oil using different extraction methods

Parameter	Light petroleum Ether (15)	(15)	Cold Pressure (2)	Hexane (2,17)	Chloroform:Methanol (2)
C _{8:0}	-	-	0.04	0.03	0.02 - 0.03
C _{14:0}	0.1	1.4	0.13	0.11 - 0.13	0.11 - 0.13
C _{16:0}	7.8	3.5 - 6.9	6.34	5.10 - 6.46	5.81 - 6.36
C _{16:1}	2.2	1.1	-	-	-
C _{16:1 cis n-9}	-	-	0.1	0.09 - 0.11	0.09 - 0.10
C _{16:1 cis n-7}	-	-	1.28	1.36 - 1.46	1.40 - 1.44
C _{17:0}	-	-	0.08	0.08 - 0.09	0.08 - 0.08
C _{18:0}	7.6	4.3 - 8.3	5.70	5.88 - 4.14	4.00 - 5.74
C _{18:1^a}	67.9	67.3 - 76.5	71.6	71.21 - 77.6	71.22 - 73.91
C _{18:2}	1.1	0.4 - 3.5	0.77	0.65 - 4.05	0.66 - 0.71
C _{18:3}	0.2	-	0.20	0.18 - 0.22	0.17 - 0.20
C _{20:0}	4.0	2.7 - 4.7	3.52	2.50 - 3.62	2.70 - 3.60
C _{20:1}	1.5	2.3	2.24	2.22 - 2.40	2.25 - 2.46
C _{22:0}	6.2	4.6 - 7.4	6.21	5.70 - 6.73	6.28 - 6.38
C _{22:1 cis}	-	-	0.12	0.12 - 0.14	0.12 - 0.14
C _{24:0}	1.3	0.4 - 3.2	-	-	-
C _{26:0}	-	-	1.21	1.08 - 1.18	1.06 - 1.23

^aMixture of *cis* and *trans* C_{18:1}

Vitamins

M. oleifera seed oil is high in the natural antioxidants - tocopherols (128) with homologues (α -, β -, γ -, and δ -tocopherol) (42). Extraction methods influence the quantities of tocopherols extracted (2). α -Tocopherol is the primary vitamer with biological activity, while the other vitamers have been shown to have decreased activity. γ -tocopherol has 10% of the activity of α -tocopherol, and δ -tocopherol has 1% of the activity of α -tocopherol (38,115). α -tocopherol content of *M. oleifera* leaves averages 90.0 mg/kg, However, α -, γ - and δ -tocopherols are detected up to levels of 105.0, 39.5 and 77.6 mg/kg of oil, respectively as shown in Table VII (2,82).

Table VI. Relative percent composition of triglycerides of *M. oleifera* seed oil extracted with light petroleum ether or by enzymatic extraction

Parameter	%
Monounsaturated	
POB	2.1
PSO	2.0
PPL	1.5
PPO	1.3
Polyunsaturated	
OOO	36.7
POO + SOL	12.4
SOO	11.4
OOA	7.7
POL	5.0
OOGa	4.1
OOLn	0.8
OLA	0.6
OOL	0.5
Others (Not yet identified)	13.9

Source: (15)

Table VII. Tocopherol composition of *M. oleifera* seed and extraction solvent

Parameter (mg/kg)	Cold pressure		n-Hexane		Chloroform:Methanol	
	(2)	(114)	(2)	(114)	(2)	(114)
α -tocopherol	5.1	101.5	15.6	98.8	2.4	105.0
γ -tocopherol	25.4	39.5	4.5	27.9	5.5	33.5
δ -tocopherol	3.6	75.7	15.5	71.2	12.7	77.6

Lutein and zeaxanthin are the dominant carotenoids in the human retina (112). They represent about 36% and 18% of the total carotenoid content of the retina, respectively (101). *M. oleifera* contains high amounts of lutein, 24.8 mg/100 g edible fresh leaves (39). Carotenoid types – α -carotene and β -carotene are up to 4.5 and 34 mg/100 g, respectively, while lycopene is not present in *M. oleifera* (44). In fresh leaves of *M. oleifera* of Tanzania, vitamin C (ascorbic acid) content can be as high as 204 mg/100 g fresh weight (113). On the other hand, in Pakistan vitamin C contents range between 36 and 43 mg/100 g (4). In terms of cooking stability, when 20 mg samples are cooked in 400 ml of water, the vitamin C content loss can be as high as 98.5%. However, smaller losses of vitamin C are observed when *M. oleifera* sample is cooked in less water. Since most leafy vegetables are cooked prior to consumption, it is recommended that *M. oleifera* leaves be cooked in small amounts of water for short periods to minimise loss of vitamin C (113). Essential Oils

The total neutral essential oil from *M. oleifera* leaves is composed of 44 constituents and include: toluene, 5-*tert*-butyl-1,3-cyclopentadiene, benzaldehyde, 5-methyl-2-furaldehyde, benzeneacetaldehyde, linaloloxide, 2-ethyl-3,6-dimethylpyrazine, undecane, α -isophorone, benzylnitrile, 2,6,6-trimethyl-2-cyclohexane-1,4-dione, 2,2,4-trimethyl-pentadiol, 2,3-epoxycarane, *p*-menth-1-en-8-ol, 2,6,6-trimethylcyclohexa-1,3-dienecarbaldehyde, indole, tridecane, α -ionone, 1,1,6-trimethyl-1,2-dihydronaphthalene, α -ionene, β -damascenone, β -ionone, ledene oxide, 2-*tert*-butyl-1,4-dimethoxybenzene, (*E*)-6,10-dimethylundeca-5,9-dien-2-one, 4,6-dimethyl-dodecane, 3,3,5,6-tetramethyl-1-indanone, dihydro-actiridioid, 2,3,6-trimethyl-naphthalene, megastigmatrienone, 1-[2,3,6-trimethyl-phenyl]-2-butanone, 1-[2,3,6-trimethyl-phenyl]-3-buten-2-one, isolongifolene, hexahydrofarnesylactone, farnesylacetone, methyl palmitate, *n*-hexadecanoic acid, [6*E*,10*E*]-7,11,15-trimethyl-methylene-1,6,10,14-hexadeca-tetraene, (*E*)-phytol, docosane, 1-docosene, teracosane, pentacosane and hexacosane (23).

Antinutritional Factors

One of the major problems in nutritional exploitation of tree leaves is the presence of antinutritional and toxic factors (6). The leaves of *M. oleifera* contain about 1.4% tannins, whereas condensed tannins are not present. Total phenols in leaves ranges from 2.7 - 3.4% (6,100). The *M. oleifera* leaves contain 5.0% saponins as diosgenin equivalent with phytate contents in the leaves of 3.1% (6, 100). Activity of trypsin inhibitors and lectins is not detected in the *M. oleifera* leaves. Other antinutritional factors present in *M. oleifera* leaves are flatus factors (sucrose + raffinose + stachyose) at 5.6% (107). Nitrate (0.5 mmol per 100 g) and oxalate (4.1%) are also present in *M. oleifera* leaves (100). The presence of these antinutritional factors in leaves of *M. oleifera* decreases the bioavailability of other nutrients. However, extraction using 80% ethanol decreases the contents of some of these antinutritional factors (6).

M. oleifera seeds have water soluble lectin which is mainly active with rabbit cells at pH 4.5. Heating (100°C), pH 7.0, fructose and porcine thyroglobulin treatments diminish the hemagglutinating activity of the water

soluble lectin. This is explained on the possible involvement of carbohydrate binding sites in the hemagglutinating activity. Heat treatment is responsible for protein denaturation and pH 7.0 treatment is explained on the basis of the coalescence of the protein molecules due to equilibrium of positive and negative charges (43).

Antioxidants And Phytochemicals In *Moringa*

Phytochemicals and antioxidant constituents in plant material have raised interest among scientists, food manufacturers, producers, and consumers for their roles in the maintenance of human health (44). The *M. oleifera* plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (22,81,88). *M. oleifera* leaves have a high total antioxidant capacity, total polyphenol content and flavanols. Season and agroclimatic locations have a profound effect on the antioxidant activity of *M. oleifera* leaves (4, 110). Significant differences are observed in the antioxidant activities of the *M. oleifera* extracts from different locations and seasons in Pakistan (4). Extracts from *M. oleifera* have higher antioxidant activity (83%) than α -tocopherol (72%) in linoleic-lipid models. This indicates that *Moringa* plant extract is a potential source of dietary antioxidants (45).

The stem bark contains two alkaloids, namely moringine and moringinine (21). The *M. oleifera* oil also contains high levels of sterols (Table VIII). There are no significant differences in sterol composition of the oils extracted due to extraction methods. The comparison of data between variety seed 1 (*Mbololo*) and seed 2 (*periyakulam 1*) shows that there are significant differences in all sterols apart from cholesterol, brassicasterol, campesterol, campestanol and ergostadienol (2). The notable quantities of sterols from *Moringa* oil include β -sitosterol (up to 50%), stigmasterol (up to 23.10%) and campesterol (up to 15.81%).

Table VIII. Sterol composition of *M. oleifera* seed oil based on different extraction methods

Parameter (mg/kg)	Cold pressure		n-Hexane		Chloroform:Methanol	
	(2)	(114)	(2)	(114)	(2)	(114)
Total sterols in oil (%, w/w)	0.5	-	0.6	-	0.5	-
β -Sitosterol	45. 6	49.2	43. 7	50.1	44.1	50.0
Stigmasterol	23. 1	17.3	23. 1	16.9	22.5	16.8
Campesterol	15. 8	14.0	15. 3	15.1	14.6	14.1
Δ^5 -Avenasterol	8.5	12.8	11. 6	8.8	10.4	11.4
Clerosterol	2.1	1.0	1.2	2.5	1.8	0.8
28, Isovenasterol	0.3	1.0	0.3	1.4	0.4	1.1
Cholesterol	0.2	0.1	0.1	0.1	0.1	0.1
24, Methylene cholesterol	0.1	0.9	0.1	0.9	0.1	1.0
Brassicasterol	0.1	ND	0.1	0.1	0.1	0.1
Stigmastanol	0.8	1.1	0.6	0.9	0.7	0.8
$\Delta^{7,14}$	0.5	-	0.4	-	0.4	-
Stigmastadienol $\Delta^{7,14}$ Stigmastanol	0.4	0.8	0.9	0.4	0.5	0.5
Campestanol	0.4	ND	0.3	0.4	0.3	0.4
Ergostadienol	0.3	ND	0.4	0.4	0.3	0.3
Δ^7 , Avenasterol	0.5	0.9	ND	1.1	1.2	1.0

^sND: Not detected

In terms of compartmentalization of the phytochemicals in tissues, *M. oleifera* from a wide variety of sources and *M. stenopetala* from a single source have been analyzed for glucosinolates and phenolics (flavonoids, anthocyanins, proanthocyanidins, and cinnamates). *M. oleifera* and *M. stenopetala* seeds only contains 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate at high concentrations. Roots of *M. oleifera* and *M. stenopetala* have high concentrations of both 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate and benzyl glucosinolate. Leaves from both species contains 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate and three monoacetyl isomers of this glucosinolate. Only 4-(α -L-rhamnopyranosyloxy)-benzylglucosinolate is found in *M. oleifera* bark tissue. *M. oleifera* leaves contains quercetin-3-*O*-glucoside and quercetin-3-*O*-(6"-malonyl-glucoside), and lower amounts of kaempferol-3-*O*-glucoside and kaempferol-3-*O*-(6"-malonyl-glucoside). *M. oleifera* leaves also contains 3-caffeoylquinic acid and 5-caffeoylquinic acid. Leaves of *M.*

stenopetala contain quercetin 3-*O*-rhamnoglucoside (rutin) and 5-caffeoylquinic acid. Neither proanthocyanidins nor anthocyanins are detected in any of the tissues of either species (81).

Antioxidant Properties of *Moringa Oleifera* Plant Extract When Used in Food

M. oleifera plant extract is a source of natural antioxidants which imparts effects on the stability of fat in biscuits. *M. oleifera* extracts exhibits a higher percentage of antioxidant activity than synthetic antioxidants when evaluated in β -carotene–linoleic acid *in vitro* system. Biscuits treated with natural antioxidants from *M. oleifera* leaves receive higher panel scores during storage period of 6 weeks, than control and butylated hydroxyl anisole (BHA) incorporated biscuits. Addition of *M. oleifera* extracts gives an excellent antioxidant effect on the biscuit compared with the effect of BHA, as measured in both by peroxide and acid values after 6 weeks. Therefore, extracts from *M. oleifera* leaves can be effective in controlling lipid oxidation during storage (46).

Oxidative Stability of *Moringa Oleifera* Oil As A Frying Oil

Conventionally, available cooking oils can not fulfill high oxidative resistance required in deep frying to minimize causing serious health disorders due to the generation of hazardous oxidation products (17). Therefore, there is a continued search for oils that will fulfill this requirement. *M. oleifera* seed oil shows a long induction period (at 120°C), which however is reduced from 42.6 to 72.6% after degumming. The *M. oleifera* seed oil shows high stability to oxidative rancidity, although its oxidative stability is influenced by the extraction method (2).

In frying performance, repeated frying operations of the *M.oleifera* seed oil variety *Periyakulam 1* from India extracted by cold press and n-hexane shows some stability. The frying performance (by measuring the oxidative stability during frying of potatoes and cod) of *M. oleifera* seed oil compares well with virgin olive oil. When the oils are used for intermittent frying of potato slices and cod filets at a temperature of $175 \pm 5^\circ\text{C}$ for 5 consecutive days, analytical and sensory data shows that the lowest deterioration occurs in cold pressure produced oil and the highest in n-hexane extracted oil. Therefore, cold pressure oil is recommended to be the most appropriate for frying. In comparison with virgin olive oil, the results indicate that virgin olive oil has the highest resistance to thermal deterioration during frying compared with the two *M. oleifera* oils (47).

Blending of *M. oleifera* oil (MOO) with high-linoleic oils like sunflower oil (SFO) and soybean oil (SBO) improves their oxidative stability. The blending of *M. oleifera* oil with sunflower oil and soyabean oil in proportions of 20–80% results in the reduction of linoleic acid (C18:2) content of sunflower oil and soyabean oil from 67.0% to 17.2% and 56.2% to 14.6% and increase in the contents of oleic acid (C18:1) from 26.2% to 68.3% and 21.4% to 65.9%,

representing factors of 0.72, 0.72 and 1.27, 1.33, respectively. A storage stability test (180 days; ambient conditions) shows an appreciable improvement in the oxidative stability of substrate oils with increase of *M. oleifera* oil concentration, as depicted by the least oxidative alterations in peroxide value, iodine value and highest increase in induction period of the MOO:SBO (80: 20 w/w) blend. Each 20% addition of *M. oleifera* oil results in decrease of peroxide value and iodine value by factors of 0.84, 0.85 and 0.89, 0.88, respectively, and increases in induction period by factors of 1.45 and 1.37 of sunflower oil and soyabean oil, respectively. The heating performance test (180°C for 42 h; 6 h heating cycle per day), as followed by the measurement of polymer contents and total polar contents, reveals the MOO:SBO (80:20 w/w) blend is the most stable. Every 20% addition of *M. oleifera* oil in sunflower oil and soyabean oil results in reduction of the polymer contents and total polar contents of sunflower oil and soyabean oil by factors of 0.91, 0.92 and 0.94, 0.94, respectively. It is therefore, clear that proper blending of high linoleic oils with *M. oleifera* oil can result in oil blends which could meet nutritional needs with improved stability for domestic cooking and deep-frying (17).

***Moringa Oleifera* Oil As An Olive Oil Substitute**

The seed oil of *M. oleifera* has physical and chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols (23,114). The seed oil contains all the main fatty acids found in olive oil, and therefore, it can be used as a possible substitute to the expensive olive oil after some modifications. In addition, *M. oleifera* oil possesses behenic acid (C22:0), lignoceric acid (C24:0) and traces of lauric n-pentadecanoic and pentadecenoic acids (128). The characteristics of *M. oleifera* seed oil can be highly desirable especially with the current trend of replacing polyunsaturated vegetable oils with those containing high amounts of monounsaturated acids (130). High oleic acid vegetable oils are very stable even in highly demanding applications like frying (15, 131).

Behenic Acid Products

M. oleifera oil has relatively high contents of behenic acids (15). The oil can, therefore, be used as a natural source of behenic acid, which is used as an oil structuring and solidifying agent in margarine, shortening, and foods containing semi-solid and solid fats, eliminating the need to hydrogenate the oil (132). Due to its physical properties, addition of behenic acid can lighten chocolate texture and oily feel, prevent solid roux from being whitened and provide excellent mouth feel and melt-down behaviour to semi-solid and solid fats (such as margarine, shortening, and foods containing semi-solid and solid fats) (133). Behenic acid is also poorly absorbed from the diet and can be used in low calorie foods (2).

***Moringa* - Medicine**

History Of Pharmacological Use Of *Moringa Oleifera*

M. oleifera has been claimed in traditional literature to be valuable against a wide variety of diseases. *M. oleifera* is well documented for its various pharmacological importance (48, 51, 135, 136). A number of medicinal and therapeutic properties have been ascribed to various parts of this multipurpose tree (21, 22). Its various parts are identified for innumerable pharmacological properties (12, 134) and have been studied for several pharmacological actions (50). Although the oral history for the medicinal properties of *Moringa* are overwhelming, it has been subject to much less intense scientific scrutiny and therefore it is useful to review the claims that have been made and assess the quality of evidence available for the more well-documented claims. In many cases, *in-vitro* (cultured cells) and *in-vivo* (animal) trials do provide a degree of mechanistic support for some of the claims that have sprung from the traditional medicine lore.

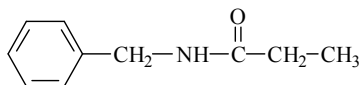
There is a view that the use of *Moringa* in modern medicine has stalled because many of the reports of efficacy in human beings are not supported by placebo controlled, randomized clinical trials, nor have they been published in high visibility literature (22). However, *M. oleifera* is now incorporated in various marketed formulations, such as Rumalaya and Septilin, Orthoherb , Kupid Fort and Livospin which are available for a variety of disorders (50).

Anti-Bacteria And –Fungi Properties of *Moringa Oleifera*

Various parts of *M. oleifera* are identified for innumerable pharmacological properties *viz.* antimicrobial activity (12, 134). The juice from the leaves and stem bark of *M. oleifera* inhibit *Staphylococcus aureus* but not *Escherichia coli* (50). The fresh leaf juice inhibits the growth of pathogenic *Pseudomonas aeruginosa* and *Staphylococcus aureus* (21, 143). The bark extract possesses anti-fungal and anti-tubercular activity (50). Niaziridin rich fraction of *M. oleifera* pods enhances the bioactivity of commonly used antibiotics such as rifampicin, tetracycline and ampicillin against gram(+) and (-) bacteria (37).

Extracts of *M. oleifera* have anti-fungal properties. Ethanol extracts of seeds and leaves of *M. oleifera* Lam exhibit anti-fungal activities *in vitro* against dermatophytes such as *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Epidermophyton Xoccosum* and *Microsporium canis* (23). *M.oleifera* contains an antibiotic principle, pterygospermin (C₂₂H₁₈O₂N₂S₂, m.p. 15°C) isolated from the root-bark (31, 137). Pterygospermin is both a bactericidal and fungicidal compound (50). 4-(α -L-Rhamnosyloxy)benzyl isothiocyanate has been identified as an active antimicrobial agent from seeds of *M. oleifera* and *M. stenopetala* (53, 85). The roots of *M. oleifera* contain active antimicrobial agents 4-(α -L-rhamnosyloxy)benzyl isothiocyanate and benzyl isothiocyanate, but not pterygospermin (85). In the presence of ascorbic acid, water extracts of defatted and shell free *M. oleifera* seeds contain about 8 – 10% 4-(α -L-

rhamnosyloxy)benzyl isothiocyanate. These compounds are active against several bacteria and fungi with minimal bactericidal concentration *in vitro* of 40 $\mu\text{mol/L}$ for *Mycobacterium phlei* and 56 $\mu\text{mol/L}$ for *Bacillus subtilis* (85). An aglycone of deoxy-niazimicine (N-benzyl, S-ethyl thioformate) (Figure 5) isolated from the chloroform fraction of an ethanol extract of the root bark is responsible for the antibacterial and antifungal activities (67).



Deoxy-niazimicine (N-benzyl,S-ethyl thioformate)

Figure 5. Antibacterial and antifungal aglycon of *M. oleifera*

The aglycone shows high antibacterial activity against *shigella boydii*, *shigella dysenteriae* and *staphylococcus aureus*. The minimum inhibitory concentration (MIC) values against *B. megaterium*, *St. aureus*, *Sh. Dysenteriae* and *Sh. Boydii* range from 32 to 128 $\mu\text{g/mL}$. The aglycone also shows moderate antifungal activity against *Candida albicans* and *Aspergillus flavus* (67).

A coagulant cationic protein with pI greater than 9.6 and molecular mass less than 6.5 kDa from water and salt extracts exhibits antibacterial activity (66). The coagulant protein shows antibacterial effects of 1.1 – 4.0 log reduction as observed for *E. coli*, *B. thuringiensis* and *P. aeruginosa*. This polypeptide (named Flo) from *Moringa* possesses a bactericidal activity capable of disinfecting heavily contaminated water. The inhibition of *E. coli* growth is transitory, with resumption of growth after 3–6 h. Treatment with Flo significantly decreases the number of recovered cells, with an IC_{50} value of approximately 1 mg/mL. Flo at 6 mg/mL and above lowers viable cell count by 3 orders of magnitude, indicating that cell lysis occurs, while approximately 99% of the remaining particles are non-viable.

Flo antimicrobial specificity is observed against several Gram-positive and -negative bacteria. A bacteriostatic effect is observed against several human pathogens such as *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *L. pneumophila*, and MIC values ranges between 0.8 and 5.0 mg/mL of Flo and, between 5 and 20 mg/mL for the *Moringa sp.* seed extract. Bactericidal (killing) activities are also observed against several human pathogens, such as *Staphylococcus*, *Streptococcus*, and *Legionella species*, including strains resistant to commonly used antibiotics. Thus, Flo displays selective antibacterial effects on a range of Gram-positive and Gram-negative human pathogens (138). Based on the molecular basis and structural determinants of the antibacterial activities of the Flo. polypeptide through structure-function analysis, it shows that partly overlapping structural determinants mediate the antibacterial activities. The bactericidal activity is localized to a sequence prone to form a helix-loop-helix structural motif. Amino acid substitution shows that the bactericidal activity requires hydrophobic

proline residues within the protruding loop. Vital dye staining indicates that treatment with peptides containing this motif results in bacterial membrane damage. Assembly of multiple copies of this structural motif into a branched peptide enhances antibacterial activity, since low concentrations effectively kills bacteria such as *Pseudomonas aeruginosa* and *Streptococcus pyogenes* without displaying a toxic effect on human red blood cells. The bactericidal activity would require bacterial membrane destabilization by a hydrophobic loop. Also, Flo antibacterial activity may be associated with a motif composed of two amphipathic helices separated by a hydrophobic and kinked structure and that multimeric structures of this motif exhibit more efficient and specific antibacterial activities (138).

Anti-Viral Properties of *Moringa Oleifera*

M. oleifera extracts inhibits plaque formation of anti-herpes simplex virus type 1 (HSV-1) more than 50% at 100 µg/ml in a plaque reduction assay (55). *M. oleifera* extracts are also effective against thymidine kinase-deficient HSV-1 and phosphonoacetate-resistant HSV-1 virus strains. The extract of *M. oleifera* at a dose of 750 mg/kg body weight per day significantly delays the development of skin lesions, prolongs the mean survival times and reduces the mortality of HSV-1 infected mice. Compared to the synthetic compound acyclovir, *M. oleifera* extracts delay the development of skin lesions and has mean survival times as acyclovir. A polysaccharide from hot aqueous extract of mature pods (fruits) of *M. oleifera* with a structural repeating unit [\rightarrow 4)- α -D-Glc_p(1 \rightarrow)] has immunoenhancing properties (76).

Wound Healing Properties of *Moringa Oleifera*

In recent decades, the extracts of leaves, seeds and roots of *M. oleifera* have been extensively studied for many potential uses including wound healing (31, 139, 140). The uses of the plants in wound healing are rationalised on the basis of their antioxidant capacity (52). *M. peregrina* has been shown to inhibit the DPPH radical at 89–93%, after 15 min of incubation at a test concentration of 50 µg/mL. *M. peregrina* has been shown to have total antioxidant capacity as gallic acid equivalent of 814 mg/g of ethanol extracts in the phosphomolybdenum assay.

Anti-Inflammatory And Antihepatotoxic Activities of *Moringa Oleifera*

The leaves of *M.oleifera*, as well as the flowers, roots, gums, fruits and seeds are extensively used for treating inflammations (49). Anti-inflammatory and hepatoprotective activities of various tissues of *M. oleifera* have been reported (37). Ethanol and hexane fractions of *M. oleifera* have stronger anti-inflammatory activities. Crude *M. oleifera* ethanol extract of dried seeds inhibits the carrageenan-induced inflammation in the hind paw of mice by 85% at a

dosage of 3 mg/g body weight while the mature green seeds by 77% (99). The hexane fraction of the crude ethanol extract of the dried seeds also inhibits inflammation by 77% at the same dosage while both butanol and water fractions inhibits inflammation by only 34%. On the other hand, the ethyl acetate fraction causes a 267% increase in inflammation and is toxic to mice when orally administered at 3mg/g BW (99).

Oral administration of the *M. oleifera* extracts inhibits carrageenan-induced rat paw edema in a dose-dependent manner, with 50% inhibitory concentration - IC₅₀ (dose producing 50% inhibition) of 660 mg/kg. In the rat 6-day air pouch inflammatory models, *M. oleifera* extract is much more potent, with IC₅₀ values of 302.0 mg/kg and 315.5 mg/kg, for the inhibition of cellular accumulation and fluid exudation, respectively. The maximum inhibition obtained with 600 mg/kg can be as high as 83.8% and 80.0%, respectively. When delayed (chronic) inflammation is induced in the 6-day air pouch model using Freund's complete adjuvant, the extract is still effective though less than in acute inflammation. In contrast, a moderate dose of indomethacin (5 mg/kg) inhibited the acute, but not the delayed form of air pouch inflammation (87). In other species of *Moringa*, the roots of *M. pterygosperma Gaertn* methanolic extract exhibits oedema suppressant activity against carrageenan-induced paw oedema similar to that of indomethacin, a synthetic oedema suppressant. The aqueous and methanolic extracts of *M. pterygosperma Gaertn* have antihepatotoxic activity that is comparable to synthetic antihepatotoxic compounds such paracetamol and rifamicin. Also, aqueous and alcoholic extracts of root and flower of *M. oleifera* shows antihepatotoxic activity in paracetamol treated albino rats (108).

Antiuroliithiatic Properties of *Moringa Oleifera*

In the indigenous system of medicine in India, the aqueous extract of roots of *M. oleifera* are reported to be useful in the treatment of urinary stones (129). Ethylene glycol alone when orally fed to male Wistar rats results in hyperoxaluria as well as increased renal excretion of calcium and phosphate. But when supplemented with aqueous and alcoholic extract of *M. oleifera* root-wood, there is a significant reduction in the elevated urinary oxalate, indicating a regulatory action on endogenous oxalate synthesis. The increased deposition of stone forming constituents in the kidneys of calculogenic rats is also significantly lowered by curative and preventive treatment using aqueous and alcoholic extracts of *M. oleifera*. The mechanism underlying this effect is still unknown, but is suggested to be related to increased diuresis and lowering of urinary concentrations of stone forming constituents. This however, demonstrates that the root-wood of *M. oleifera* is endowed with antiuroliithiatic activity (31).

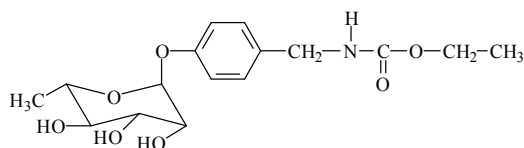
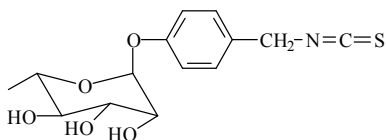
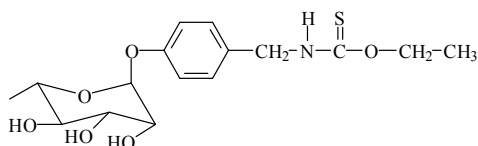
Anti-Tumor And Anti-Cancer of *Moringa Oleifera*

The use of natural products as anticancer agents has a long history that began with folk medicine and through the years has been incorporated into

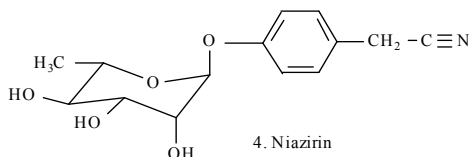
traditional and allopathic medicine. Several drugs currently used in chemotherapy have been isolated from plant species or derived from a natural prototype (56). Many reports describe various tissues of *M. oleifera* as a highly potent anti-tumor (23,31,37,49,53,99,103).

Ethanol extract (50%) of *M. oleifera* (whole plant excluding roots) shows anti-cancer activity in mice (50). Evaluation of the anticancer potential of 11 plants used in Bangladeshi folk medicine has shown that, only three extracts, among which *M. oleifera*, is considered as a potential source of anticancer compounds (56). Crude ethanol extract of *M. oleifera* dried seeds inhibits the formation of Epstein-Barr virus-early antigen (EBV-EA) induced by 12-*O*-tetradecanoylphorbol-13-acetate. At a dosage of 100 µg/mL, the extract inhibits EBV-EA formation by 100% suggesting its antitumor-promoting activity (99).

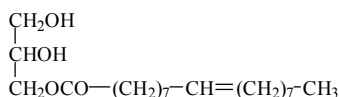
Three known thiocarbamate (TC)- and isothiocyanate (ITC)-related compounds have been isolated from the leaves of *M. oleifera* and are inhibitors of tumor promoter teleocidin B-4-induced Epstein-Barr virus (EBV) activation in Raji cells. Niaziminin isolated from *M. oleifera*, comparable to synthetic thiocarbamates shows considerable inhibition against EBV activation. The structure-activity relationships indicate that the presence of an acetoxy group at the 4'-position of niaziminin is important and indispensable for inhibition. On the other hand, among the isothiocyanate-related compounds, naturally occurring 4-[(4'-*O*-acetyl- α -*L*-rhamnosyloxy)benzyl] isothiocyanate and commercially available allyl- and benzyl-isothiocyanate significantly inhibits activation, suggesting that the isothiocyano group is a critical structural factor for activity (103). Figure 6 shows thiocarbamates and isothiocyanate related compounds isolated from the leaves and ethanol extracts of the seeds of *M. oleifera*. These include *O*-ethyl-4(α -*L*-rhamnosyloxy)benzyl carbamate (**1**), 4-(α -*L*-rhamnosyloxy)benzyl isothiocyanate (**2**), niazimicin (**3**), niazirin (**4**), glycerol-1-(9-octadecanoate) (**5**), β -sitosterol (**6**), 3-*O*-(6'-*O*-oleoyl- β -*D*-glucopyranosyl)- β -sitosterol (**7**), and β -sitosterol-3-*O*- β -*D*-glucopyranoside (**8**) (53).

1. *O*-ethyl-4(α -L-rhamnosyloxy-benzyl carbamate2. 4(α -L-rhamnosyloxy-benzyl isothiocyanate

3. Niazimicin



4. Niaziirin



5. Glycerol-1-(9-octadecanoate)

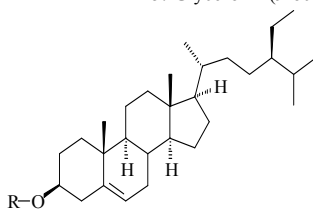
6. β -sitosterol (R=H)7. 3-O-(6-O-oleoyl- β -D-glucopyranosyl)- β -sitosterol (R=6-O-oleoyl- β -D-glucopyranosyl)8. β -sitosterol-3-O- β -D-glucopyranoside (R= β -D-glucopyranosyl)

Figure 6. Structures of antitumor and anti cancer compounds from *M. oleifera*

Four of the isolates (**2**), (**3**), (**7**) and (**8**) showed inhibitory activity against Epstein–Barr virus-early antigen (EBV-EA) activation with compounds (**2**), (**3**) and (**8**) having very significant activities (53), indicating higher potential for antitumor activities. Most compounds that inhibit *in vitro* EBV-EA activation are also known to be antitumor promoters *in vivo* (12). Niazimicin (**3**) when

tested for *in vivo* antitumor promoting activity shows potent antitumor promoting activity in the two-stage carcinogenesis in mouse skin using 7,12-dimethyl-benz(a)anthracene (DMBA) as initiator and 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) as tumor promoter (53).

Hypotensive And Spasmolytic Activities of *Moringa Oleifera*

In recent decades, the extracts of leaves, seeds and roots of *M. oleifera* have been extensively studied for many potential uses including hypotensive activity (29) and analgesic activity (31, 141). Nitrile, mustard oil glycosides and thiocarbamate glycosides have been isolated and found to be responsible for the hypotensive principles of the leaves (53). Prior to these investigations, there was no instance of the isolation of active hypotensive principles, although the antihypertensive and antispasmodic properties have long been attributed to the species (29,48).

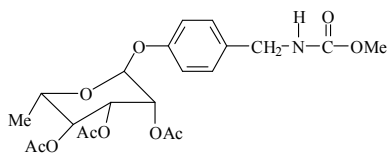
Niazinin A, niazinin B, niazimicin and niaziminin A and B have been isolated and identified from the ethanolic extracts of the fresh leaves of *M. oleifera*. They are mustard oil glycosides, rare in nature and the first examples of naturally occurring thiocarbamates (90). Two novel hypotensive carbamate glycosides designated niazimin A and niazimin B and two new hypotensive mustard oil glycosides possessing the thiocarbamate group, niazicin A and niazicin B along with a benzaldehyde glycoside have been isolated from the fresh leaves extract of *M. oleifera*. Niazimin A and niazimin B are the first natural products embodying the carbamate moiety. The amide bond in these compounds is proposed to play an important role in the hypotensive activity, since in both the carbamates and thiocarbamates it is common to those which possess hypotensive activity (91).

Bioassay-guided analysis of an ethanol extract of *M. oleifera* leaves showing hypotensive activity led to the isolation of two nitrile glycosides, niazirin and niazirinin and three mustard oil glycosides, 4-[(4'-*O*-acetyl- α -rhamnosyloxy)benzyl]isothiocyanate, niaziminin A, and niaziminin B. Niazirinin at the time of its isolation was reported to be a new compound. However, Niaziminins A and B had previously been obtained from the leaf extract as a mixture, while 4-[(4'-*O*-acetyl- α -rhamnosyloxy)benzyl]isothiocyanate was reported to be new from *M.oleifera*. The Isothiocyanate, 4-[(4'-*O*-acetyl- α -rhamnosyloxy)benzyl]isothiocyanate and the thiocarbamate glycosides niaziminin A and B shows hypotensive activity while nitrile glycosides niazirin and niazirinin do not have hypotensive activity (92).

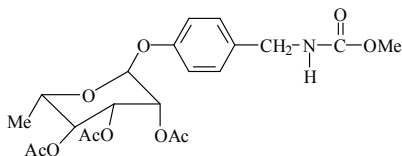
Intravenous administration of either one of the four pure compounds previously isolated from *M. oleifera* leaves (niazinin A, niazinin B, niazimicin and niaziminin A + B) in the concentration range of 1-10 mg/kg, produces hypotensive and bradycardiac effects in anaesthetized rats. Pretreatment of the animals with atropine (1 mg/kg) completely eliminates the hypotensive and bradycardiac effects of acetylcholine (ACh), whereas cardiovascular responses to the test compounds remains unaltered, ruling out the possible involvement of muscarinic receptor activation. In isolated guinea-pig atria all the compounds

(50-150 $\mu\text{g/mL}$) produce negative inotropic and chronotropic effects. Each compound inhibits K^+ -induced contractions in rabbit aorta as well as ileal contractions induced by acetyl choline (ACh) or histamine at similar concentrations. Spontaneous contractions of rat uterus is also inhibited equally by all of the isolated compounds. The direct depressant action of these compounds is probably responsible for its hypotensive and bradycardiac effects observed *in vivo*. Moreover, spasmolytic activity exhibited by the constituents of the plant provides a scientific basis for the traditional uses of the plant in gastrointestinal motility disorders (98).

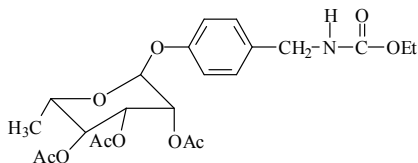
Six new and three synthetically known glycosides have been isolated from the leaves of *M.oleifera* by employing a bioassay-directed isolation method on the ethanolic extract. Most of these isolated compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides and are reported to be very rare in nature (29). Figure 7 shows compound groups of these glycosides.



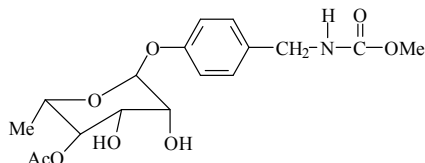
(1) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E isomer)



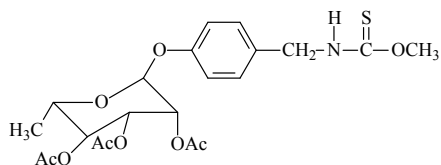
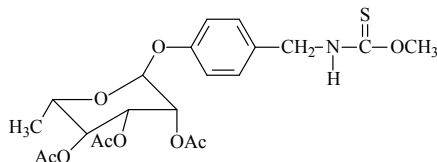
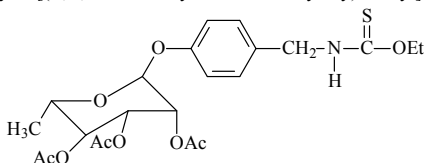
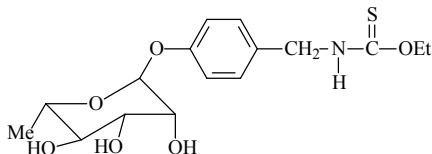
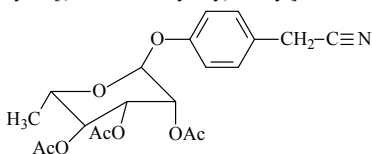
(2) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl]carbamate (Z isomer)



(3) *O*-ethyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E isomer)



(4) *O*-methyl-4-[(4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E isomer)

(5) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl]thiocarbamate (E isomer)(6) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl]thiocarbamate (Z isomer)(7) *O*-ethyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl]thiocarbamate (Z isomer)(8) *O*-ethyl-4-[(α -L-rhamnosyloxy)-benzyl]thiocarbamate (Z isomer)(9) 4-[2',3',4'-tr-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] nitrileFigure 7. Glycosides isolated from *M.oleifera*

These included 4 carbamates: (1) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E-isomer), (2) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (Z isomer), (3) *O*-ethyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E isomer) and (4) *O*-methyl-4-[(4'-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] carbamate (E isomer); 4 thiocarbamates: (5) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] thiocarbamate (E isomer), (6) *O*-methyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] thiocarbamate (Z isomer), (7) *O*-ethyl-4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] thiocarbamate (Z isomer) and (8) *O*-ethyl-4-[(α -L-rhamnosyloxy)-benzyl] thiocarbamate (Z isomer) which has also been

named Niazimicin B and; one nitrile: **(9)** 4-[(2',3',4'-tri-*O*-acetyl- α -L-rhamnosyloxy)-benzyl] nitrile (29).

Compounds **(6)**, **(7)** and **(9)** have been obtained previously through acetylation of niazinin, niazimicin (90) and niazirin (99), respectively. Compound **(6)** has earlier been obtained through acetylation of both niazinin A (Z) and niazinin B (E). Compound **(7)** is identical to the triacetylated derivative of niazimicin and niaziminins A and B (90). Compound **(8)** is the cis isomer of niazimicin and thus named Niazimicin B. Compound **(9)** had earlier been obtained through acetylation of niazirin and niazirin (92). It is important to note that unlike thiocarbamates which exist in two discrete tautomers (90, 91), carbamates **(1)** to **(4)** have only one form (29). Intravenous administration of glycosides **(5)** to **(8)** causes a fall in systolic, diastolic and mean arterial blood pressure in normotensive anaesthetized rats. Compounds **(1)** to **(4)** and **(9)** have not been tested for hypotensive activity though. The hypotensive responses of **(5)** to **(8)** are dose dependent and similar in magnitude. At 1 mg/kg, the fall in mean arterial blood pressure can be 10-20% while the next higher dose (3 mg/kg) produces 30-40% hypotension (29). These hypotensive responses are similar to those observed for previously reported compounds from the same species (90, 91, 92, 98).

The activity of the ethanolic extract of both the pods and the seeds of *M. oleifera* is equivalent at the dose of 30 mg/kg. The ethyl acetate phase of the ethanolic extract of pods has more hypotensive potency at the same dose. The bioassay-directed fractionation ethyl acetate phase of the ethanolic extract of pods led to the isolation of thiocarbamate and isothiocyanate glycosides which are also the hypotensive compounds observed in *M.oleifera* leaves. Two new compounds, *O*-[2'-hydroxy-3'-(2''heptenyloxy)]-propyl undecanoate and *O*-ethyl-4-[(α -L-rhamnosyloxy)-benzyl] carbamate along with methyl *p*-hydroxybenzoate and β -sitosterol are also isolated from the pods. The latter two compounds and *p*-hydroxybenzaldehyde shows promising hypotensive activity (89).

In essence, this identifies the rhamnosylated 4-hydroxy-benzyl isothiocyanates, carbamates and thiocarbamates as the pharmacologically active principles of the extracts. The effects of all of these compounds have been found to be similar, reversible and dose-dependent. Nitriles do not show any hypotensive effect however. Investigations on organs of several animals indicate spasmolytic properties of thiocarbamates *in vitro* along with the hypotensive effects (29). Nevertheless, with these studies no specific mechanism can be supposed for the hypotensive and spasmolytic effects of these thio-carbamates (57).

Cardiovascular Effects of *Moringa Oleifera*

The aqueous extract of stem bark of *M. pterygosperma* has been investigated for its effect on various pharmacological parameters. In cardiovascular profile, at lower concentrations the extract produces a dose dependent positive inotropic effect and at higher concentrations (0.1-1 μ g) a dose dependent negative inotropic effect on the isolated frog heart is observed. It

also produces a dose dependent hypotensive effect on dog blood but fails to elicit any effect on isolated guinea-pig ileum, rat stomach fundus or frog rectus abdominis muscle (102).

Anti-Ulcer Activity Of *Moringa Oleifera*

Leaves of *Moringa* plant are traditionally known for or reported to have various biological activities, including gastric ulcers. The methanol fraction of *M. oleifera* leaf extract possesses significant protective actions in acetylsalicylic acid, serotonin and indomethacin induced gastric lesions in rats. A significant enhancement of the healing process in acetic acid - induced chronic gastric lesions is also observed with the extract-treated animals (107). *M. pterygosperma* (flower buds) shows some decrease in the ulcer index of aspirin-induced gastric ulcers in rats. The methanolic extract of *M. pterygosperma* (4.0 g/kg) significantly increases the hexosamine, fucose and decreases the sialic acid content of carbohydrates and protein concentration. Thus, *M. pterygosperma* flower buds have protective effects on the gastric mucosa from damage to some extent by increasing the mucin although this increase is considered non significant. The methanolic extract of *M. pterygosperma* produces a 43% inhibition of ulcer formation (58).

Thyroidal Hormone Regulation of *Moringa Oleifera*

The thyroidal hormone regulation in rats by *M. oleifera* leaf extracts is sex and dose-specific dependence (59). Following the administration of the *M. oleifera* leaves extracts to rats, serum triiodothyronine (T3) concentration and hepatic lipid peroxidation (LPO) decreases with a concomitant increase in the serum thyroxine (T4) concentration in female rats, while in males no significant changes are observed. This phenomenon suggests that *M. oleifera* leaf extract is more effective in females than in the males. The antiperoxidative effects are exhibited only by the low dose and the decrease in serum triiodothyronine (T3) concentration is nearly the same for both low and high doses. This suggests that the lower concentration of this plant extract may be used for the regulation of hyperthyroidism.

Inhibition of Heavy Metal Poisoning With *Moringa Oleifera*

The therapeutic efficacy of oral administration of seed powder of *M. oleifera* (500 mg/kg, orally, once daily) post arsenic exposure (100 ppm in drinking water for 4 months) in rats has been investigated (49). Animals exposed to arsenic(III) shows a significant inhibition of δ -aminolevulinic acid dehydratase (ALAD) activity, decrease in reduced glutathione (GSH) level and an increase in reactive oxygen species (ROS) in blood. On the other hand, a significant decrease in hepatic ALAD, and an increase in δ -aminolevulinic acid synthetase (ALAS) activity is observed after arsenic exposure. These changes

are accompanied by an increase in thiobarbituric acid reactive substances (TBARS) level in liver and kidney. Activities of liver, kidney and brain superoxide dismutase (SOD) and catalase also shows a decrease on arsenic exposure. Administration of *M. oleifera* seed powder post arsenic exposure, exhibits significant recovery in blood ALAD activity while, restoring blood GSH and ROS levels. A significant protection in the altered ALAD and ALAS activities of liver and TBARS level in liver and kidney is also observed after *M. oleifera* administration. There is a marginal but significant depletion of arsenic from blood, liver and kidneys. This means that post arsenic exposure administration with the seed powder of *M. oleifera* has a significant role in protecting animals from arsenic-induced oxidative stress and in the depletion of arsenic concentration (49).

The co-administration of *M. oleifera* seed powder with arsenic protects animals from arsenic induced oxidative stress and reduce body arsenic burden (49). Exposure of rats to arsenic (2.5 mg/kg, intraperitoneally for 6 weeks) increases the levels of tissue reactive oxygen species (ROS), metallothionein (MT) and thiobarbituric acid reactive substance (TBARS) and is accompanied by a decrease in the activities in the antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx). Also, Arsenic exposed mice exhibits liver injury as reflected by reduced acid phosphatase (ACP), alkaline phosphatase (ALP) and aspartate aminotransferase (AST) activities and altered heme synthesis pathway as shown by inhibited blood δ -aminolevulinic acid dehydratase (δ -ALAD) activity. Co-administration of *M. oleifera* seed powder (250 and 500 mg/kg, orally) with arsenic significantly increases the activities of SOD, catalase, GPx with elevation in reduced GSH level in tissues (liver, kidney and brain). These changes are accompanied by approximately 57%, 64% and 17% decrease in blood ROS, liver metallothionein (MT) and lipid peroxidation respectively in animal co-administered with *M. oleifera* and arsenic. There is a reduced uptake of arsenic in soft tissues (55% in blood, 65% in liver, 54% in kidneys and 34% in brain) following co-administration of *M. oleifera* seed powder (particularly at the dose of 500 mg/kg). This points to the fact that administration of *M. oleifera* seed powder could be beneficial during chelation therapy with a thiol chelator (26).

Hypoglycaemic Activity of *Moringa Oleifera*

M. oleifera has been used as an hypoglycaemic agent, a compound that has ability to decrease the level of glucose in the blood and used in the treatment of diabetes mellitus. A 95% ethanolic extract of the bark of *M. oleifera* demonstrates hypoglycemic activity in alloxandiabetic albino rats. A single dose 250mg/kg daily of the extracts has potential to lower blood glucose within 1 week confirming the hypoglycaemic activity of the *M. oleifera* herbal (60). In oral glucose tolerance test (GTT), the inorganic constituents of the stem bark (ash consisting of mineral elements) shows more pronounced action of glucose tolerance factor than its corresponding organic parts. The probable mechanism is through the release of insulin from β cells of islets of Langerhans in presence of some specific mineral elements. However, though improvement in glucose

tolerance factor (especially significant in the case of latent or early mild diabetes) is not observed, no significant blood glucose lowering effect of the *M. oleifera* herbal ash samples is observed in moderately high or high diabetic animal models. This is due to the fact that, minerals are not hypoglycaemic agents as such and most essential trace mineral elements act primarily as catalysts or co-actors in enzyme systems (60). In nondiabetic rabbits, *M. stenopetala* plant extract, though less potent than glibenclamide, lowers blood glucose concentration. The hypoglycaemic effect increases with time and with an increase in the dose of the extract (116).

Hypocholesterolemic Activity of *Moringa Oleifera*

The leaves of *M. oleifera* possesses cholesterol-reducing effects and are used to treat patients with heart disease and obesity (28). Administration of the crude leaf extract of *M. oleifera* along with high-fat diet decreases the high-fat diet-induced increases in serum, liver, and kidney cholesterol levels by 14.4% (115.0–103.2 mg/100 ml of serum), 6.4% (9.4–8.8 mg/g wet weight) and 11.1% (1.1–1.0 mg/g wet weight), respectively. The effect on the serum cholesterol is significant but not on serum total protein. However, the crude extract significantly increases serum albumin. Rabbits fed fruit of *M.oleifera* (200 mg/kg/day, p.o.) along with standard diet and hypercholesterolaemic diet for 120 days shows a lowering of serum cholesterol, phospholipid, triglyceride, VLDL, LDL, cholesterol to phospholipid ratio and atherogenic index, but an increase in the HDL ratio (HDL/HDL-total cholesterol) (50). The mechanism of the hypocholesterolemic activity of *M. oleifera* is not clearly elucidated at present but it is suggested that β -sitosterol may be the bioactive phytoconstituent in the leaves of *M.oleifera* responsible for the hypocholesterolemic activity. β -sitosterol is a plant sterol with a structure similar to that of cholesterol, except for the substitution of an ethyl group at C24 of its side chain. It is believed to lower cholesterol by lowering plasma concentrations of LDL (28).

Generally, high cholesterol to phospholipid ratio is associated with atherosclerosis. Atherogenic index indicates the deposition of foam cells or plaque or fatty infiltration or lipids in heart, coronaries, aorta, liver and kidneys. The higher the atherogenic index, the higher is the risk of the above organs for oxidative damage. Treatment with *M. oleifera* in normal rabbits decreases the HDL levels. However, HDL levels significantly increases in *M. oleifera*-treated hypercholesterolaemic rabbits. Increase in HDL ratio is one of the desirable criteria of an ideal hypocholesterolaemic agent since the higher the ratio, the lower the atherosclerotic risk. *M. oleifera*-treated hypercholesterolaemic rabbits shows decrease in lipid profile of liver, heart and aorta while similar treatment of normal animals do not produce significant reduction in heart. *M. oleifera* increases the excretion of faecal cholesterol. Thus, this demonstrates that *M. oleifera* possesses hypolipidaemic and anti-atherosclerotic activities (50).

Activities Of *Moringa Oleifera* On The Central Nervous System

The scanty understanding of the physiological activity of *M. oleifera* on the central nervous system has centered on opiod, behavioral, sedative-hypnotic, analgesic and anticonvulsant activities. In mice, *M. oleifera* methanol extracts significantly potentiates the sleeping time induced by pentobarbitone sodium, diazepam and meprobamate. The methanolic extract of *M. oleifera* roots shows significant CNS depressant action in a dose-dependent manner. Methanol extracts of the root at increasing concentrations can prolong the sleeping time induced by standard sedatives such as pentobarbitone, meprobamate and diazepam (61).

The extract also exhibits analgesic properties and potentiates analgesia induced by morphine and pethidine. Pretreatment with the methanolic extracts causes significant protection against strychnine- and leptazol-induced convulsions. Methanol extracts not only significantly increases the average survival time but also decreases the percentage mortality in a dose-dependent manner on strychnine and leptazol-induced convulsions. This behavioral outcome in mice indicates the CNS depressant nature of *M. oleifera* extracts. The effect of the methanolic extracts on the prolongation of the sleeping time is due to its ability to increase the 5-Hydroxytryptophan (5-HTP) a precursor to the neurotransmitter serotonin, levels in mice brain. This increase explains the analgesic and anticonvulsive activities of the methanolic extracts. The increase of brain gamma-aminobutyric acid (GABA) levels after treatment with methanolic extracts explains another reason for its anticonvulsive activity. The reduction of awareness and depressant action is suggestive of the overall CNS depressant action of the methanolic extracts (61).

Other Medicinal Related Activities Of *Moringa Oleifera*

In addition to the above medicinal properties, *M. oleifera* also has other medicinal attributes under claim such as those used in the treatment of ascites, rheumatism, venomous bites and circulatory stimulant (17, 142). Various parts of this plant were used in tribal medicine for diseases like sores, dysentery, pneumonia (61). A bioenhancing property of *M. oleifera* pods extract is reported and that niaziridin rich fraction of *M. oleifera* pods facilitates the absorption of drugs, vitamins and nutrients through the gastro-intestinal membrane thus increasing their bio-availability. Therefore, niaziridin can be used in combination therapy with drugs and nutrients resulting in reduced drug associated toxicity, reduced cost and duration of chemotherapy (37). The extracts of leaves, seeds and roots of *M. oleifera* is being extensively studied for many potential uses including infertility (31).

Animal And Aquaculture Feed Use Of *Moringa Oleifera*

Moringa Oleifera - Nutrients For Animal Feed

Scarcity of animal feed resources, particularly during the dry season, is a major constraint to livestock production in the tropics. Animal feed supplements are too expensive for most farmers. Therefore, alternative feed sources are receiving a lot of attention in research. *M. oleifera* is a multipurpose tree, the leaves of which are used as animal feed in many places (72), but its potential as an animal feed supplement has only began to be actively documented in the recent years.

The protein, oil, vitamins, minerals, antinutritional factors and their constituents in *M. oleifera* have been described in the section under food. The non-protein nitrogen (NPN) is completely degraded in the rumen (6). However, the *in vitro* rumen crude protein degradability after 24 h of incubation averages 48.6% for the *M. oleifera* leaves. Much higher values of protein degradability are reported for seed cakes (144), although the values for *M. oleifera* falls within most common forages such as tannin-containing tree forages with protein degradability between 16 – 40% (145).

One of the factors responsible for the low rumen protein degradability of *M. oleifera* could be the low solubility. Solubility of the proteins averages 24% of the crude protein for the leaves (6). Similar results are known for *M. peregrina*. Also, lower solubility of *M. peregrina* proteins compares with that of soya (146). The protein insoluble in acid-detergent fiber (ADIP; protein unavailable to animals) averages 9.8% in acid detergent fibre (ADF) of the *M. oleifera* leaves. This amount of protein is unavailable to the animal. The protein potentially digestible in the intestine (PDI) averages 47.0% of the total crude protein for *M. oleifera* leaves. However, about 95.0% of the total nitrogen in *M. oleifera* leaves can either be available in the rumen or in the post rumen. High values of protein and PDI observed in *M. oleifera* leaves suggest that these leaves are a good source of protein supplement for ruminants (6).

The rate at which *M. oleifera* leaves are fermented *in vitro* is highly comparable with sorghum and barley straws. The shorter lag period and higher rate of digestion of neutral detergent fibre (NDF), suggests that the fiber quality of *M. oleifera* leaves is better than that of sorghum or barley straw. The rate of gas production from cell solubles of sorghum and barley straws is much higher than that of the *M. oleifera* leaves. Since there is a direct relationship between gas production and short chain fatty acid production (which are sources of energy for rumen microbes), *M. oleifera* leaves provides a sustained release of energy *in vivo* which is known to increase the efficiency of microbial protein synthesis (6).

The leaves of *M. oleifera* have negligible amounts of tannins and total phenolics (6). Condensed tannins, activity of trypsin inhibitors and lectins have not been detected in *M. oleifera*. Therefore, these antinutritional factors are considered not to be able to produce any adverse effects in animals. However, lectins have been reported in aqueous extracts at pH 4.5 (43). The saponins are observed to be at comparable contents as in soyabean meal. Phytates are found

to be at a level of concern as they range from 1 to 5%, the levels in legumes at which the phytates are known to decrease the availability of minerals in monogastrics (147). Other antinutritional factors reported for *M. oleifera* leaves are flatus factors (sucrose + raffinose + stachyose) which are known for producing flatulence in monogastrics. Nitrate and oxalate are also reported in *M. oleifera* leaves (148). The leaves of *M. oleifera* are quite rich in minerals, and the presence of oxalates and phytates at the reported concentrations are likely to decrease the minerals' bioavailability. However, the ethanol extract of *M. oleifera* leaves is free of tannins, saponins, lectins and trypsin inhibitors (6).

General Performance Of Animals Fed On *Moringa Oleifera*

Increased replacement levels of sunflower seed cake with *M. oleifera* leaves increases the digestibility of DM (dry matter) and NDF (neutral detergent fibre) (72). In a trial with goats, the inclusion of 9%, 27% and 36% of *M. oleifera* leaves in the ration that includes sunflower seed cake results in dry matter intakes (DMI) of 251, 335 and 311 g/day, respectively. This means that significantly higher DMI and metabolisable energy intake (MEI) are observed (72). In cows the supplementation of hay foliage with *M. oleifera* increases DMI and milk production (25). When cotton seed cake is substituted by *Moringa* leaf meal at levels of 10, 20 or 30% of DM, milk yield in *Bos indicus* cows significantly increases by 1.4, 0.9 and 0.8 kg cow day⁻¹ respectively. However, there are no effects of substituting cotton seed cake with *Moringa* leaf meal on total solids, fat and crude protein (CP) content of the milk (73).

Goats supplemented with *M. oleifera* leaf meal exhibits a significant higher body weight gain than nonsupplemented (batiki grass only) goats averages. Also, goats supplemented with 20% and 50% of *M. oleifera* leaf diets has higher digestibility of DM, crude protein (CP), neutral detergent fibre (NDF), organic matter (OM) and energy than the goats on batiki grass only and supplemented with 80% *M. oleifera* leaves (7). This suggests that *M. oleifera* leaves could be used as a substitute for the conventional supplemental feed. However, the levels of inclusion are still a matter of further research.

***Moringa Oleifera* In Aquaculture Feed**

The increasing cost of fish feed and the scarcity of fish meal have focused research on reducing the cost of the most expensive item in fish feeds, namely, the protein source. In order to attain a more economically sustainable and environmentally friendly production, research interest has been directed towards the evaluation and use of unconventional protein sources, particularly from plant products such as seeds, leaves and other agricultural byproducts (40, 75, 86). Therefore, developing a strategy to replace fish meal and fish oil in feeds should become a priority (104).

In Tilapia, a reduction in growth performance is observed when their diets are supplemented with *Moringa* leaf (40, 75). Diets with higher inclusion levels of *Moringa* leaves (20 and 30% *Moringa* leaf meal) significantly depresses

growth performance of tilapia fish (40). However, no difference in growth performance are observed in tilapia fish fed a methanol extract of *Moringa* leaf meal. The depressed growth performance of the tilapia fish on *Moringa* inclusion diet is attributed to the presence of toxic substances or antinutritional factors in the *Moringa* leaves. Specifically, relatively high total phenolics (0.7% and 1%), nonhaemolytic saponin (1.5% and 2.3%) and phytic acid (0.5% and 0.8%) in inclusion levels of *Moringa* leaves (20 and 30% *Moringa* leaf meal as protein base) feed formulations, as well as NDF (3.8% and 5.7%) and ADF (3.0% and 4.5%) in the aforementioned diets may contribute to the poorer growth performance (40). As a result, the use of plant-derived materials such as leaf meals as fish feed ingredients is limited by the presence of such antinutritional compounds (93).

In a bid to mitigate the antinutritional effects of *Moringa* on fish growth performance, the inclusion of methanol-extracted leaf meal (containing 11%, 22% and 33% methanol-extracted *Moringa* leaf meal) has potential as there is no significant effect on the growth performance of fish. This observation makes a basis to conclude that extraction of the antinutrients/antimetabolic components using 80%-methanol would permit a higher incorporation of *Moringa* leaf meal residue up to 33% in tilapia diets without any adverse effects on their growth (75).

The inclusion of raw, methanol extracted residues and methanol extracts of the *Moringa* leaf meal reduces the plasma cholesterol content significantly in Tilapia (74). The hypocholesterolaemic response of tilapia fed the *Moringa* - containing diets is attributed to the individual or combined effect of a high neutral detergent fibre (NDF) content, a high concentration of saponins and a substantial reduction in fishmeal (75). The fish on diets containing 13% raw *Moringa* leaf meal and containing 11% methanol-extracted *Moringa* leaf meal has significantly lower hepatosomatic indices when compared to those on a fishmeal based diet. The reduction in hepatosomatic indices is also observed in reduced liver weights in relation to the body weight of the fish (75). This observation is attributed to the reduced fat absorption, which results in a reduced fat retention in the whole body and liver in the fish.

Potential Industrial Utilisation Of *Moringa Oleifera*

Traditionally, *M. oleifera* seeds have been used to clarify turbid waters in rural areas of Asia and Africa (29). Of the identified 14 species of the moringaceae family, *M. oleifera* has gained considerable importance as flocculants in nearly all tropical and subtropical regions (8). Further, the coagulating property of the seed powder of this plant has been used for various aspects of water treatment such as turbidity, alkalinity, total dissolved solids, and hardness (30, 51, 54, 63). The seeds of six more frequent and cultivated species of *Moringa* have been found to contain flocculant components. Numerous studies so far confirms that *M. oleifera* seeds possess effective coagulation properties (49, 54, 63, 65). Branches of the *M. oleifera* when lopped and thrown into turbid and contaminated wells, over an episode of time, the previously dirty water becomes clear. The desiccated *M. oleifera* seeds are

recognized to clear water (1g/L) (149). Besides reducing turbidity of contaminated water, *M. oleifera* seeds also reduce the levels of microorganism during the same process where the bacteria are concentrated in the coagulated sediment. Coagulation and bacterial reduction using *M. oleifera* seeds has been observed to reduce turbidity up to 80-99.5% paralleled by a bacterial reduction of 1-4 log units (90-99.99%) within the first one to two hours of treatment. (97). A parasite, *Schistosoma mansoni cercariae* can be removed from clear and turbid water by flocculation with *M. oleifera* seeds by more than 90% (106).

Seed kernels of *M. oleifera* are known to remove lead, iron and cadmium ions from contaminated water by a biosorption process through the metal – protein interactions(26,51). *M. oleifera* pods (MOP) shows sorption potential for the removal of organics (e.g. benzene, toluene, ethylbenzene and cumene (BTEC)) from aqueous media and methyl parathion pesticide (MP) from surface and ground waters (68,69). *M. oleifera* alone or in combination can effectively be used and replace alum for dewatering of chemical sludge. In comparison to alum, *M. oleifera* shows comparable conditioning effects as alum (105).

Palm oil mill effluent (POME) is a voluminous, high biochemical oxygen demand (BOD) liquid waste, generally discharged at 75–85°C, highly polluting, but also extremely difficult to treat by conventional methods (14). *M. oleifera* seeds after extraction of oil has been shown as a potential natural coagulant for the POME pretreatment with minimum suspended solids and organic matter present in the treated POME. Compared to alum, the performance of *M. oleifera* seeds extract (MOSE) in coagulation–flocculation process, is an effective coagulant removing 95% suspended solids and 52.2% reduction in the chemical oxygen demand (COD) (16).

Activated carbon is a general term, which covers carbon material mostly derived from charcoal with an exceptionally high surface area and large amount of microporosity and absorbing properties. The steaming of waste husks of *M. oleifera* have demonstrated potential to produce activated carbon through the process of pyrolyzation and gasification. Steam activated husks exhibits a well-developed micropore volume of 0.57 cm³/g and a corresponding apparent surface area of 734 m²/g, as determined by BET N₂ adsorption hysteresis. In an assessment of aqueous phase adsorptive performance, activated *M. oleifera* carbon is comparable to commercial powdered activated water treatment carbons (PACs) and exhibits a Langmuir monolayer coverage constant (Q₀) of 1.89 mmol/g for phenol adsorption from the aqueous phase (18).

M. oleifera waste natural materials (WNM) can be converted into anion-exchangers through consecutive chemical reactions with thionylchloride, N,N-Dimethylformamide (DMF), dimethylamine and formaldehyde as cross linking agents. The final products obtained are weak-base anion-exchangers with tertiary amines as major functional exchange groups. (64).

M. oleifera possesses a gummy polymer which has been partially characterized. The gummy plant polymer from *M. oleifera* is highly polar and contains carbon, hydrogen, oxygen, phosphorus and calcium and is soluble in highly polar aprotic solvents (70). However, purified, whole-gum exudates from *M. oleifera* contains L-arabinose, D-galactose, D-glucuronic acid, and L-rhamnose, D-mannose and D-xylose and a tentative structure is assigned to the average repeating-unit of the gum (150). Although the gum is not structurally

characterized completely, the presence of phosphorus and the fire retardancy property of the gum are the main reasons behind the investigation of antioxidant property of the plant polymeric gum (70). This is because phosphoric acid esters are known to be heat stabilizers in polymers.

Imparting fire retardancy to polymers has, of late, become an important concern. One of the traditional ways of imparting fire resistance to polymers is the addition of fire retardant additives. A renewable polymer, collected as a gum from *M. oleifera*, has been dry blended with various rubbers and also with different commodity polymers. The adhesive strength of the blend of *M. oleifera* plant polymer with some rubbers is found to be higher than that of commercial adhesives. The limiting oxygen index values of the *M. oleifera* plant polymer used as the flame retardant (FR) additive in the rubber compounds is higher than all tested commercial FR additives used in the rubber compounds. In addition, rubber and/or commercial polymers may be made biodegradable by blending them with this *M. oleifera* polymer (71).

Potential Use Of *Moringa Oleifera* In Crop Production

In the last decade, large-scale cultivation of *Moringa* has been initiated (6, 27) and interest in research on agronomic aspects of *Moringa* is growing (3, 118,119,120). *Moringa* has a high fresh green matter production potential. Seventy five (75) day cutting frequency of *Moringa* produces the highest fresh matter yield, 100.7 and 57.4 Mg ha⁻¹year⁻¹ during the first and second year, respectively (3). High biomass production of *Moringa* of up to 99 tons of dry matter ha⁻¹ year⁻¹ as a result of eight cuttings after sowing 1 million seeds ha⁻¹ and managing the plantation with irrigation and fertilization has been achieved (151). The crude protein content of *Moringa* ranges between 193–264 g CP kg⁻¹ DM (3,6,7,118). The seed cake, produced after extraction of active ingredients of seed contains even higher levels of protein and this makes *Moringa* a potential good fertilizer for use in agriculture (14).

The leaves of *M. oleifera* can be extracted in 80% aqueous ethanol on a large scale and the extract used successfully to increase growth of plants, namely *Jatropha curcas* and peanut (6). The extract can be used in the form of a foliar spray to accelerate the growth of young plants. Use of the growth hormone spray from *M. oleifera* extract is known to cause the plants to be firmer, more resistant to pests and disease, produce more and larger fruit (151). Interest is growing to isolate hormone/growth promoters from leaves of *M. oleifera*. Nodulation of black-gram (*Vigna mungu L.*) shows to increase vigorously with the application of 80% ethanol extract of *M. oleifera*. The nature of the active agent present in the ethanolic extract is still unknown. However, the cytokinins have been shown to be present in the fruits of *M. oleifera* (6).

Other Uses Of *Moringa Oleifera*

The many other uses of *Moringa* include: alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), ornamental plantings, biopesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum) (22,47). Nonfood uses of behenic acid include applications as surfactants and detergents, plastics and plastic additives, cosmetics, photographing and recording materials. This shows the potential of the *M.oleifera* plant as an economic benefit for various industries (2). Further research and work on this plant is paramount to realize full benefits from it.

Future Research Prospects

The classification of *M.oleifera* has still some form of controversy, in the sense that it is still an area gathering more data in an effort to rest this case. Therefore, more research efforts into this area would go a long way in addressing this controversy. Although research on the use of *M.oleifera* oil in food applications is beginning to grow, there is still a lot of room required to bring this product into the food industry application arena. Its good antioxidant capacity, its thermal oxidative stability and presence of good amounts of fatty acids like behenic needs further explorations. The medicinal properties of *M.oleifera* have indeed been studied for a while now. Studies of *M.oleifera* extracts leave behind a big trail of evidence for various pharmacological properties of *M.oleifera*. While there has been good effort in isolating the pharmacological active compounds, more research is still required especially with the advent of more sophisticated analytical techniques available now. Much effort in research is also required in finding the mechanisms of action of such isolated active compounds. This will help in moving more towards *in vivo* research particularly in human beings, which has not been possible at present apart from what is obtained from traditional medicine. There has been overwhelming evidence for the water purification (coagulation/flocculation) properties of *M.oleifera* and *M.oleifera* extracts. Research is now beginning to concentrate on isolating and characterizing the active compounds in this area. The mechanisms of action of such isolated compounds is also being investigate, but it is obvious that more research is still required in isolation, characterization and mechanisms of action of coagulation/flocculation active compounds.

This chapter has revealed that the gum from *M.oleifera* as a polymer has great potential for renewable polymer applications. However, so far, research in this area seems to be in its infancy stage. Research in the agronomical aspects of *M.oleifera* is beginning to grow, although more still needs to be done as evidenced by the limited literature in this area. The agronomical data of *M.oleifera* will not only assure the sustainability of the above applications, but

will also assure its application in production of other crops. Utilization of *M.oleifera* in production of other crops, is in fact another area that requires a lot of research as this area is also in its very infancy. Research efforts also needs to be directed towards uncovering analytical techniques for isolation of active compounds in *M.oleifera* as this is the heart of research in order to have accurate and reliable research data.

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Chapter 25

Nutritional Assessment of Moringa (*Moringa spp.*) from Ghana, Senegal and Zambia

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The objective of this work was to assess the nutritional value of *Moringa spp.* leaves from Sub-Saharan Africa countries, Zambia, Ghana and Senegal. Our data showed that moringa leaves, can be used as nutritional supplements for malnourished people, particularly children and pregnant/lactating women in Sub-Saharan Africa. These findings also confirm and further support the use of moringa leaves as a plant-based affordable mechanism that can provide significant source of elements, particularly calcium, potassium and iron, proteins, and antioxidants to the diet.

Good nutrition is the cornerstone for survival, health and normal development for current and succeeding generations (1). Yet, this seemingly simple observation is in stark contrast to populations in many developing countries where malnutrition, especially in children, is a major societal problem. Of the 12 million deaths of children under the age of five that occurs annually, 55% of them are due to malnutrition (2). There are additionally approximately 146 million children who are at high risk of dying because they are considered to be underweight. Underweight is a leading crisis in developing countries because mortality rate increases exponentially with declining weight (1).

One low cost affordable strategy to improve the health and nutrition locally is to identify edible plants that are culturally acceptable and naturally high in those vitamins, minerals, and other nutritional factors lacking in the traditional foods that are available and consumed in the vulnerable regions.

Moringa (*Moringa oleifera*) is an important multipurpose tree, known as “The Miracle Tree”, because of its high nutritional value. It is also called by many names including drumstick tree, horse radish tree and mother’s best friend. Moringa is a deciduous tree or shrub, fast-growing, drought-resistant, average height of 12 meters at maturity (3). Of particular note is that this plant can grow in environments and soils that many other plants, trees and crop plants cannot thrive and/or survive. As many undernourished populations living in rural areas coincidentally are situated in or have access in general to difficult soils (eroded, denuded, infertile) in contrast to the rich alluvial soils where agricultural crops can easily thrive, having a plant that can grow, survive and produce highly nutritious leaves, pods, and seeds can be significant (Figure 1).

Nutritional analyses of moringa showed that the leaves contained high levels of calcium (2%) and iron (28 mg/100 g dry leaves) (3-5). Other reports have shown that moringa can contain seven times more vitamin C than oranges, four times more calcium and two times more proteins than milk, four times more vitamin A than carrots and three times more potassium than bananas (3).

While there is a wealth of information on basic potential nutritional value of moringa, the data is far from complete relative to the variation of nutritional quality and genetic differences within and between the many varieties of moringa in Sub-Saharan Africa. Furthermore, information is very limited on the nutritional value of moringa coming from Zambia, Ghana and Senegal.

The objective in this work was to assess the nutritional value of *Moringa spp.* leaves from Sub-Saharan Africa countries, Zambia, Ghana and Senegal. The use of moringa at the household level in these countries based upon science-based evidence could be a key and affordable strategy to help decrease the nutritional deficit and malnutrition that is currently found in Zambia, Ghana and Senegal as well as in so many other countries. In addition, the development of moringa-based products (dried leaves, moringa powder and other value-added products using moringa in foods) could provide economic opportunities at the family and village level for local and regional sales as well as encourage the sustainable production and increase use of the moringa trees in the landscape.

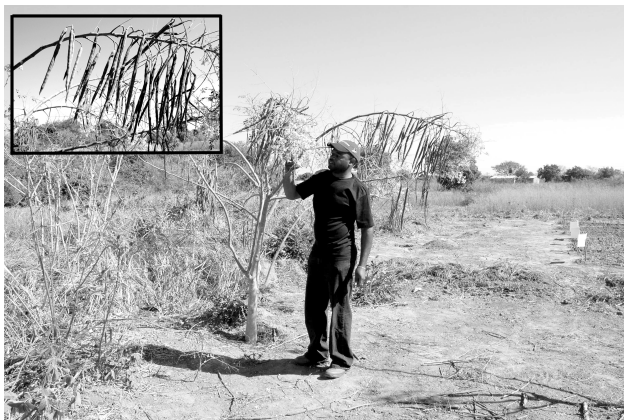


Figure 1. A young *Moringa oleifera* tree of the PKM 2 variety characterized by producing large amount of pods (upper left) (Zambia, near Lusaka).

Materials and Methods

Plant Material

Moringa (*Moringa oleifera*) leaves from Zambia were collected from the regions of Nampundwe, Mumbwa; Chikupi; Mungu, and Lusaka areas (PKM 1 and 2) and harvested at two different times (June 2006 and August 2006). The samples from Nampundwe belonged to the species *M. ovalifolia*. The samples from Ghana were collected from Dodowa and Volta Dam region in August 2006. All moringa samples from Senegal were collected from Massar (Niaye area) in January 2007. Leaves were collected manually and sun-dried at each location. All the procedures were run at least in duplicate. While this study sought to document the nutritional content from each country using the same genetic materials, we recognize that differences in the actual environment where each moringa site was established, rates of growth, sampling times and methods all would contribute to differences in final nutritional assessment. However, despite these differences, overall broad comparisons between the nutritional content was planned and relative ranges and values that may be seen across such diverse ranges and environments were actually of greater interest to better assess the robustness of the nutritional impact of such a plant.

Quality Control Analysis

The dry moringa leaves from Zambia, Ghana and Senegal were submitted to foreign matter analysis and moisture content analysis. Each sample was separated in three groups, the leaves and fine particles, and then weight recorded. The leaf samples were then ground to a fine powder for subsequent analysis. To determine the mineral content and contamination with sand and earth, total ashes and total insoluble ashes were evaluated for each sample using methods describe by the Food Chemical Codex (6).

Total Phenols Analysis

A total of 100 mg of the grounded leaves were placed in a 25 ml volumetric flask. Different concentrations of methanol/water were tested to determine the highest recovery of total phenols. We observed that the total phenols from moringa were mostly water soluble since the highest level of total phenols was extracted with 100% of water (Figure 2).

The moringa extracts (100 mg dry powder/25 ml water) were sonicated for 25 minutes. The total phenols were measured using the Folin Ciocalteu' reagent and absorbance was measured at 752 nm. The results were expressed as grams of Gallic acid equivalents on a dry basis (g gallic acid/100 g DW) (7).

Antioxidant Activity Analysis

The grounded leaves (100 mg) were extracted using the same method that was used with the total phenols. ABTS method was used to evaluate the antioxidant activity and the absorbance was measured at 734 nm. The results were expressed as g of Trolox (a water soluble analog of vitamin E) on a dry weight basis (g Trolox /100 g DW) (8).

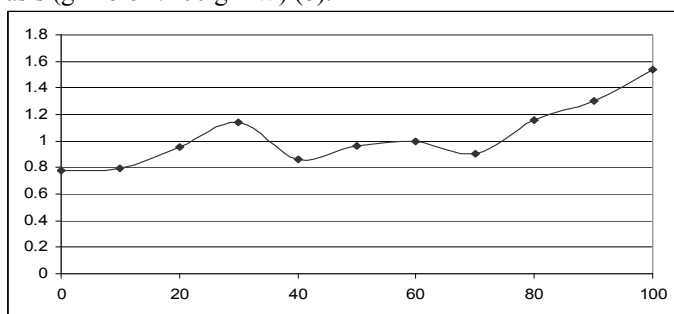


Figure 2. The relationship between extraction solvent (increasing concentration of water in methanol) and the recovery of the total phenolic content from moringa extracts.

Protein Analysis

The protein content was determined using the Bradford reagent with modifications (9). A total 50 mg of the grounded leaves were placed in a tube along with 10 ml of Bis-Tris- Hydrochlorine-Glycerol. This was mixed until all the material had dissolved and then transferred to a centrifuge tube for centrifugation for 2 minutes. Following, the supernatant was transferred to another cuvet where 1.5 ml of the Bradford reagent was added to each. After 20 minutes the absorption was measured at 595 nm. The results were expressed as g of protein (Albumin) on a dry weight basis (g Albumin/100 g DW).

Elemental Analysis

Foliar samples were submitted to the Agricultural Analytical Services Lab (Pennsylvania State University Soil and Plant Testing Laboratory) to determine the concentration 11 elements in moringa including: phosphorus, potassium, calcium, magnesium, manganese, iron, copper, boron, aluminum, zinc and sodium. The results were expressed for the macronutrients (phosphorus, potassium, calcium, magnesium) as g of element per 100 grams on a dry weight basis (g element/100 g DW). For the micronutrient (manganese, iron, copper, boron, aluminum, zinc and sodium), the results were expressed as mg of element per 100 grams on a dry weight basis (mg element/100 g DW).

Results and Discussion

Quality Control Analysis

For the samples from Zambia, the dry moringa leaves were found to be light to dark green. However, the Zambia's samples from Chikupi for June and August contained some yellowing and chlorosis and the sample from Binga for August contained some brown necrotic and yellowing leaves (Tables I and II). Leaf size ranged from 1cm to 1.6 cm in the samples from Binga to 2.3-2.7 cm in the indigenous moringa from Mumpundwe for both harvesting periods (Tables I and II). As observed from the Zambian moringa, the leaves from both Ghana and Senegal were light to dark green in color. However, the coloring for the leaves from Ghana appeared a bit darker when compared with the ones from Zambia. The sizes of leaves for Ghana and Senegal were medium with a range of 1.6 (Volta Dam & PKM-1-Senegal) to 1.8 cm (Local Variety-Senegal) again when compared to those of Zambia (Table III).

Table I. Selected Quality Control Parameters for *Moringa* spp. Samples from Zambia, June 2006.

<i>Variety</i>	<i>Color</i>	<i>Size</i> ¹	<i>Leaf %²</i>	<i>F. Matter</i> ³	<i>Fine Part.</i> ⁴	<i>Moist.</i> ⁵	<i>Ashes</i> ⁶	<i>AIA</i> ⁶
PKM-1	Dark green	2.6 ± 0.2	86	14	0.2	7.7 ⁷	11.7 ⁷	0.7 ⁷
Nampundwe	Light green	2.7 ± 0.2	86.4	13.6	0.2	7.0 ⁷	11.1 ⁷	0.7 ⁷
Chikupi	Yellow, dark green	1.73 ± 0.5	84.6	15.2	0.23	7.5 ⁷	10.1 ⁷	0.7 ⁷
PKM-2	Dark green	1.8 ± 0.2	80.3	19.4	0.3	6.9 ⁷	11.2 ⁷	0.9 ⁷
Mungu	Dark green	2.1 ± 0.1	85.5	14.8	0	6.6 ⁷	11.7 ⁷	0.95 ⁷ 0.05 ⁷
Binga	Light green	1 ± 0.2	80.2	19.8	0	6.9 ⁷	-	

NOTE: ¹Size in mm, mean ± standard error; ²Percent of leaves (g/100 g dry weight, DW); ³Foreign matter in percent; ⁴Fine particles in percent; ⁵Moisture; ⁶total ashes, and acid Insoluble ashes (AIA) in percent (g/100 g DW); ⁷Standard error less than 0.1.

All of the moringa samples from the three countries were composed mostly of leaves with a range from 73% to 86% (Tables I-IV). Concerning the samples from Zambia, those collected in August contained higher levels of foreign matter (stems and small twigs) when compared to the ones collected in June (Tables I and II). Regarding Ghana, the sample from Volta Dam contained a larger amount of foreign matter (25%) when compared to the sample from Dodowa (16%) (Table III). The samples from Senegal all contained a high amount of foreign matter with a range of 17% to 26% (Table IV). All the samples from Zambia, Ghana and Senegal contained low levels of fine particles (dust and sand) (Tables I-IV).

Table II. Selected Quality Control Parameters for *Moringa* spp. Samples from Zambia, August 2006.

<i>Variety</i>	<i>Color</i>	<i>Size</i> ¹	<i>Leaf</i> <i>%</i> ²	<i>F.</i> <i>Matter</i> ³	<i>Fine</i> <i>Part.</i> ⁴	<i>Moist.</i> ⁵	<i>Ash</i> ⁶	<i>AIA</i> ⁶
PKM-1	Dark, light green	1.8 ± 0.4	87.5	12.2	0.1	7.8 ⁷	11.2 ± 0.2	1.3 ± 0.1
Nampun dwe	Dark, light green	2.3 ± 0.3	85.2	13.2	0.2	9.6 ⁷	9.1 ⁷	1.0 ⁷
Chikupi	Green, some yellow	1.7 ± 0.3	73.2	25.6	0.2	8.3 ⁷	9.5 ⁷	0.8 ⁷
PKM-2	Dark & light green	1.8 ± 0.7	84.3	15.6	0.0	7.3 ⁷	10.2 ⁷	0.9 ⁷
Mungu	Dark & light green	2.1 ± 0.4	68.2	31.5	0.2	8.4 ⁷	9.4 ⁷	0.7 ⁷
Binga	Green, brown and yellow	1.6 ± 0.3	72.5	27.5	0.2	8.2 ⁷	9.7 ⁷	0.8 ⁷

NOTE: ¹Size in mm, mean ± standard error; ²Percent of leaves (g/100 g dry weight, DW); ³Foreign matter in percent; ⁴Fine particles in percent; ⁵Moisture; ⁶total ashes, and acid Insoluble ashes (AIA) in percent (g/100 g DW); ⁷Standard error less than 0.1.

The moisture content of the samples from Zambia, Ghana and Senegal ranged from 5.8% to 10.6%. In general, the recommended maximum values for moisture content in such dried leaves ranges from 10 to 12% according to international standards for many herbs and dried plants (Tables I-IV) (10). The samples from Ghana contained the highest amount of moisture with a range from 10.3% (Dodowa) to 10.6% (Volta Dam). In contrast, the leaves from Senegal had the lowest moisture content with a range of 5.9% (Local Variety) to 6.72% (PKM-1) (Tables III-IV). The dried leaves from Zambia were in between ranging from 6.6 % (Indigenous-Mungu Area-June) to 9.6% (Indigenous-Nampundwe-August) (Tables I-III).

Table III. Selected Quality Control Parameters for *Moringa oleifera* Samples from Ghana.

<i>Variety</i>	<i>Color</i>	<i>Size</i> ¹	<i>Leaf</i> % ²	<i>F.</i> <i>Matter</i> ³	<i>Fine</i> <i>Part.</i> ⁴	<i>Moist.</i> ⁵	<i>Ashes</i> ⁶	<i>AIA</i> ⁶
Dodowa	Darker green	1.7 ± 0.3	82.2	15.7	0.0	10.3 ⁷	7.3 ⁷	0.7 ⁷
Volta Dam	Darker green	1.6 ⁷	72.8	24.5	0.0	10.6 ⁷	6.4 ⁷	0.3 ⁷

NOTE: ¹Size in mm, mean ± standard error; ²Percent of leaves (g/100 g dry weight, DW); ³Foreign matter in percent; ⁴Fine particles in percent; ⁵Moisture; ⁶total ashes, and acid Insoluble ashes (AIA) in percent (g/100 g DW); ⁷Standard error less than 0.1.

The total ashes from Zambian leaves ranged from 9.1% (Indigenous-Nampundwe- August) to 11.7% (PKM-1, Indigenous-Mungu Area-June) (Tables I and II). The samples from Senegal have a higher value of total ashes ranging from 11.1% (PKM-1) to 13.0% (Local Variety) (Table IV). These high percentages suggest that both the Zambia and Senegal have a high amount of total minerals in the samples.

Table IV. Selected Quality Control Parameters for *Moringa oleifera* Samples from Senegal.

<i>Variety</i>	<i>Color</i>	<i>Size</i> ¹	<i>Leaf</i> % ²	<i>F.</i> <i>Matter</i> ³	<i>Fine</i> <i>Part.</i> ⁴	<i>Moist.</i> ⁵	<i>Ashes</i> ⁶	<i>AIA</i> ⁶
PKM-1	Dark- light green	1.6 ⁷	79.8	17.4	0	6.7 ⁷	11.1 ⁷	1.4 ⁷
Local Variety	Dark- light green	1.8 ⁷	73.5	23.5	0.2 ⁷	5.9 ⁷	13.1 ⁷	1.4 ⁷

NOTE: ¹Size in mm, mean ± standard error; ²Percent of leaves (g/100 g dry weight, DW); ³Foreign matter in percent; ⁴Fine particles in percent; ⁵Moisture; ⁶total ashes, and acid Insoluble ashes (AIA) in percent (g/100 g DW); ⁷Standard error less than 0.1.

The samples from Ghana have a lower percentage of total ashes with Dodowa having a 7.3% and Volta Dam with a 6.4% (Table III). This suggests that the content of minerals will be lower in the Ghana samples when compared to those of Zambia and Senegal. However, all samples do have considerably high percentages since the value for total ashes for dried botanicals usually ranges from 3.5% to 16% (19).

The acid insoluble ashes are a classic determination of cleanliness in botanical products; usually a maximum of 1-1.5% is accepted for food products. Most of the samples from both Zambia and Ghana contained less than 1% (Tables I-III). However, the samples from Senegal had a percentage of acid insoluble ashes that was a little higher than 1% with a value of 1.4% for both varieties (PKM-1 & Local Variety) (Table IV).

Total Phenols, Antioxidant Activity & Protein Analysis

Moringa leaves from Zambia exhibited high levels of total phenols and antioxidant activity suggesting that these samples were a rich source of antioxidants components (Tables V and VI). Total phenols ranged from 3.4% (Binga-August) to 5.6% (Indigenous-Nampundwe-June) which is an intermediate level of total phenols (Tables V and VI). The antioxidant activity ranged from 4.9% (PKM-1, June) to 7.7% (Indigenous-Mungu, June) (Tables V and VI), the results were expressed as vitamin E equivalents. Analyzing these data it appears that the collection time (June vs. August) appeared to have little effect in the above mentioned parameters. This was a concern as nutritional composition of leaves may also be related to leaf age, seasonal accumulation patterns and more.

Leaf samples from Ghana contained lower levels of total phenols and antioxidant activity (Table VII). The sample from the Volta Dam only contained 3.3% of total phenols and the sample from Dodowa was even lower at 2.6%. The antioxidant activity not surprisingly was also lower than the majority of the samples from Zambia (Tables V-VII). The sample from Volta Dam had an antioxidant activity of 5.7% while the sample from Dodowa's had a little lower activity of 5.0% (Table VII).

In contrast to the samples from Ghana and Zambia, the samples from Senegal had a level of total phenols that was lower than those of Zambia but higher compared to the two samples from Ghana. The variety PKM-1 had a total phenol content of 3.6% and the Local Variety contained 3.1% (Table VIII).

The protein content of all the samples from Zambia, Ghana and Senegal are noticeably high (Tables V-VII). For Zambia the range was from 13.0% (Indigenous-Nampundwe, Mungu Area-June, and Indigenous-Chikupi-August) to 17.0% (PKM-2, August) (Tables V and VI). Ghana's two samples yielded 10.2 % (Dodowa) and 13.1% (Volta Dam) (Table VII). Samples from Senegal, contained very similar amounts of protein with PKM-1 having 13.0% and Local Variety having 14.30% (Table VIII).

The recommended dietary allowance (RDA) of protein for a child between 1 and 3 years old is 13 g/day and for children between the ages of 4 and 8 years it is 19 g/day (11). Consequently, a serving of 100 grams of grounded leaves from any of the varieties from Zambia and Senegal and from Ghana the variety from Volta Dam will satisfy all the daily requirements of protein for children ages 1 to 3. Furthermore, to satisfy the RDA of protein for children ages 4 to 8 146 grams and up of dried leaves from any of the same varieties needs to be given. For the other variety from Ghana (Dodowa) 128 grams or 187 grams of fresh grounded leaves depending on the age of child would need to be given to the same child to satisfy the RDA. That is, though the total protein content varied between areas, moringa was found to be nutritionally significant in all sites.

Table V. Total Phenol, Anti-Oxidant and Protein Activity in Different *Moringa* spp. Samples from Zambia, June 2006.

<i>Variety</i>	<i>Total Phenols</i> ¹	<i>Antioxidant Activity</i> ²	<i>Protein</i> ³
PKM-1	4.9	7.6	14.3
Nampundwe	5.6	7.2	12.7
Chikupi	3.8	4.8	14.0
PKM-2	4.3	6.3	13.5
Mungu	4.9	7.7	13.0
Binga	3.8	6.0	13.4

NOTE: ¹g gallic acid equivalents/ 100g dry weight; ²g Trolox equivalents/100g dry weight; ³g protein/100 g DW.

Table VI. Total Phenol, Anti-Oxidant and Protein Activity in Different *Moringa* spp. Samples from Zambia, August 2006.

<i>Variety</i>	<i>Total Phenols</i> ¹	<i>Antioxidant Activity</i> ²	<i>Protein</i> ³
PKM-1	3.5	5.3	15.3
Nampundwe	3.8	6.0	15.4
Ckikupi	4.3	5.4	13.0
PKM-2	5.4	5.8	17.0
Mungu	4.7	6.3	14.3
Binga	3.4	5.2	13.0

NOTE: ¹g gallic acid equivalents/ 100g dry weight; ²g Trolox equivalents/100g dry weight; ³g protein/100 g DW.

Table VII. Total Phenol, Anti-Oxidant and Protein Activity in Different *Moringa oleifera* samples from Ghana.

<i>Variety</i>	<i>Total Phenols</i> ¹	<i>Antioxidant Activity</i> ²	<i>Protein</i> ³
Dodowa	2.6	4.5 ± 0.2 ⁵	10.2
Volta Dam	3.3	5.1 ± 0.3	13.1

NOTE: ¹g gallic acid equivalents/ 100g dry weight; ²g Trolox equivalents/100g dry weight; ³g protein/100 g DW.

Table VIII. Total Phenol, Anti-Oxidant and Protein Activity in different *Moringa oleifera* Samples from Senegal.

<i>Variety</i>	<i>Total Phenols</i> ¹	<i>Antioxidant Activity</i> ²	<i>Protein</i> ³
PKM-1	3.6	7.4	13.2
Local Variety	3.1	7.0	14.3

NOTE: ¹g gallic acid equivalents/ 100g dry weight; ²g Trolox equivalents/100g dry weight; ³g protein/100 g DW.

Elemental Analysis

The moringa from Zambia and Senegal contained high amounts of total minerals (Tables IX-XIV). The PKM 1 and 2, and Nampundwe samples were high in calcium (2.7-2.9%), potassium (2%) and iron (31-34 mg/100g) (Table IX). The other samples showed lower levels of elements, particularly calcium (Binga, 2% and Mungu, 1.7%). The sample from Binga was high in iron (35 mg/100g). The samples from Senegal (PKM-1) were also excellent sources of calcium (3%), potassium (2-1.5%), magnesium (0.3-0.4%), iron (31-50 mg/100g), manganese (13-16 mg/100g) and copper (0.9-0.8 mg/100mg) for June and August, respectively (Tables IX and XIV). In contrast the samples from Ghana contained lower levels of many of these minerals (Table XI).

Nutritionally, 20 grams of dried grounded leaves of *Moringa oleifera* from the variety PKM-1 from Zambia, would provide 100% of the RDA'S (11) for calcium, iron, magnesium and manganese for a child between the ages of 1 to 3 (Table XV). From only around 30 grams of the same variety, PKM-1, moringa would provide the same nutritional value to a child from 4 to 8 years old. For copper, 50 grams of fresh grounded leaves from the variety PKM-1 would be enough to provide the RDA for children ages 1 to 8.

For the variety PKM-1, only 17 grams of dry leaves of moringa would be needed to provide 100% of the Recommended Daily Allowances for calcium, iron, magnesium and manganese for a child between the ages of 1 to 3 (Table XV). Furthermore, for a child ages 4 to 8 years, 27 grams of this same variety will be enough to also provide 100% of the RDA for all the same nutrient.

Although, the difference between the quantities needed to provide 100% RDA for calcium, iron, magnesium and manganese using the Zambia sample and the ones using the Senegal sample are not drastically different, it is still noteworthy to recognize that overall; The Senegalese moringa had a higher nutritional value than that coming from Zambia and Ghana. For example, to satisfy all the daily requirements of iron for a child between the ages of 1 and 3 years, only 9 grams of the leaves of PKM-1 from Senegal, 14 grams of the leaves from Zambia and 30 for the leaves from Ghana grams would be needed.

Moringa samples from Ghana had a lower nutritional value when compared to those from Zambia and Senegal. For calcium 16.6 grams of grounded leaves of the Zambian sample would be sufficient for the RDA of children 1 to 3 years old. However, the Ghanaian samples, although still a good sources of nutrients, would require about 30 g of dry moringa samples to provide the adequate levels

of calcium and iron (Tables IX and XV). Consequently, to provide 100% the RDA'S for calcium, iron, magnesium and manganese for a child between the ages of 1 to 3 using the samples from Dodowa, 30 grams of dry leaves would be needed, and for a child between the ages 4 to 8, 50 grams would be needed. For copper, 88 grams of the dry leaves will be need for children (1 to 8 years) to meet their daily requirement. While the variety PKM-1 from Zambia, only 50 grams of dry leaves would provide the requirement of copper (Table XV).

Our results show that the samples from Zambia and Senegal are excellent sources for the Recommended Daily Requirements of calcium, iron, magnesium, manganese and copper for children and also would be other vulnerable populations such as pregnant and lactating women. With 100 grams of the PKM-1 variety from Zambia, one can satisfy the RDA's of calcium, iron, magnesium and manganese for pregnant and lactating women between the ages of 14 to 18 years old. For the same population, using the PKM-1 variety from Senegal you would only need 60 grams of fresh grounded leaves to provide 100% of the same RDA's (Table XII). To satisfy the RDA's for copper, one would need from the Senegal's PKM-1 118 grams of fresh grounded leaves for pregnant women between the ages 14 to 18 and around 150 grams for lactating women between the same ages.

While we recognize that these observations relative to RDA's are based upon assumptions relative to dietary/nutritional recommendations and are thus mathematically calculated not taking into account body weight and other factors, but rather 'age' only, the data can still be viewed in a comparative manner to other sources of the same nutrients and in a relative rather than absolute manner using the same assumptions. While these estimates may vary from that recommended by the governments of Ghana, Senegal and Zambia's public health programs, these data are relevant by providing comparative values of nutrition.

Figures on RDA are provided as estimates only (Table XIV), since it has to be noticed that the daily consumption of a certain amount of moringa powder (e.g. 50-100 g or higher) would be too much to be consumed in a single day, particularly for young children. These work suggest that moringa leave powder can be used to supplement the diet.

Moringa sample contained varying levels (14% to 24%) of twigs and small stems (Table I-IV). The elemental analysis of the macronutrients showed that the twigs contained similar amounts of elements as compared with the leaves. In the PKM1, variety from Zambia, the leaves contained higher levels of potassium and magnesium, and lower levels of calcium and phosphorus. For micronutrients, the twigs always contained lower levels of manganese, iron, copper, boron, and aluminum, while zinc remained unchanged. The samples from the Mungu area showed a similar trend (Table X). Though the elemental content of the stems were a reflection of the nutritional status of the leaves since the stems from Ghana showed also lower levels of calcium, potassium and iron, among others. This data suggests that a certain percentage of stems can be allowed in the fresh grounded leaves without decreasing the nutritional value.

Table IX. Elemental Composition of the Leaves of Different *Moringa* spp. Varieties from Zambia

<i>Variety</i>	<i>P (%)</i>	<i>K (%)</i>	<i>Ca (%)</i>	<i>Mg (%)</i>	<i>Mn¹</i>	<i>Fe¹</i>	<i>Cu¹</i>	<i>B¹</i>	<i>Al¹</i>	<i>Zn¹</i>	<i>Na¹</i>
First Harvest, June 2006											
PKM-1	0.3	2.0	2.9	0.3	13.3	31.0	0.9	2.7	31.6	1.8	3.2
Nampundwe	0.3	2.0	2.7	0.3	13.5	33.9	0.9	2.6	34.5	1.8	2.9
Chikupi	0.4	2.7	1.5	0.4	8.2	27.9	0.7	3.0	30.3	1.8	7.2
PKM-2	0.3	2.0	2.8	0.2	13.9	33.8	0.9	2.3	32.5	1.8	2.5
Mungu	0.2	1.7	3.0	0.4	15.5	29.3	0.7	2.2	27.8	2.0	12.6
Binga	0.3	1.7	2.1	0.3	11.3	34.9	0.8	1.5	31.9	1.9	2.3
Second Harvest, August 2006											
PKM-1	0.3	1.5	3.0	0.4	16.4	49.9	0.8	2.9	50.9	2.0	2.8
Nampundwe	0.3	1.7	2.3	0.3	15.1	35.1	0.7	2.2	41.3	2.4	3.4
Chikupi	0.3	2.3	1.9	0.4	5.5	36.2	1.0	0.8	47.4	2.6	13.0
PKM-2	0.3	1.6	2.7	0.3	15.3	32.7	0.7	2.5	38.7	2.2	3.0
Mungu	0.3	2.2	2.0	0.4	5.8	36.5	0.6	0.8	45.2	2.2	13.7
Binga	0.3	1.7	2.3	0.3	6.3	59.4	0.8	0.6	62.4	1.9	37.0

NOTE: ¹mg/100g DW.

Table X. Elemental Composition of the Stems of Different *Moringa* spp. Varieties from Zambia

<i>Variety</i>	<i>P (%)</i>	<i>K (%)</i>	<i>Ca (%)</i>	<i>Mg (%)</i>	<i>Mn¹</i>	<i>Fe¹</i>	<i>Cu¹</i>	<i>B¹</i>	<i>Al¹</i>	<i>Zn¹</i>	<i>Na¹</i>
First Harvest, June 2006											
PKM-1	0.17	2.24	2.69	0.62	9.2	18.1	0.6	1.9	22.2	1.8	8.2
Second Harvest, August 2006											
Mungu	0.23	2.53	1.30	0.47	2.1	28.2	0.4	1.0	36.5	1.9	14.9
PKM-1	0.18	1.49	2.35	0.83	6.7	27.0	0.5	1.7	29.3	2.1	5.1

NOTE: ¹mg/100g DW.

Table XI. Elemental Composition of the Leaves of Different *Moringa oleifera* Varieties from Ghana

<i>Variety</i>	<i>P (%)</i>	<i>K (%)</i>	<i>Ca (%)</i>	<i>Mg (%)</i>	<i>Mn¹</i>	<i>Fe¹</i>	<i>Cu¹</i>	<i>B¹</i>	<i>Al¹</i>	<i>Zn¹</i>	<i>Na¹</i>
Dodowa	0.2	1.1	1.6	0.5	6.1	24.0	0.5	6.4	24.9	1.4	30.8
Volta	0.3	1.8	0.9	0.3	8.0	15.7	0.8	2.4	18.9	2.0	15.1
Dam											

NOTE: ¹mg/100g DW.

Table XII. Elemental Composition of the Stems of Different *Moringa oleifera* Varieties from Ghana

Variety	P (%)	K (%)	Ca (%)	Mg (%)	Mn ¹	Fe ¹	Cu ¹	B ¹	Al ¹	Zn ¹	Na ¹
Volta Dam	0.20	2.37	0.61	0.15	15.1	17.2	0.5	1.9	29.5	2.2	16.0

NOTE: ¹mg/100g DW.

Table XIII. Elemental Composition of the Leaves of Different *Moringa oleifera* Varieties from Senegal

Variety	P (%)	K (%)	Ca (%)	Mg (%)	Mn ¹	Fe ¹	Cu ¹	B ¹	Al ¹	Zn ¹	Na ¹
PKM-1	0.32	0.97	2.91	0.66	11.25	75.55	0.85	4.70	119.70	3.70	246.45
Local											
Variety	0.33	1.21	3.11	0.56	10.45	51.05	1.65	6.60	86.65	3.00	289.10

NOTE: ¹mg/100g DW.

For the use of *M. oleifera* leaves as nutritional supplement, we propose the following initial standards for moringa, thus providing to users and international community recommendations for consistent and defined products (Table XV). The development of clear grades and standards for moringa should provide a foundation upon which researchers, processors, and producers can objectively define this product and then compare final quality and nutritional attributes.

Table XIV. Estimated amount of *Moringa oleifera* Leaf Powder from Ghana, Senegal and Zambia to Provide Recommended Dietary Allowances of Selected Elements

Recommended Dietary Allowances (RDAs) ¹	Ghana Dodowa	Senegal PKM-1	Zambia PKM-1
K 3,000 mg (Children 1-3y)	272.7 g	309.3 g	200.0 g
K 3,800 mg (Children 4-8y)	345.5 g	391.8 g	253.3 g
Ca 500 mg (Children 1-3y)	31.3 g	17.1 g	16.6 g
Ca 800 mg (Children 4-8y)	50.0 g	26.9 g	26.6 g
Mg 80 mg (Children 1-3y)	16.0 g	12.1 g	20.0 g
Mg 130 mg (Children 4-8y)	26.0 g	19.7 g	32.5 g
Mn 1.2 mg (Children 1-3y)	19.7 g	10.7 g	7.3 g
Mn 1.5 mg (Children 4-8y)	24.6 g	13.3 g	9.2 g
Fe 7 mg (Children 1-3y)	29.2 g	9.3 g	14.0 g
Fe 10 mg (Children 4-8y)	41.6 g	13.2 g	20.0 g
Cu 0.34 mg (Children 1-3y)	68.0 g	40.0 g	42.5 g
Cu 0.44 mg (Children 4-8y)	88.0 g	51.8 g	55.0 g
Zn 3 mg (Children 1-3y)	214.3 g	81.1 g	150.0 g
Zn 5 mg (Children 4-8y)	357.1 g	1.35 g	250.0 g

SOURCE: Modified from United States Department of Agriculture, National Agriculture Library. Washington DC (11).

Table XV. Recommended Quality Standards for *Moringa oleifera* Dried leaves for its use as a Nutritional Supplement

<i>Characteristic</i>	<i>Requirement</i>
Color	Dark/light green
Foreign non plant materials %, (m/m)	1
Twigs an small stems %, (m/m) maximum	20
Foreign materials (other than twigs/stems)	1
Fine particles %, (m/m) maximum	1
Moisture content %, (m/m) maximum	12
Total ashes %, (m/m) minimum	10
Total insoluble ashes %, (m/m) maximum	2
Total phenols %, (m/m) minimum	4
Antioxidant activity %, (m/m) minimum	6
Total Proteins %, (m/m) minimum	10
Calcium % (m/m) minimum	2.3
Iron % (m/m) minimum	0.04

Conclusions

Our data showed that moringa leaves can be used as nutritional supplements for malnourished people, particularly children and pregnant/lactating women in Sub-Saharan Africa. These findings also confirm and further support the use of moringa leaves as a plant-based affordable mechanism that can provide significant source of elements, particularly calcium, potassium and iron, proteins, and antioxidants to the diet. Moringa leaves can be an affordable avenue to improve health and nutrition in Sub-Saharan countries. While the objective of this research was to better understand the nutritional characteristics of moringa across several regions in Africa and using both similar as well as distinct varieties and sources, no studies were conducted to evaluate the safety and toxicity of this plant product, particularly for infants and young children. Given its long history of food use in many countries, including the USA and the absence of adverse reports indicating toxicity, it is reasonable to suggest that there is a high probability that the plant would be safe for human consumption even in a concentrated formula. Studies evaluating the palatability and toxicity under a concentrated nutraceutical formula are needed and recommended. While the issue of taste and food preparation were also not factors included in this study, it is important to note that the leaves of moringa are noted for their varying degree of bitterness. Future studies examining the bitterness, methods to reduce this characteristic as well as identifying the plethora of foods that can be prepared with moringa is also warranted.

This study also showed that there was variation in the nutritional values, and the results suggest the necessity to conduct quality analyses to assess the nutritional value of moringa leaves coming from different sources, over time and

over regions. Simple methods to assess moringa leaves nutritional status (e.g. moisture, ashes, proteins, phenols and antioxidants) were developed to define and control quality of the leaves and thus optimize their nutritional content. These methods can be easily transferred to Sub-Saharan countries, as each screen was used for its low cost and ease, with the exception of the mineral analyses. With 100 grams of the variety from PKM-1 from Zambia or Senegal, the Recommended Daily Allowance of calcium, iron, magnesium, manganese, anti-oxidants and proteins for a child between the ages 1 to 3 can be satisfied. For a child ages 4 to 8 only 50 grams would be needed for the same outcome. Pregnant women aged 14 to 18 years would need about 130 grams of the PKM-1 to satisfy the same RDA's. For lactating women, this amount would increase to 160 grams.

This study also suggests that *Moringa oleifera* leaves, particularly those from Zambia and Senegal, can serve as an excellent nutritional supplement to malnourished children in Sub-Saharan Africa as well as pregnant and lactating women. The moringa from Ghana can also serve only higher amounts would be needed to be consumed for the same impact. In all cases, moringa being native to Africa can be used by individuals, families, and communities as a local affordable source of nutritionally important and often presently limited nutrients. The attribute that the moringa can thrive or survive under harsh adverse soils provides another practical and strategic advantage. As Moringa can be an affordable avenue to improve health and nutrition in sub-Saharan countries, we recommend that it be further studied and integrated into national health and well-being programs.

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Chapter 26

Saro (*Cinnamosma fragrans* Baillon) essential oil: Application in Health and Medicine

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The richness of medicinal plants of the island Madagascar is unique in the world and has largely been understudied. Saro, *Cinnamosma fragrans*, is produced from a Madagascan plant which is locally well-known for its antimicrobial activities and also for its powerful anti-poisoning effects. While searching for local strategies to enhance the value of this renewable natural resources for the benefit of local populations and biodiversity conservation this plant appeared as a very promising candidate with an essential oil that could be used in many new ways. This paper presents research on the plants essential oil composition including the variation found across environments where the plant is endemic as well as the results of associated biological tests conducted on the essential oils' toxicity, skin and eye tolerance and on mutagenesis potential. The properties of the Saro essential oil have been tested and show very interesting results as an antibacterial and antiviral agent and as a health protection enhancer.

Madagascar, an island as large as the state of Texas, is located in the southern Indian Ocean. The island was separated from Africa about 65 million years ago. This isolation has created an amazing diversity of plants and animals. The island hosts up to 13,000 plant species of which 85% are endemic (1), while endemism (96%) is even higher in the case of trees and large shrubs (2), the endemic richness of species in Madagascar is thus unparalleled in the world (3).

Saro, or *Cinnamosma fragrans* (*Canellaceae*), is an aromatic shrub or small tree, up to five meters high, evergreen and very aromatic. This small family includes only nine species in four genus, *Winterana* and *Cinnamodendron* in America, *Warburgia* in western Africa and *Cinnamosma* endemic to Madagascar. The genus includes, *C. madagascariensis*, in the eastern forests of the island between 900 to 1500 meters of altitude, with a variety *C. madagascariensis namorensis*, in the littoral forest at low altitude. *C. macrocarpa*, in the eastern forest between 600 to 900 meters altitude but much rarer; and *C. fragrans*, in the western forest (4).

Leaves are full alternate transparent when young becoming hard when adult. Flowers are white small (5 to 6 mm) and hermaphrodites. The fruit is an ovoid berry dark brown when mature, having 15 to 25 seeds. Saro is often found in trophophyle forests, close to the water or in rocky shady areas, and growing in chalky or silicitic soils. Flowering occurs between September and November and fruits develop during the rainy season which occurs December and May (5).

Some initial research was conducted on Saro (6) though research came to a halt due to the sociopolitical isolation of the island from 1972 until recently. Initial work also had been conducted on the vanilloides present in this plant which had originally suggested potential applications as memory enhancers, appetite regulation and emotions, among others (7). These new applications may be surprising to scientists but the plant has been well-known by the local populations and its uses have been described by different authors since 1910 (8-10).

Although, it has incredible natural wealth, Madagascar is still one of the poorest nations of the world. The United Nations has declared Madagascar a world major priority for conservation because of the threats on its biodiversity (11). Thus, the discovery of new products with new potential applications can drive the commercialization process and add value to the natural resources and by generating income for local populations which in turn can contribute to sustainable development. The sustainable production of essential oils, through sustainable harvesting of the leaves is an alternative to other destructive agricultural practices, like slashing and burning (12). Thus, the ability to be able to continually harvest leaves of this plant for its essential oils would be a key strategy to contribute to sustainable development.

The first humans arrived to the island 2000 years ago. The newcomers had to discover the properties of plants to treat themselves and since then the local population (80%) still use medicinal plants (13). Saro is one of these plants and its' local name reflects the fact that it has many therapeutic uses including anti poisoning. Saro's local name is "Mandravasaroatra" which literally translates to "which keeps bad things away" or "annihilates diseases". The plant *C. fragrans* has different other names following tribes and in the west it is also called

“Motrobeatinainy” which means “large fire in the belly” as all its aerial parts have a hot and spicy taste (14).

Roots are used against coughing, asthma and dysenteries. Liquid expressed from leaves are known for their powerful treatment of wounds and abscesses. The most characteristic use of this plant is as a tonic and antitoxic against poisoning. Leaf decoctions are also used for weak overweight children and for underweight children, for difficult and exhausting labor or for weak persons to protect them from diseases. The Tanala tribe uses Saro during traditional sportive fights between men (Tolona) and traditional rodeos (Tolon’omby). *Cinnamosma fragrans* leaves are boiled with leaves of “voafotsy” (*Aphloia theiformis*) a traditional tea which has been found to contain high levels of interesting antioxidants (15). Fighters drink the decoction before battle. The plant is also known to treat nervous and sensorial system diseases (16).

Isolated and unpublished work has been conducted in Saro, though the composition of the oil and their pharmacological properties are less known. In view, of the ethnobotanical uses of this plant by the Malagasy people, the aim of this work was to study the chemical diversity of Saro essential oil from different regions of Madagascar and to evaluate its safety and pharmacological activities.

Material and Methods

Leaves of Saro were collected manually during the rainy season and processed. The leaves were steam distilled over a 4 h period and captured using a Clevenger-type apparatus. Distillations were performed less than 24 h after harvesting. The essential oils were dried over anhydrous sodium sulphate until the last traces of water were removed and then stored in dark glass bottles at 4°C. The botanical identity of *C. fragrans* was confirmed and a herbarium voucher was deposited at the Madagascar Academia. Study sites ranged from the extreme north of the island around Antsiranana to Manja at the southern extreme of the western domain. A special emphasize was placed on this latest site to compare different specimens with some at around 500 km northern on the site of Antsalova between 18°32’10’’ to 18°41’21’’ south and 44°11’16’’ to 44°15’30’’ east.

The Saro essential oils (EO) were analyzed by GC/MS using a Hewlett-Packard GC/MS System. Rtx-WAX column (30 m x 0.25 mm x 0.25 film thickness) was used with hydrogen (H₂) as the carrier gas. GC oven temperature was kept at 50°C for 10 min and programmed to 250°C at a rate of 5°C/min, an then kept constant at 250°C for 10 min. The FID and injector temperature was at 255°C. The results were expressed as relative (area) percentage.

Different toxicity tests were conducted to evaluate safety of Saro essential oil. These include primary eye tolerance tests, cytotoxicity and mutagenity potential. Each test was conducted in compliance with the Principle of Good Laboratory Practice regulation by internationally recognized laboratories.

Primary eye tolerance tests were conducted by Eurotox Registered Toxicologists at the “Institute d’Expertise Clinique” (Clinical Expertise Institute), Lyon, France following the Good Laboratory Practises published in the J.O.R.F. (French Republic Official Texts) of December 1996. The method

used was that described by Luepke (1985) which allows checking of irritation potential of product through application on hen's egg chorioallantoic membrane at day 10 of incubation when the embryo has no cerebral activity and when the membrane does not have any sensorial receptors (17, 18).

For mutagenic activity, the risk of carcinogens and mutagens compounds which might be presented in the oil was also evaluated according to the international guidelines (OECD 471 and commission directive N° B13/14). Tests have been conducted at the CIT (International Center of Toxicity) Safety and Health Research Laboratories, 27005 Evreux, France. This test evaluates the potential of the Saro essential oil to induce reverse mutation in *Salmonella typhimurium*, knowing that the bacterial reverse test is able to identify substances that cause point mutations, by affections of DNA base-pairs (19, 20). Five strains of *S. typhimurium*: TA 1535, TA 1537, TA 98; TA 100 and TA 102 were supplied for the study by B.N. Ames Laboratory (University of California, Berkeley or Oakland Research Institute, USA).

Cytotoxicity tests have been conducted at the "Institut d'Expertise Clinique" (Clinical Expertise Institute), Lyon, France, on isolated cultivated rabbit cells registered at the American Tissues Culture Company (ATCC, CCL60) following the Good Laboratory Practices for cosmetic products as published by the French legislation (J.O.R.F., 2004) which prohibits animal testing. The test was based on the evaluation of the cytotoxicity of the product by the determination of the concentration inducing 50% of cell mortality (C.I. 50) through direct application on rabbit eye cell fibroblast monolayer and neutral red release as described in Official Journal of the French Republic (J.O.R.F., 1999).

Antimicrobial activity was evaluated using Mueller-Hinton agar plates by the disc diffusion technique. Solution of the essential oils were prepared with Tween 80 (1 Vol. Saro + 1 Vol. Tween) and kept at 4° Celsius, then diluted in sterile distilled water at 10 mg/ml. 0.1 µl of the bacterial suspension was applied to Mueller-Hinton agar plates for sensitivity testing. Sterile antibiogram discs were moistened with 10 µl of pure Saro oils and its decimal dilutions and then applied to the surface of the agar plates. After incubation of the plates for 24h the diameters of the inhibition zones were measured with a caliper and expressed in mm. The results were expressed as +++ high activity, ++ medium activity, + low activity, - no activity. Tests were conducted at the National Centre for Research on the Environment (CNRE, Antananarivo, Madagascar). Additional antimicrobial tests on microbes of gynecological origin (*Neisseria gonorrhoe*, *Gardenerella vaginalis*, and *Candida albicans*) were conducted by Dr. Berengère Arnal-Schnebelen (22).

Results and Discussion

Physicochemical Properties of the Essential Oil

The essential oil of Saro is a yellow and mobile liquid with a slight and characteristic fresh and eucalyptus aroma. The oil is less dense than water (0.9452), and the refractive index was 1.4636 (Table I). The essential oil is characterized by high levels of 1,8-cineole (40-55%), with minor amounts of α -pinene (4-7%), β -pinene (5-8%) and linalool (4-9%). The Saro EO also contained at lower levels terpenyl acetate (1-4%), α -terpineol (2-5%) and terpinen-4-ol (2-6%) (Table II).

Prior studies showed the presence of at least two EO chemotypes of *C. fragans*, the first one rich in linalool (49.2 to 95.8% (22) with only traces of 1,8-cineole. The second EO chemotype was the opposite, being rich in 1,8-cineole with a range from 37.1% to 71.6% (22, 23).

Saro essential oil, produced in the Northwestern region of Madagascar is of the second type and regular analyses of the essential oil chemical composition for over a year has shown it to be stable with a level of 1,8-cineole between 40 to 55 % (Table II).

Table I. Physicochemical properties of Saro essential oil

<i>Character</i>	<i>Value</i>
Aroma	Slight and characteristic fresh and eucalyptus aroma
Color	Light yellow
Density	0.9452
Refractive index	1.4636

Table II. Essential oil composition of Saro essential oil

<i>Essential oil components</i>	<i>Percent (as % of total EO)</i>
Sabinene	1 -3
α Pinene	4-7
β Pinene	5-8
1,8-Cineole	40-55
Linalool	4-9
Terpinen-4-ol	2-6
α Terpineol	2-5
Terpenyl acetate	1-4
β -Caryophyllene	2 - 5

Biological Activities of the Essential Oil

The primary eye tolerance tests showed that the Saro essential oil has eye irritation levels of 2.8 on a scale of 9 and thus, can be considered as a low irritant oil, since its value is within 1 and 5 of the scale. The study appeared to be valid according to the criteria specified in the international guidelines and the Saro essential oil did not show any mutagenic activity in the bacterial reverse mutation test with *S. typhimurium*.

Values of the test reference quality control satisfied the standards and following the published J.O.R.F. classification Saro essential oil can now be stated as having a “negligible cytotoxicity”.

Saro essential oil appears also interesting in terms of tolerance. However, high doses 1,8-cineole is epileptogen and is also an enzyme inductor of hepatic microsomes presenting when taken internally unknown interactions with other drugs (24, 25).

Although some pharmacists recommend the use of similar essential oils (with 1,8-cineole like ravintsara) for young children and pregnant woman (26), we disagree. We do not recommend the use of Saro essential oil for children without medical advice, and this EO should not be given to pregnant or lactating women.

Table III. Microbial activity of essential oil against bacteria and the application of Saro essential oil against potential human diseases.

<i>Bacterial strains</i>	<i>Saro activity</i>	<i>Suggested uses for potential human diseases.</i>
<i>Alcalescens dispar</i>	++	Dysenteric syndrome
<i>Bacillus cereus</i>	+++	Food borne illnesses
<i>Candida albicans</i>	++	Vaginal mycosis, perionyxis, skin infection.
<i>Cryptococcus neoformans</i>	+++	Skin and nails wounds,
<i>Enterobacter cloacae</i>	+++	Gastro intestinal toxicity
<i>Escherichia coli</i>	++	Urinary tract infections, meningitis, peritonitis, mastitis, septicemia, gastrointestinal toxicity and Gram-negative pneumonia.
<i>Gardenerella vaginalis</i>	++	Vaginal infections
<i>Klebsiella pneumoniae</i>	+++	Nosocomial infections, opportunistic pathogen: chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy and rhinoscleroma.
<i>Listeria ivanovii</i>	-	Gastrointestinal toxicity
<i>Listeria seeligeri</i>	++	Gastrointestinal toxicity
<i>Listeria welshimeri</i>	+	Gastrointestinal toxicity
<i>Neisseria gonorrhoe</i>	+++	ORL infections, genitals infections.
<i>Pasteurella multcida</i>	+++	Gastro intestinal toxicity
<i>Salmonella typhi</i>	++	Typhoid
<i>Shigella sonnei</i>	+++	Gastro intestinal toxicity
<i>Staphylococcus aureus</i> (Cocci Gram+)	+++	Skin infections, abscesses, pneumonia, meningitis, endocarditis, toxic shock, syndrome, septicaemia.
<i>Streptococcus pneumoniae</i>	+++	Skin infections, pneumonia, endocarditis, pericarditis, gastrointestinal toxicity, osteo-articular infections
<i>Trycophyton rubrum</i>	+++	Nail mycoses
<i>Trycosporon mucoides</i>	+++	Intertrigos, onyxis

NOTE: Listed in alphabetical order by microbe. Results shown as =+++; high activity; ++: medium ; +: low ; -: without activity.

For gynecologic infection prevention or treatment, when it is not only used as an external pubian massage and taken internally, caution should be taken that it might affect the Doderlein flora, and natural beer yeast should be taken simultaneously to help regenerate the Doderlein flora (21).

Tests conducted on Saro essential oil demonstrated powerful properties even when compared to the most interesting essential oil known in Aromatherapy.

The antimicrobial results suggest that the oil could have activities against vaginal infections, candidiasis, listeriosis, diarrhea, common infections and even animals diseases like pork pledge and chicken cholera.

Suggested Applications

Saro essential oil showed high activity against a broad range of bacteria (Table III). The oil was particularly active against both gram positive (e.g. *Staphylococcus aureus*, *Bacillus cereus*, and *Streptococcus pneumoniae*) and negative bacteria (e.g. *Shigella sonnei*). The oil showed moderate activity against *Escherichia coli*, *Salmonella typhi*, *Gardenerella vaginalis*, *Alcalescens dispar* and *Candida albicans*. The oil showed low or no activity for different members of the genus *Listeria* (Table III). These results showed that the oil could have potential application as antimicrobial.

Possible applications of Saro essential oil are vast considering its properties: antiviral, antibacterial, antimycosis, antiparasitic, immune modulant, analgesic, antispasmodic, among other uses. The antimicrobial properties might be related to the high content of 1,8-cineole in the Saro essential oil coupled with α -pinene as these compounds have expectorant and mucolytic properties (27). Saro's use as a general tonic (immunomodulation) should increase the attractiveness for this use as well our finding showing that the Saro essential oil can be considered non-toxic, with the possibility of becoming an organic certified product.

Application in gynecology might be relevant especially as art of a larger holistic approach to serious infections and in the prevention of recurrences. This essential oil could also be a good complement for preventive and curative treatments of HPV (Human papilloma virus) and HSV (herpes simplex virus). Saro essential oil uses could be effective for minor infections and especially to prevent them in topic application (27).

The antalgic properties of Saro essential oil might also be useful in relaxing massage oils as they will be associated with its anti inflammatory properties (21, 27).

Conclusions

Madagascan Saro essential oil was found to have an attractive odor with a fresh eucalyptus type aroma, being the oil dominated by 1,8-cineole. The oil was found to be safe for human use, with low irritation levels, no mutagenicity and cytotoxicity. This EO also showed antimicrobial activity against several mold and bacteria. Due to its favorable organoleptic and chemical profiles, its safety and effective microbial activity, this oil may have application in the cosmetic, perfumery and pharmaceutical industries. The identification of new uses and application of essential oils, can ultimately assist rural communities in Madagascar by generating interest and market access to their products only if these new applications find a sustainable market.

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Chapter 27

Essential Oil Bearing Plants from Kenya: Chemistry, Biological Activity and Applications

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Essential oils are aromatic volatiles that are recovered from different plant tissues using a variety of distillation and extraction technologies. Kenya, being a country with diverse plant genetic resources, is endowed with plant species containing essential oils, many of which have not been studied. A review of research on the chemical constituents and biological activities of Kenyan essential oil bearing plants is presented and shows that the use of these indigenous natural resources are under-recognized and underutilized. Potential applications in cosmetic, food, agricultural and pharmaceutical industry, among others, are discussed.

Essential oils include aromatic volatile compounds which when appropriately recovered from flowers, seeds, leaves, stems barks and woods of herbs, bushes, shrubs and trees, provide a wide range of subtle and distinct aromas and fragrances. The essential oils are complex mixtures of many chemical constituents such as monoterpenes, sesquiterpenes and phenylpropenes among others (*1*). These compounds are widely used in perfumery, cosmetics, foods, beverages, pharmaceutical and industrial products as well as in aromatherapy. The essential oils are recovered using a variety of distillation and extraction technologies which impact the recovery efficiency as well as the quality and chemical composition. Many essential oils are recognized as containing chemical constituents that are bioactive possessing antimicrobial (2-

9), larvicidal (10,11), anthelmintic (12,13), insect attractant (14,15) and repellent (11,16-19) and antifeedant activity (17-22). Essential oils have also been shown to have important pharmacological effects. These include sedative, antiseptic, expectorant, diuretic, stimulant, hypotensive, bronchodilatory, anti-inflammatory, spasmolytic, local anaesthetic and cholesterol lowering effects (23-26).

Kenya is a nation rich in plant genetic resources. It is estimated that Kenya is home to some 7,000 higher plant species of which 1,100 are thought to be rare (27). Among these are wild species of vegetables, fruits, forage, grasses, legumes, cereals, oilseed crops and medicinal and aromatic plants. The rich plant diversity is due to the various ecosystems found throughout Kenya including vegetation in moorland on top of high mountains, moist highland forests, dry forests, evergreen and semi-evergreen woodlands, savannahs, coastal forests and woodlands, riverside vegetation, mangrove and desert. Kenya, being the home to several essential oil bearing plant species coupled with the diverse range of environments is in an excellent position for the collection and production of essential oil plants. Prospects for the development of a local essential oil industry have been strengthened by green consumerism promotion, environmental concerns, expanding markets, the growing need for aromatherapy and need for import substitution.

The commercial production of essential oils in Kenya started with cedarwood oil as a by-product in pencil industry after the First World War (28). The oil is very useful as a modifier of other oils and a fixative in perfumes. Kenyan geranium oil was also known the world-over for its quality and usefulness in perfumery industry from 1930s until the sixties (28-30). Waste shavings from the pencil industry in Kenya was a source of cedarwood oil. The production of cedarwood oil stopped in the mid-1970s when this industry closed. Other essential oils produced on small-scale by 1940 were those of *Leptospermum citratum* and *Malaleuca bracteatum* (Myrtaceae) (31). The production of essential oils in Kenya had reached 6.5 tones in 1938 and was progressing well even during the difficult years of the Second World War. Much later, between 1966 and 1967, Kenya also exported citronella oil to USA (31). Today, Kenya imports almost all its essential oils or their constituents for its perfumery, cosmetic soaps, food products, soft drinks and pharmaceutical products. In 1987, for example, the essential oil based products import into Kenya amounted to over 402.9 tonnes at the cost of 1 million US dollars. These included concentrates of essential oils in fats, fixed oils or waxes; mixtures of essential oils; and distillates. These essential oils were mostly imported from European countries. During the same period, about 265 tonnes of essential oil based products worth about 0.6 million US dollars were exported from Kenya. These figures excluded the imported finished goods containing essential oils, such as cosmetics and pharmaceuticals. The exported essential oils or related products recorded arose from either re-exports of the imported products or reformulated products. In addition, in the same year, 175.5 tonnes of various spices worth over 30,000 US dollars were imported into the country, while the exports of spices fetched about 42,000 US dollars (32).

The present chapter discusses the research on the chemistry and biological activities of essential oils indigenous or introduced into Kenya over a period of

the last three decades and the potential commercial opportunities for these natural products.

Research

Research on essential oil-containing plants started during the early 1930s. Some plants analyzed for their oils included *Plectranthus* species, *Mentha piperita* (peppermint), *Cymbopogon nardus* (citronella grass), *Cymbopogon citratus* (lemon grass) and *Brachylaena hutchinsii*. This last plant yielded an essential oil with a persistence odor which offered possibilities for use in soap, perfumery and as a perfume fixative (28). The general screening of the Kenyan flora for the essential oil yielding plants has been in progress since 1978 largely in the School of Pharmacy, University of Nairobi, Kenya. Out of more than 200 plants belonging to more than 38 families screened for essential oils, several families such as Apiaceae (Umbelliferae), Asteraceae (Compositae), Lamiaceae (Labiatae), Myrtaceae, Poaceae (Graminea) and Verbenaceae, among others have been considered important enough to warrant further research in order to establish their commercial viability. We review those Kenyan plants and their respective essential oil composition in the following section that have been researched. A summary of these plants and the major essential oil constituents are also summarized in Table I. Table II summarizes traditional uses and methods of preparation of the plants.

Apiaceae (Umbelliferae)

Anethum graveolens L. (Dill): The essential oil (3.3%) of the mature dried fruit of this plant (33) contains mainly carvone and phellandrene. The oil (2.5-5%) from other countries has been reported to contain (*S*)-(+)-carvone (18-81%), dihydrocarvone (0.1 to 62%) and (*R*)-(+)-limonene (10-50 %) (34). Dill is exotic to Kenya and the seeds are used as spices. Although the leaves are used in many countries, in Kenya they are rarely used. The oil has spasmolytic and antimicrobial effects (34). Dill water is a product containing this oil which is used as a carminative, antispasmodic and as gripe medicine for infants.

Coriandrum sativum L. (Coriander): The major constituent of the dried fruit essential oil (0.5%) of this species is linalool (33). Coriander has been introduced to Kenya, probably from the eastern Mediterranean region. Oil (0.1-2%) from other countries contains linalool (45-85%), α -pinene (1-15%), limonene (0-4%), γ -terpinene (trace-15%), p-cymene 0-15%), camphor (0-10%), geraniol (0-7%) and geranyl acetate (1-20%) (34). In Kenya, fruits are used as a spice in cooking and fresh leaves are sold in the market for flavoring food and salads. The oil has mild spasmodic effects, is appetizing and has digestive effects. Coriander fruit is used for dyspepsia and for loss of appetite. In India it is used for treatment of coughs, bladder problems, fever, dysentery and eye problems. In Central and South America it is used for menstrual problems (34). The oil from this spice is a flavoring agent and carminative (33).

Foeniculum vulgare Hill (Fennel): Anethole and fenchone are the main constituents of the essential oil (4.0%) of the fruit of *F. vulgare*. Teuscher has reported that the main constituents of the oil (3-8.5%) of bitter fennel are *trans*-anethole (50-70%), (+)-fenchone (15-30%), estragole (methylchavicol) (2-6%) (34). Fennel is native to south and south western Europe. In Kenya, cultivation of fennel had earlier been established from imported fruits. The plant is currently not being cultivated on a commercial basis resulting in importation of all the fennel used in the country. Fennel has been found growing in the wild in Kenya as an escape from cultivation. Fennel water is a product from this common spice and it is used as a carminative and flavoring agent (33). Fennel is used for gastrointestinal pains, bloating, flatulens, catarrhs of the upper respiratory tract and for conjunctivitis (34). In East Africa, boiled or roasted roots are used for the treatment of gonorrhoea (35).

Heteromorpha trifoliata (Wendl.) Eckl. & Zey.: The semi-dried leaves and flowering parts of *H. trifoliata* yielded 0.8 and 2.0% oil, respectively. Of the sixty compounds identified, α -pinene (22%), germacrene-D (18%) and sabinene (12%) were the major constituents (36). The Maasai people of Kenya use the ground stems of this indigenous plant as a tonic for babies and small children. Ash from the leaves is mixed with fat and given to infants as a purgative (37). Roots are generally used as a purgative and to cure syphilis. The leaves are used for treating snake bites (35). It has also been reported that the plant is used to treat abdominal and mental disorders, intestinal worms, shortness of breath and coughs (38).

Pimpinella anisum L. (Anise): The essential oil of *P. anisum* (2.7%) contains anethole as the major compound (33). This plant is exotic to Kenya, its native origin probably being the eastern Mediterranean region. Teuscher reports that the oil (1.5-6%) contains mainly *trans*-anethole (80-95%) and estragole (1-4%). It has appetite stimulating and digestion promoting properties. Anise is used as a spice and the oil serves as a flavor in toothpastes, mouthwashes, soaps, perfumes creams and lotions (34). The products of this oil are anise spirit and anise emulsion, which have carminative and expectorant actions (33).

Asteraceae (Compositae)

Artemisia afra Willd. (African wormweed): The major components in *A. afra* oil (1.5%) from semi-dried leaves were 1,8-cineole (67%), terpinen-4-ol (7%) and borneol (5%) (39). The oil from Zimbabwe contains mainly artemesia ketone (6.3-41.9%) and 1,8-cineole (0.1-27.9%) (40). In East Africa the aerial parts of *A. afra* are used to treat indigestion as well as sore throats and fever in children. The roots are used for treating intestinal worms and the leaves are chewed as emetic (35). In South Africa fumes from boiled leaves are inhaled for blocked nasal passages (41).

Blumea brevipes (Oliv. & Hiern) Willd.: The semi-dried flowers of *B. brevipes* yielded 0.7% oil whose major ingredients were terpinen-4-ol (28%), germacrene-D (15%) and sabinene (8%) (42). The plant is administered as an afterbirth and in treatment of sexual disorders (38).

Helichrysum odoratissimum (L.) Less.: The oil from fresh flowers of *H. odoratissimum* (0.7%) had an agreeable aroma and consisted mainly of α -pinene (43%), (E,E)-farnesol (17%) and α -humulene (15%) (43). The roots are used as a purgative and for treatment of coughs. Leaves are used as an anthelmintic and for burn wounds, while the extract of pounded leaves is used to treat conjunctivitis (35).

Matricaria chamomilla L. (Chamomile): The oil (1.2%) from the semi-dried flowers of *M. chamomilla* contained mainly bisabolols and chamazulenes (33). Recently, chamomile tea has gained popularity in Kenya as people turn more to the use of natural products. The total extract is used to make an imported powder which is used for soothing baby gums when they are teething.

Microglossa pyrhopappa (A. Rich) var. *pyrhopappa*: Analysis of the essential oil (0.4%) of semi-dried aerial parts of *M. pyrhopappa* revealed that this oil had 61% sesquiterpene hydrocarbons and 13% hydrocarbon monoterpenes with β -caryophyllene (20%), γ -gurjunene (12%) and limonene (9%) as the major components (44).

Psidia punctulata Vatke: Analysis of the essential oil (0.5%) of *P. punctulata* semi-dried leaves gave germacrene-D (27%), β -phellandrene (20%) and β -caryophyllene (10%) as the major compounds (45). The leaf decoction of this plant is used for treatment of abdominal pains and colds (35). The Maasai use the leaves as an insecticide for fleas in sheep, goat kids and calves. The roots are used to treat gout (37).

Sphaeranthus bullatus Mattf.: The essential oil isolated from the partially dried leaves (0.5%) contain thymol (22%), bornyl acetate (8%) and germacrene-D (8%) as the major constituents (46). The plant is used as a tonic and for treatment of stomachaches (35).

Sphaeranthus cyathuloides O. Hoffm.: The oil (2.9 %) of fresh leaves of *S. cyathuloides* was found to contain an oil with an agreeable aroma and it was rich in *trans*-dihydrocarvone (67%) and *cis*-dihydrocarvone (26%) (47).

Sphaeranthus suaveolens O. Hoffm.: Oil of semi-dried *S. suaveolens* leaves (0.5%) had *cis*-pinocamphone (64%) as the major constituent (46). The flower heads, which are very fragrant, are used for treatment of afterbirth pains by the Kikuyu people of Kenya (48). The Maasai use the powdered leaves in water to bath newborn babies to make their skin smooth (37). A decoction of the leaves is used for treatment of malaria and coughs (35).

Tarconanthus camphoratus L.: The semi-dried leaves of *T. camphoratus* produced 0.8% oil whose major compounds were α -fenchyl alcohol (29%), 1,8-cineole (16.5%) and α -terpineol (9%) (49). Leaves are traditionally used by the Maasai as a deodorant by placing them in the armpits or around the neck as they look after their cows. They also use the plant for treatment of headaches and tape worms (37). Leaves are used for treating respiratory conditions (38).

Burceraceae

Boswellia neglecta S. Moore (*Boswellia hildebrandtii*): Frakincense or Olibanum is an oleo-gum-resin obtained by incision from the bark of *B. neglecta*. The oleo-gum-resin contains 3-8% of essential oils consisting of

terpenes and sesquiterpenes, about 60-70% of resin and 27-35% of gum. The hydrodistilled essential oil of *B. neglecta* from Ethiopia contained mainly α -thujene (19.2%), α -pinene (16.7%) and terpinen-4-ol (12.5%) (50). Frankincense has been used for many millennia as an incense and also as a symbol of worship in orthodox churches. It is also used in fumigating preparations (51). The root decoction of *B. neglecta* is used as a diuretic and for treatment of gonorrhoea (35).

Commiphora holtziana Engl. ssp. *holtziana* (*Commiphora erythrea*): Gum-Opopanax or Sweet Myrrh is the sun dried exudates from the bark of *C. holtziana*. It has been used in northern Africa and in Asia for centuries for incense, perfumes, for embalming and fumigation, muscular pain and to heal wounds. The plant grows in parts of Somalia and the local people collect the oleo-gum-resin and ship it to Aden, a port city in Yemen, from where it is exported to Europe, India and China (52). Somalia produces most of the world's true and sweet myrrh, both of which are occasionally used as flavouring agents. Gum opopanax is also indigenous to the northeastern part of Kenya bordering Somalia, where the gums are used as acaricides and insect repellants. The oil is obtained by hydrodistillation of the oleo-gum-resin which is sold in open-air local markets and exported. Both the oil and the oleo-gum-resin are easily distinguishable from those of true myrrh due to different resin and oil colour and aroma. However, both fetch very low prices when exported due to the lack of quality control and inconsistent product characteristics. The oil (3.5-8%) contains sesquiterpene hydrocarbons such as δ -elemene, α -cubenene, α -copaene, *cis*- α -bergamotene, β -elemene (51,53), germacrene D and germacrenone derivatives (54).

Commiphora myrrha (Nees) Engl. (Synonyms *C. molmol* (Engl.). Engl.; *C. molmol* (Nees) Engl; var. *molmol* Engl.; *C. cispidata* Chiov.; *C. coriacea* Engl.; *C. playfairii* (Oliv.) Engl. var. *benadirensis* Chiov; *Balsamodendron myrrha* Nees; Common myrrh; Myrrha): True myrrh, the oleo-gum-resin from *C. myrrha*, is an important article of commerce in the sparsely populated north eastern Kenya. There is no traditional or current practice of cultivation of the plant, rather, it is wild harvested. Myrrh contains 7-17% essential oil, 25-40% of resin and 57-61% of gum. The oil is obtained by water distillation of the oleo-gum-resin and it contains monoterpenes and sesquiterpenes and is a carminative, astringent and mouth gargle (51). *Commiphora myrrha* and *C. holtziana* from Kenya contain sesquiterpenes, notably furanosesquiterpenes based on eudesmene, elemene and germacrene. The oleo-gum-resin of *C. myrrha* is used traditionally in the treatment of intestinal complaints, body wounds, boils and excessive menstrual bleeding. It is also used to kill ticks on cattle. Indeed, the oleo-gum-resin hexane extracts yielded furanosesquiterpenes which were moderately toxic to *Rhipicephalus appendiculatus*, the tick that transmits East Coast Fever (causative agent *Theileiria parva*) in cattle (55). Myrrh is used as an incense for ceremonial and religious purposes, in toothpastes and in the perfume industry. There appears to be chemotypes of *C. myrrha*, but this requires further research.

The oleo-gum-resins form the most variable ingredients in perfumes of oriental types and blend well with many oils. They are used in perfumes to impart a 'rounding-out' effect and alluring tonalities. Oleo-gum resins could be

used in Kenya in the perfume, soap and cosmetic industry as well as food and beverage industry (52).

Euphorbiaceae

Croton sylvaticus Hochst.: The essential oil of semi-dried *C. sylvaticus* leaves (0.14%) contained mainly the sesquiterpenes β -caryophyllene oxide (35%) and α -humulen-1,2-epoxide (12%) (56). The aqueous extract of the stem bark of *C. sylvaticus* reduced exploratory activity, prolonged ether anesthesia, relaxed the muscles and had analgesic effects on mice (57). The plant is used for treatment of swellings caused by kwashiorkor, for tuberculosis and as a purgative (35).

Synadenium compactum N. E. Br. var *compactum*: The oil from the dried stem bark of *S. compactum* var *compactum* (0.2%) contained 55 constituents, of which α -selinene (30%) and β -selinene (19%) were major (58). Decoctions of the leaves and the white latex of this plant are widely used for treatment of East Coast Fever in cattle (35). The plant is rare in the wild and it is endemic to central Kenya, where the plants are used for marking farm boundaries (48).

Lamiaceae (Labiatae)

Several species from the Lamiaceae family have very high potential for commercial production of essential oils in Kenya. Most members of this family are perennial herbs.

Ocimum basilicum L (Basil). The oil of *O. basilicum* leaves (0.2-1.9%) from plants collected from different geographical locations exhibited chemical variation. Both leaves and flowering tops exhibited the same composition. While the first chemovariety contained mainly geranial (50%) and neral (31%), the second had mainly camphor (32%) and linalool (29%), and the third exclusively linalool (over 95%) (59). Many chemotypes of *O. basilicum* and other *Ocimum* spp. are known to exist. This shrub is widely used in Kenya to repel mosquitoes. Vapour from boiling leaves is used for nasal and bronchial catarrh, while a decoction of the roots is used for treatment of constipation and stomach pains (35).

Ocimum fischeri Gürke: *O. fischeri* oil (3.8%) contained linalool (36.8%) and fenchone (40.7%) as the major ingredients (60). Fenchone is used in room sprays and in bath preparations and soaps. Linalool is widely used in perfume, cosmetic, soap and flavor industry. This oil could be useful in flavor, soap/detergent and perfume industries due to its composition (60).

Ocimum gratissimum L (Synonym *Ocimum suave* Willd.) (Tree basil). The leaves of *O. gratissimum* produced an oil (0.2-2.9%) whose constituent was mainly eugenol (70-95%). However, the oil of the flowering tops had significantly less eugenol and varied chemically. One variety contained mainly eugenol (64%), β -bisabolene (10%) and *Z*- γ -bisabolene (7%), while the second contained mainly *Z*- β -ocimene (34%) and eugenol (33%) (59). In Kenya, an infusion of the leaves is used instead of tea or coffee by some people who do not

consume any drink with caffeine for religious reasons. The leaves are used for treatment of abdominal pains, sore eyes, ear problems, coughs and blocked nasal passages. An infusion of the leaves is also used as a disinfectant and as an insecticide (35). The plant is sweet smelling but very bitter. The Maasai use the roots as an emetic against 'malaria' and also chew the plant as tobacco (37).

Ocimum keniense Ayobangira: The semi-dried leaves of *O. keniense* yielded 4% oil which contained 1,8-cineole (38%) and methyl chavicol (24%) as the major compounds. The essential oil from this species appears promising for use in the cosmetic industry. Cultivation of the plant would be of interest because it grows naturally in poorly drained, waterlogged black-cotton soils (61) and because the essential oil is so high relative to other aromatic plants. Thus, this species could be grown in soils which may not be suitable for other crops.

Ocimum kilimandscharicum Gürke: The Kenyan *O. kilimandscharicum* is a high yielding source of essential oils (5.3%) of which one chemotype was reported to contain about 70% camphor (62). It is cultivated commercially in India from seeds initially obtained in Kenya during the World War II. The oil is a natural source of camphor as it crystallizes out on cooling (63,64). The leaves are used to relieve blocked nasal passages and severe colds, for abdominal pains, sore eyes and coughs. An infusion of the leaves is used as a disinfectant and insecticide (35) The plant is also planted where new beehives are established to both to attract bees and to provide nectar (48).

Plectranthus amboinicus (Lour.) Spreng: The essential oil contains limonene, linalool, myrcene, thymol, borneol, camphene, α -amorphene and β -cubebene (65). A root decoction is used for treatment of stomach pain (35).

Plectranthus barbatus Andr.: This species contains a wide range of mono- and sesquiterpenoids including humulene and β -caryophyllene, δ -selinene, borneol, camphene, β -phellandrene, α -ionone and α -thujene (65). The leaves are used as a purgative and for stomachaches. Leaves are soaked in warm water and the water is used to bath babies with measles (35).

Plectranthus marrubioides Benth: The semi-dried leaves of *P. marrubioides* yielded about 4% oil containing mainly 64% camphor, and less amounts of α -phellandrene (8.5%) and ocimene (4.2-9.3%). Owing to the high content of camphor, this oil could therefore be useful in making medicinal preparations such as a local anesthetic, remedy for muscular and rheumatic pains and for respiratory conditions (66).

Plectranthus sylvestris Gürke oil is reported to contain β -caryophyllene and germacrene D (65). The plant is used for treatment of abdominal pain, malaria, headaches and aching chests (35).

Plectranthus tenuiflorus (Vatke) Agnew: The partially dried leaves of *P. tenuiflorus* yielded oil (1.6%) that contained carvacrol (14%), α -terpinene (10%), *p*-cymene (11%) as the major constituents, while the hydrocarbon sesquiterpenes (α -cubenene, β -cubenene, δ -cardinol, γ -cardinol, α -copaene) amounted to about 26% (67).

Rosmarinus officinalis L. (Rosemary): Rosemary is an exotic or introduced plant in Kenya whose native origin is southern Europe particularly the coastal regions of the Mediterranean Sea. The semi-dried leaves of rosemary grown in Kenya yielded 2.0 % of oil which contained mainly of 1,8-cineole and geranyl acetate (33). Teuscher has reported that the main constituents of rosemary oil

are 1,8-cineole (3-60 %), α -pinene (1-57 %), camphene (1-57 %), camphor (0-35 %), bornyl acetate (1-21 %), verbenone (0-28 %), p-cymene (0.5-10 %) and myrcene (0.5-12 %) (34). The leaves are used as spices due to their appetizing and digestive properties. The oil has antibacterial and fungistatic activity and it is used in the perfume and liqueur industry (34).

Saturea biflora L.: Citral was the main component of the essential oil (1.5%) of partially dried leaves of *Saturea biflora* (33). Owing to the presence of citral, this plant has potential for use in the soap industry. Citral is easily converted in the laboratory to α - and β -ionones, which are used in the perfume industry.

Thymus vulgaris L. (Thyme): The main component of the essential oil (2.3 %) of partially dried leaves of thyme grown in Kenya was thymol. Thyme oil from other countries is reported to contain thymol (up to 85%) and p-cymene (up to 45%). Thyme is used as a spice due to its appetite stimulating and digestion promotion properties. The oil has strong antimicrobial activity and is also used in the liqueur industry and as a perfume in soaps, deodorants and hair lotions (34).

Myrtaceae

Eucalyptus citriodora Hook f. (Lemon gum): The oil of the fresh leaves of *E. citriodora* (2.2-8.3%), a tree introduced into Kenya from Australia (68), has been well researched in Kenya. Results indicate that there are two chemical varieties that were introduced. The chief essential oil constituents of one variety are citronellal (65-88%), citronellol (2-25%) and isopulegol (2-19%). In contrast, the second variety has as its major essential oil constituents citronellol (32-52%), citronellal (38-59%) and 1,8-cineole (2-19%) (68,69). The essential oil is regarded as a perfumery oil. This plant is the second most commercially important *Eucalyptus* species in the essential oil trade and is only second to *E. smithii* Baker (69). The aromatic oil is used in soaps, creams and lotions due to its desirable odor properties, and as with many other essential oils is also used extensively in many technical preparations to mask other industrial malodors. A highly active mosquito repellent compound, p-menthane 3,8-diol has been isolated from the waste water of distillation of *E. citriodora*. There are now many commercial mosquito repellents based on this waste water (70-73).

Eucalyptus globulus Lab.: The oil of the fresh leaves of *E. globulus* yielded 2.0 % of an essential oil that contains mainly 1,8-cineole (33). This plant is used for treatment of malaria and it also repels insects (48).

Papilionaceae

Rynchosia minima DC.: Very small yield of oil (0.1%) has been obtained from *R. minima*. β -caryophyllene (30%), germacrene B (18%) are the major components(74). The oil exhibited significant inhibition against *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus luteus*. The plant is used for the treatment of afterbirth pains, headaches and colds (48) in Kenya.

Poaceae (Gramineae)

Cymbopogon afronardus Stapf: One of the indigenous wild grasses in the East African countries is *C. afronardus* Stapf. Recent scientific work using GC and GC/MS indicates that the semi-dried leaves oil (yield 0.4 %) which has a highly pleasant aroma contains mainly sesquiterpene alcohols (intermedeol and 5-epiparadisol 40.9%, together) and ketones (6S:7R-bisabalone 39.5% and 6R:7R-bisabalone 4.4%). The oil also had mild antibacterial activity (75). Although the only known uses of this grass is thatching of houses, the oil has a potential in cosmetic, perfumery and soap industry. However, unless programs are designed and implemented to preserve this species, it is likely to become extinct as pressure for cultivation of food crops increases.

Cymbopogon citratus Stapf and *C. nardus* Rendle.: The essential oils of Citronella grass (*C. nardus*) and Lemon grass (*C. citratus*) are used worldwide in toiletry soaps, perfumes and cosmetics. These essential oils have found many uses including in flavoring chewing gums, and confectionery products. In addition, these oils are also extensively used in masking disagreeable odors in industrial products such as detergents, polishes, paints, domestic air fresheners, bleaches and in insect repellents. Their pleasant aroma is mainly due to citronellal, citronellol, geraniol, nerol and their acetates. These species are widely cultivated as commercial crops in such countries as India, China, Sri Lanka, Indonesia, Argentina, Guatemala, Ghana and Zimbabwe (31,52). Studies in Kenya have highly recommended the commercial production of *C. nardus* and *C. citratus* oils (yield 0.9 and 0.5%, respectively) both of which contain mainly citronellal and geraniol (33,76). Preliminary studies in Kenya indicate that clonal propagation is used to expand the germplasm available for cultivation and that as perennial crops several harvests per year can be achieved. These grasses do not interfere with other crops and because of their deep and extensive rooting system have the ability to prevent soil erosion if planted along contours and hedges.

Rutaceae

Clausena anisata (Willd.) Benth.: Semidried leaves of *C. anisata* from Kenya yielded an oil (1.5 %) which was rich in anethole (33). The plant is native to tropical Africa and has an anise-like odor. The oil from other countries has a yield of 3.5 to 8% and contains anethole as the main component (75-90%) as well as anisaldehyde, anisketone, estragole and α -elemene. Extracts of the leaves and the essential oils are used in the bakery and confectionery industries (34)

Santalaceae

Osyris lanceolata Hochst & Steudel (African or East African Sandalwood): The essential oil yield from dry sandal wood was 4.0 % of oil which contained sesquiterpenes and sesquiterpene alcohols (33). This indigenous plant is currently under presidential protection due to piracy of the plant resulting in an extinction threat. About 150 tonnes of logs of the plant, including roots, are pirated every month to produce 750-800 kg of the oil. The oil is very interesting due to presence of high amounts of santalols (about 35%) and (+)-z- α -santalyl acetate (77). The international sandalwood of commerce comes largely from *Santalum album* and other *Santalum* species, though there are several aromatic woods that are known locally as sandalwood.

Verbenaceae

Lippia species are used extensively in Africa for medicinal purposes and they have all been found to be rich in essential oils. The essential oils from the reported eight known *Lippia* species in Kenya have been studied extensively. Whereas *L. grandifolia* is the most common in West Africa, *L. ukambensis* Vatke and *L. javanica* Vatke are the most widely distributed in East Africa and particularly in Kenya. The remaining *Lippia* species are limited in their ecological distribution. *Lippia dauensis* is rare and is located in the dry areas of Kenya (78).

Lippia carviadora Meikle: Carvone (about 60%) was the major compound in the essential oil of partially dried leaves of *L. carviadora* Meikle with the yield of 3%. Other essential oil constituents included limonene (2.4-32.7%), carviny acetate (0-19.3%), and *p*-cymene (1.1-5.7%) (79,80).

Lippia carviadora var. *minor* Meikle: A very small yield of oil (0.2%) was obtained from the semi-dried leaves of *Lippia carviadora* var. *minor* and it contained mainly the sesquiterpenes β -cubenene (32%) and β -elemene (13.7%) (79,80).

Lippia dauensis (Chiov.) Chiov.: *L. dauensis* oil was obtained from plants collected in the north-eastern arid region of the country. The semi-dried leaves and flowering tops of the plant produced 2.4% oil containing β -ocimene (24.7%), 2-methyl-6-methylene-7-octen-4-one (15.7%), myrcene (12.9%), *cis*-tagetone (11.0%), and 2-methyl-6-methylene-2,7-octadien-4-ol (9.4%). The

latter is a very important sex pheromone produced by male bark beetles (*Ips confusus*). This study from Kenya was the first to report the isolation of this sex pheromone from plants (79,80).

Lippia grandifolia A. Rich. (synonyms *Lippia multiflora*; *Lippia adoensis*. Gambian tea bush): The oil of *L. grandifolia* (0.7%) revealed 30 constituents of which contained linalol (46%), thymol (15%), β -cubenene (11.7%) and *p*-cymene (10.4%) were the major constituents. *Lippia grandifolia* is the commonest species of *Lippia* in West Africa and its leaves are taken as a tea. It is also used against fever, hypertension, conjunctivitis, as a nasal drop, as a bee attractant by producers of bees and for treatment of gastroenteritis. It also helps to remove the placenta after delivery and it controls hypertension. It has a tranquilizing and muscle relaxing effects on animal models (79,81).

Lippia javanica (Burm. f.) Spreng: The essential oil of semi-dried leaves of *L. javanica* (1.6%) contained on average myrcenone 32%, *cis*-ocimenone 32%, *trans*-ocimenone 16% as its major constituents. *Cis*-ocimenone changed into a more polar reddish-brown compound unless the oil was stored under freezing temperature conditions (79,80). In Kenya, an infusion of the leaves of *L. javanica* are used for treatment of stuffed nose, fever, malaria, tapeworm and for indigestion (36). The leaves are used to repel termites and other insects (48).

Lippia somalensis Vatke: The semi-dried leaves of *L. somalensis* yielded 0.7% of an oil whose major chemical constituents included 1,8-cineole (31.9%), δ -3-carene (11.5%), myrcene (11.0%) and β -cubebene (9.0%) (79,80).

Lippia ukambensis Vatke (*L. kituiensis* Vatke): The partially dried leaves of *L. ukambensis* yielded 1.8% of an essential oil. Two chemotypes were identified on the basis of camphor and 1,8-cineole. The camphor chemotype had camphor (37.3%) and *trans*-sabinene hydrate (18.9%) as its' major ingredients with only traces of 1,8-cineole. The other chemotype had 23.7% of 1,8-cineole and only 1.1% of camphor. Cultivation experiments, followed by analysis of the varieties transferred from the natural habitat to a completely different climatic and geographical area strongly suggested that the two varieties were true chemotypes. The former was named *L. ukambensis* chemotype camphor and the latter was named *L. ukambensis* chemotype cineole. The two chemotypes had identical morphological and histological features and the local people are not aware of the differences (79,80). It is worth noting that the *L. ukambensis* chemovariety cineole is almost extinct now. *Lippia ukambensis* is used as a termite repellent and it is therefore used for building traditional granaries (48). In Kenya the plant is used as an inhalant to treat respiratory diseases, but in Tanzania it is used to treat abdominal pains.

Lippia wilmsii H. H. W. Pearson: Limonene (36%), piperitone (27%) and piperitenone (9%) were the major compounds in the essential oil of the semi-dried leaves of *L. wilmsii* H. H. W. Pearson (1.1-2.2%) (79,80).

Zingiberaceae

Zingiber officinalis Rosco (Ginger): Ginger is a commonly used spice in Kenya which probably originated from India. The semi-dried rhizome *Z. officinalis* grown in Kenya yielded 3.1%, which contained zingiberene and zingiberol as the main constituents (33). Other countries have had oil yields ranging from 1.0 to 4.3% and which contain (-)- zingiberene (7-50%), (-)- β -sesquiphellandrene (2-12%), α -curcumene (0.2-19%), 1,8-cineole (trace- 13%) and p-cymene. This spice stimulates appetite, promotes digestion and has ulcerogenic activity. The oil has antihyperlipidemic, antiemetic, antitumorigenic, antimicrobial, nematocidal mulluscidal and antischistosomal activities. The rhizome is also used for treatment of migranes (34).

Biological Activities

Antibacterial and Antifungal Activity

The leaf oil of *Lippia grandifolia* showed antibacterial activity against *Staphylococcus albus*, *Bacillus cereus* and *Escherichia coli*, as well as antifungal activity against *Colletotrichum coffeanum*, *Microsporum audouinii* and *M. canis*. *Colletotrichum coffeanum* is the causative agent of coffee berry disease (CBD). The fungus attacks coffee flowers as well as green and ripe berries. Every year, losses to farmers in Africa by CBD is significant and work to identify successful control strategies are of interest. Preliminary work on small plots in Kenya suggested that intercropping coffee with *L. grandifolia* or *L. Javanica* could prove reasonably effective in controlling CBD (78). More work needs to be done on this potential application. The oil of chemovarieties of *Ocimum gratissimum* that had high content of eugenol showed significant activity against *Fusarium verticillioides*, while the chemovariety with low eugenol content had low activity. The oil of *O. basilicum* had moderate antifungal activity (against *Fusarium verticillioides*) (59). Presence of antifungal activity is consistent with other researchers' work which has shown that *O. basilicum* oil has antifungal and antibacterial activity (82,83). The effects of *Cymbopogon citratus* L. essential oil on the growth, morphogenesis and aflatoxin production of *Aspergillus flavus* has been reported (83).

Therapeutic potential of essential oils

A review of pharmaceutical and therapeutic potentials of essential oils, some of which are found in Kenya and their individual constituents has also been reported. At its sublethal doses, the oil of *Cymbopogon citratus* was found to completely inhibit aflatoxin B₁ production from *Aspergillus flavus*. These findings show the potential of this oil as an effective inhibitor of biodegradation and storage contamination fungi and also in fruit juice preservation. The essential oil from *Foeniculum vulgare* has hepatoprotective activity due to the

presence of d-limonene and β -myrcene. The major sesquiterpene alcohol of *Matricaria chamomilla* is a promising inducer of apoptosis in highly malignant glioma cells. Treatment of human leukemia HL-cells with 1,8-cineole, which is found in high concentrations in *Eucalyptus globulus* and in *Lippia ukambensis* var *cineole*, indicated an induction of apoptosis. The essential oil of *Anethum graveolens* has potential in prevention and treatment of atherosclerosis because it reduces blood triglyceride levels. The essential oil of *Ocimum gratissimum* induced an immediate and significant hypotension and bradycardia. This oil may also have potential as a therapeutic measure in shigellosis (84).

Anti-free Radical and Antimicotoxicogenic Activity

The essential oil of *Ocimum gratissimum* displayed potent free radical scavenging capacity, which can be attributed to high eugenol content (59). Literature shows that antioxidant compounds are used for controlling the mycotoxin fumonisin production (85,86). This essential oil with high eugenol content, an antioxidant compound and potent phenolic acid, showed a strong antimicotoxicogenic activity by inhibition of fumonisin production (59).

Mosquito Larvicidal Activity and Repellence

The oils of some *Lippia* species (*L. carviadora*, *L. dauensis*, *L. grandifolia*, *L. javanica*, *L. somaliensis*, and *L. ukambensis*, *L. wilmsii*) showed mosquito larvicidal activity. *Lippia dauensis* showed the greatest activity with 93.8% mortality at 100 ppm and 100% mortality at 150 ppm. *Lippia grandifolia* had an LD₅₀ of 150 ppm and the two chemotypes of *L. ukambensis* had an LD₅₀ between 170 and 250 ppm. Although this activity is lower than that of synthetic organophosphates, it is much higher than that reported for other essential oils such as *Ocimum sanctum*. Larvicidal activity could be due to the presence of thymol, linalool, camphor, α -pinene, limonene ocimene and 1,8-cineole in some of these oils (11,75,78).

The essential oils of some plants from Kenya were evaluated for mosquito repellence against *Anopheles gambiae* on the forearms of human volunteers. The oils of *Croton pseudopulchellus*, *Mkilua fragrans*, *Endostemon teresticaulis*, *Ocimum forskolei*, *Ocimum fischeri*, *Plectranthus longipes*, *Conyza newii* and *Plectranthus marrubioides* were all found to be more potent than DEET, the synthetic repellent. The activity of each of the oils of *Lippia javanica*, *Lippia ukambensis* and *Tetradenia riparia* was similar to that of DEET, but the oil of *Tarchonanthus camphoratus* was less potent (87,88).

Maize Weevil Repellent Activity

The oils of *L. dauensis*, *L. ukambensis*, *L. grandifolia*, *L. javanica* and *L. somaliensis* exhibited repellency against maize weevil (*Sitophilus zeamais*), an important post-harvest pest. *Sitophilus* infestation starts in the field and can be carried into storage. *Lippia dauensis* and *L. grandifolia* showed slightly higher activity than N,N-diethyltoluamide (DEET). The oil of *L. ukambensis* chemovariety camphor was 1.5 times more active than DEET, but *L. ukambensis* chemotype cineole showed lower activity than DEET. The results suggest that the high content of camphor in *L. ukambensis* camphor chemotype maybe responsible for the high activity compared to that of *L. ukambensis* 1,8-cineole chemotype. *Lippia* species should therefore be studied further for their potential in grain pest control, especially in storage (76).

Plectranthus barbatus contains an essential oil that exhibits anti-allergic activities through passive cutaneous anaphylaxis inhibition (65).

Applications

Cymbopogon citratus, *C. nardus*: Studies in Kenya have highly recommended the commercial exploitation of *C. nardus* and *C. citratus* (33,76) which are grown extensively world-wide in tropical areas for perfumery, toilet, cosmetic and confectionery industry. Studies indicate that they are clonally propagated easily and can be harvested many times during the year. These grasses do not also, seem to interfere with other crops and have the ability to prevent soil erosion if planted along contours and hedges.

Eucalyptus citriodora: This plant is the second most commercially important *Eucalyptus* species as applicable to essential oil trade. It is considered to be the best natural source of citronellal (69). This plant grows easily in Kenya and can be exploited for use in soaps, creams and lotions, as well as for making mosquito repellants. In addition to the essential oil from the leaves, *E. citriodora* yields good timber for construction, which is also termite resistant. *Lippia* species: Preliminary work indicated that *Lippia* species could be easily cultivated (8, 11, 78-80). From these studies, it was concluded that most of *Lippia* species could be commercially utilized. For example *L. ukambensis* and *L. somaliensis* oils could be utilized in aromatherapy while the others, due to their pleasant and agreeable aroma could be useful in food, beverage, soap, cosmetic and perfume industries. *Lippia somaliensis*, for example, has a mango-like aroma and organoleptic studies indicated that *L. ukambensis* and *L. dauensis* had an interesting odor that needs to be industrially evaluated for possible applications. These oils could also be used to enhance user acceptability, due to their agreeable aroma, of certain technical preparations such as insecticide, sprays, vanishes, lubricants and paints. It has been suggested that intercropping of *L. grandifolia* and/or *L. Javanica* with coffee crops would be reasonably effective in the control of Coffee Berry Disease (CBD) and allow the simultaneous development of a new crop for the harvest and distillation of this perennial species. The oils/plants are non-toxic to man (75) and are well known to have a short-lived residence time in the soil and environment in contrast to

the inorganic and organometallic fungicides that are currently in use for control of CBD (11,78)

Plectranthus marrubioides: Kenya imports all the camphor used in the local industry. The camphor content in the essential oil of *P. marrubioides* would be a good source for use in the pharmaceutical industry, especially for preparations such as local anesthetics, remedies for rheumatic and muscular pains and for respiratory conditions. These potential uses justify cultivation of this plant on a commercial scale. The plant is easily propagated from cuttings. It can withstand adverse weather conditions and can also co-exist with other plants as an undergrowth in forests (66).

Ocimum kilimandscharicum: This plant is a natural source of camphor. Some farmers in western Kenya have recently been trained on cultivation, extraction and processing of products based on the oil, for treatment of flu, colds and muscle pains. This work is a joint effort between University of Nairobi and International Centre of Insect Physiology and Ecology (ICIPE), Kenya. Several essential oil based products from *Ocimum* spp. are now in the Kenyan market. This cultivation and production should be scaled-up.

Research revealed that plants such as Fennel (*Foeniculum vulgare*), Dill (*Anethum graveolens*), Rosemary (*Rosmarinus officinalis*), and Chamomile (*Matricaria chamomilla*) and Majoram (*Majorana hortensis*) could be grown in Kenya, and the yield of essential oil was found to be usually much higher than that reported in other countries where the plants of the same species grow wild or under cultivation (33,76).

Other possible use of essential oil bearing plants would be their utilization as insecticides, fungicides and repellents in agriculture especially by intercropping with crop plants. They can also be used in forest and wildlife management since it is known that wild and domestic animals prefer to feed on plants containing sesquiterpenes while rejecting feed containing oxygenated monoterpenes.

Table I. Major Chemical Constituents of Essential Oil Bearing Plants from Kenya

Family and Botanical name	Plant part	EO (%)	Major constituents	Ref.
Apiaceae (Umbelliferae)				
<i>Anethum graveolens</i> L. (Dill) ^b	Fruits SD	3.3	carvone, phellandrene	33,34
<i>Coriandrum sativum</i> L. (Coriander) ^b	Fruits SD	0.5	linalool	33,34
<i>Foeniculum vulgare</i> Hill (Fennel) ^b	Fruits SD	4.0	anethole, fenchone	33,34
<i>Heteromorpha trifoliata</i> (Wendl.) Eckl. & Zey. ^a	Leaves SD Flowering parts SD	0.8 2.0	α -pinene, germacrene-D, sabinene	36
<i>Pimpinella anisum</i> L. (Anise) ^b	Fruits SD	2.7	anethole	33,34
Asteraceae (Compositae)				
<i>Artemisia afra</i> Willd. (African wormweed).	Leaves SD	1.5	1,8-cineole, terpinen-4-ol, borneol	39,40, 42
<i>Blumea brevipes</i> (Oliv. & Hiem) Willd ^a	Flowers SD	0.7	terpinen-4-ol, germacrene D, sabinene	42
<i>Helichrysum odoratissimum</i> (L.) Less. ^a	Flowers F	0.7	α -pinene, (E, E)-farnesol, α -humulene	43
<i>Matricaria chamomilla</i> L. (Chamomile) ^b	Flowers SD	1.2	bisabolols, chamazulenes	33

Table I. Continued.

Family and Botanical name	Plant part	EO (%)	Major constituents	Ref.
Asteraceae (Compositae)				
<i>Microglossa pyrhopappa</i> (A. Rich)	Aerial parts	0.4	β -caryophyllene, γ -gurjunene, limonene	44
Agnew var. <i>pyrhopappa</i> ^a	SD			
<i>Psidia punctulata</i> (DC.) Vatke ^a	Leaves SD	0.5	germacrene-D, β -phellandrene, β -caryophyllene	45
<i>Sphaeranthus bullatus</i> Mattf. ^a	Leaves SD	0.5	thymol, bornyl acetate, germacrene-D	46
<i>Sphaeranthus cyathuloides</i> O. Hoffm. ^a	Leaves F	2.9	<i>trans</i> -dihydrocarvone, <i>cis</i> - dihydrocarvone	47
<i>Sphaeranthus suaveolens</i> (Forsk) DC. ^a	Leaves SD	0.5	<i>cis</i> -pinocamphone	46
<i>Tarconanthus camphoratus</i> L. ^a	Leaves SD	0.8	α -fenchyl alcohol, 1,8-cineole, α - terpineol	49
Burceraceae				
<i>Boswellia neglecta</i> S. Moore (<i>Boswellia hildebrandtii</i>) (Olibanum, Frankinsence) ^a	Bark exudates SD	3-8	α -thujene, α -pinene, terpinen-4-ol	50
<i>Commiphora holtziana</i> Engl. ssp. <i>Holtziana</i> (<i>Commiphora erythrea</i>) (Opopanax, Sweet myrrh) ^a	Bark exudates SD	3.5-8	δ -elemene, α -cubenene, α -copaene, <i>cis</i> - α -bergemotene, β -elemene, germacrene-D	51,53, 54
<i>Commiphora myrrha</i> (Nees) Engl (Myrrh) ^a	Bark exudates SD	7-17	terpenes, sesquiterpenes	51

Table I. Continued.

Family and Botanical name	Plant part	EO (%)	Major constituents	Ref.
Euphorbiaceae				
<i>Croton sylvaticus</i> Hoscht. ^a	Leaves SD	0.14	β -caryophyllene oxide, α -humulen-1,2-epoxide	56
<i>Synadenium compactum</i> N. E. Br. var. <i>compactum</i> ^a	Stem bark D	0.2	α -selinene, β -selinene	58
Lamiaceae (Labiatae)				
<i>Ocimum basilicum</i> L. ^a	Leave SD, Flowers SD	0.2-1.9	geranial, neral, camphor, linalool	59
<i>Ocimum fischeri</i> Gürke ^a	Leaves SD	3.8	linalool, fenchone	60
<i>Ocimum gratissimum</i> L. ^a	Leave SD, Flowers SD	0.2-2.9	eugenol, Z- β -ocimene	59
<i>Ocimum keniense</i> Ayobangira ^a	Leaves SD	4.0	1,8-cineole, methylchavicol	61
<i>Ocimum kilimandscharicum</i> Gürke ^a	Leaves SD	5.3	camphor	62
<i>Plectranthus amboinicus</i> (Lour.) Spreng.	-	-	limonene, linalool, myrcene, thymol, borneol, camphene, α -amorphene and β -cubebene	65
<i>Plectranthus barbatus</i> Andr.	-	-	humulene and β -caryophyllene, δ -selinene, borneol, camphene, β -phellandrene, α -ionone, α -thujene	65
<i>Plectranthus marrubiioides</i> Benth. ^a	Leaves SD	4	camphor, α -phellandrene, ocimene	66
<i>Plectranthus sylvestris</i> Gürke	-	-	caryophyllene and germacrene D	65

Table I. Continued.

Family and Botanical name	Plant part	EO (%)	Major constituents	Ref.
Lamiaceae (Labiatae)				
<i>Plectranthus tenuiflorus</i> (Vatke)Agnew. ^a	Leaves SD	1.6	carvactrol, α -terpinene, <i>p</i> -cymene	67
<i>Rosmarinus officinalis</i> L. (Rosemary) ^b	Leaves SD	2.0	1,8-cineole, geranyl acetate	33,34
<i>Saturea biflora</i> L. ^a	Leaves SD	1.5	citral	33
<i>Thymus vulgaris</i> L.(Thyme) ^b	Leaves SD	2.3	thymol	33,34
Myrtaceae				
<i>Eucalyptus citriodora</i> Hook f. ^b	Leaves F	2.2-8.3	citronellal, citronellol, isopulegol, 1,8-cineole	68,69
<i>Eucalyptus globulus</i> Lab. ^b	Leaves SD	2.0	1,8-cineole	33
Papilionaceae				
<i>Rynchosia minima</i> DC. ^a	Flowering parts SD	0.1	β -caryophyllene, gemacrene B	74
Poaceae (Gramineae)				
<i>Cymbopogon afronardus</i> Stapf ^a	Leaves SD	0.4	intermedeol, 5-epiparadisol, 6S:7R-bisabalone, 6R:7R-bisabalone	75
<i>Cymbopogon citratus</i> Stapf. (Lemon grass) ^b	Leaves SD	0.9	citronellal, geraniol	33, 76
<i>Cymbopogon nardus</i> Rendle (Citronella grass) ^b	Leaves SD	0.5	geraniol, citronellal	33,76
Rutaceae				
<i>Clausena anisata</i> (Willd.) Benth. ^a	Leaves SD	1.5	anethole	33,34
Santalaceae				
<i>Osyris lanceolata</i> Hochst. & Steudel (East African Sandal wood) ^a	Wood SD	4.0	sesquiterpenes, sesquiterpene alcohols	33,77

Table I. Continued.

Family and Botanical name	Plant part	EO (%)	Major constituents	Ref.
Verbenaceae				
<i>Lippia carvioidora</i> Meikle ^a	Leaves SD	3.0	carvone, limonene, carviny acetate, <i>p</i> -cymene	79,80
<i>Lippia carvioidora</i> var. <i>minor</i> Meikle ^a	Leaves SD	0.2	β -cubenene, β -elemene	79,80
<i>Lippia dauensis</i> (Chiov.) Chiov ^a	Leaves SD	2.4	β -ocimene, 2-methyl-6-methylene-7-octen-4-one, myrcene, <i>cis</i> -tagetone	79,80
<i>Lippia grandifolia</i> A. Rich. ^a	Leaves SD	0.7	linalol, thymol, β -cubenene, <i>p</i> -cymene	79,81
<i>Lippia javanica</i> (Burm. f.) Spreng ^a	Leaves SD	1.6	myrcenone, <i>cis</i> -ocimenone, <i>trans</i> -ocimenone	79,80
<i>Lippia somalensis</i> Vatke ^a	Leaves SD	0.7	1,8-cineole, δ -3-carene, myrcene, β -cubebene	79,80
<i>Lippia ukambensis</i> Vatke ^a	Leaves SD	1.8	camphor, 1,8-cineole	79,80
<i>Lippia wilmsii</i> H.H. Pearson ^a	Leaves SD	1.1-2.2	limonene, piperitone, piperitenone	79,80
Zingiberaceae				
<i>Zingiber officinalis</i> Roscoe (Ginger) ^b	Rhizome SD	3.1	zingiberine, zingiberol	33,34

NOTE: a: indigenous. b: exotic D: dry. F: fresh. SD: semi-dried.

Table II. Traditional Uses of Essential Oil Bearing Plants from Kenya

<i>Family, Botanical name</i>	<i>Local names (Language)</i>	<i>PP</i>	<i>Use and method of preparation</i>
Apiaceae (Umbelliferae)			
<i>Coriandrum sativum</i> L. (Coriander)	Ndania (Kikuyu)	L, F	Fresh leaves or fresh or dried fruits are used for flavouring food (48)
<i>Foeniculum vulgare</i> Hill (Fennel)	-	R	Leaves are used to treat snake bites, roots for treatment of syphilis and as a purgative. Ground stems are mixed with fat and given to babies and children as a tonic. Ash from leaves is mixed with fat and given to infants (35, 37)
<i>Heteromorpha trifoliata</i> (Wendl.) Eckl. & Zey	Luguguni (Zigua), Olkuyeyini, Olkuyaini (Maasai)	L,R ,S	
Asteraceae(Compositae)			
<i>Artemisia afra</i> Willd. (African wormweed)	Fifi (Shambaa), Ushemeli (Sukuma)	A,L	Heated aerial parts are fermented and used to treat sore throats and fever in children. Aerial parts are taken by adults for treating indigestion. A decoction of boiled roots is taken two to three times daily for treatment of intestinal worms. For emetic action, the leaves are chewed and the juice swallowed (35)

Table II. Continued.

Family, Botanical name	Local names (Language)	PP	Use and method of preparation
Asteraceae (Compositae)			
<i>Helichrysum odoratissimum</i> (L.) Less.	Mulavatwa (Kamba), Inamalaba (Kakamega/Luhya)	L,R	Leaves are used as anthelmintics. Pounded leaves are applied to burn wounds to facilitate healing. Leaves and branches are pounded, soaked in water and the extract is used as eye drops for conjunctivitis. Crushed roots are steeped in hot or cold water and the extract is taken as a purgative and for coughs (35)
<i>Psidium punctulata</i> (DC.) Vatke	Kasha (Pare), Pagasha (Shambaa), Olabai, Olabaai, (Maasai), Mwenda thigo (Kikuyu), Masonzoia (Kamba)	L	Pounded leaves are taken for colds and a decoction of pounded leaves is taken for abdominal pains. Leaves are crushed in water and smeared on the animal or the extract is sprayed on the animal shed as an insecticide for fleas in sheep, goat kids and calves. Soup containing roots is taken orally for treating gout (35, 37, 48)
<i>Sphaeranthus bullatus</i> Matff.	Cheptogomda (Kipsigis), Oleturot (Maasai)	L	A leaf decoction is used as a tonic and for stomachaches (35, 37)
<i>Sphaeranthus suaveolens</i> (Forsk) DC.	Njugu ya iria (Kikuyu)	L	A leaf decoction is used to treat malaria and coughs. Pounded leaves are used in the water for bathing water to make newborn baby skin smooth
<i>Tarconanthus camphoratus</i> L.	Mariricua (Kikuyu), Oleleshua, Oleleishwa, Osendu (Maasai)	F, L,S	Leaves are used to treat afterbirth pains (35, 37, 48) The wood is used for charcoal production. Patients inhale the smoke of burnt flowers for treatment of headaches. Dry leaves are crushed and put in water, sieved and the extract is given to children for treatment of tapeworms. Fresh leaves are put under armpits or around the neck as a perfume (37, 48)

Table II. Continued.

Family, Botanical name	Local names (Language)	PP	Use and method of preparation
Burceraceae			
<i>Boswellia neglecta</i> S. Moore	Muhodja (Digo)	E, R	One cupful of a root decoction after every meal is used as a diuretic and for treatment of gonorrhoea. Frankincense is used in incense and fumigating preparations (35, 51)
<i>Boswellia hildebrandtii</i> (Olibanum, Frankincense)			
<i>Commiphora holtziana</i> Engl. ssp. <i>holtziana</i> (<i>Commiphora erythrea</i>) (Opopanax, Sweet myrrh)	-	E	Gums are used as acaricides and insect repellants. Oleo-gum resins could be used in Kenya in the perfume, soap and cosmetic industry as well as food and beverage industry (51, 52)
<i>Commiphora myrrha</i> (Nees) Engl (Myrrh)	Habag-hady (Somali), Didim (Somali)	E	The oleo-gum-resin is used in the treatment of intestinal problems, body wounds, boils and excessive menstrual bleeding. It is also used to kill ticks responsible for East Coast Fever in cattle. Myrrh is used as incense for ceremonial and religious purposes, it is used in toothpastes, in the perfume industry, as a carminative, astringent and mouth gargle (35, 51, 55)

Table II. Continued

Family, Botanical name	Local names (Language)	PP	Use and method of preparation
Euphorbiaceae			
<i>Croton sylvaticus</i> Hoscht.	Msandusi, Minduzi, Msunduzi, (Digo)	L,R RB	A leaf infusion is taken orally as a purgative, and the leaf decoction is used as a body wash to reduce Kwashiorkor swellings. Pounded roots are used to make poultices for the same swellings. The root bark decoction is taken orally for treating tuberculosis (35)
<i>Synadenium compactum</i> N. E. Br. var. <i>compactum</i>	Kinenge, Kineke, (Aholi), Kyatha, (Kamba), Watha (Kikuyu)	L, B	A leaf or branches decoction is given to cattle for treatment of East Coast Fever. The sap/exudates from the leaves or branches can also be rubbed directly onto the swollen glands of cattle with ECF. This plant is used in making farm boundaries. It is rare in the wild and endemic to central Kenya (35, 48)
Lamiaceae			
<i>Ocimum basilicum</i> L.	Chivumbani (Digo), Bwar (Luo), Jumba yaza (Nyamwezi), Kirumbasi (Swahili)	A,B,L ,R	Aerial parts are used when fresh as mosquito repellents. The vapour from boiled leaves and branches is inhaled for nasal and bronchial catarrh. A root decoction is taken orally for constipation and for stomach pain in pregnant women (35, 48)
<i>Ocimum gratissimum</i> L.	Kirumbasi (Swahili), Makandu (Kamba), Yoiyoia (Marakwet), Olemoran, Olemuran (Maasai)	L,R	Leaves are used to treat abdominal pains, sore eyes, ear problems and coughs. The leaves are rubbed between palms and sniffed for a blocked nasal passages. A leaf infusion is used as a disinfectant and insecticide. The roots are used as an emetic and the leaves are smoked like tobacco (35, 37)

Table II. Continued.

Family, Botanical name	Local names (Language)	PP	Use and method of preparation
Lamiaceae			
<i>Ocimum kilimandscharicum</i> Gürke	Esilokha (Lunyore), Mwenye (Kamba), Mwonyi (Kakamega/Luhya), Mzugwa, (Shambaa), Okita (Luo)	L	Leaves are pounded and soaked in hot water and the extract is taken orally for abdominal pains. Vapours of boiled leaves are inhaled or the leaves are rubbed between palms for colds and coughs. Leaves are put in hot water and then pressed on sore eyes as treatment. Pounded leaves are soaked in warm water and the water is used to bath babies with measles to heal the skin (35)
<i>Plectranthus amboinicus</i> (Lour.) Spreng.	Reza (Giriama)	R	A root decoction is taken for stomach pain (35)
<i>Plectranthus barbatus</i> Andr.	Maiya (Kamba), Muigoya, Maigoya (Kikuyu), Makuri, Mukuri (Kikuyu)	L	Leaves are crushed and the juice is drunk as a purgative and for stomach pain. Pounded leaves are put in water for bathing for measles patients to heal the skin. The plant is used for baiting bee into new beehives (35, 48)
<i>Plectranthus syhvestris</i> Gürke	Muoro (Meru), Nginga (Taita)	L, R	Leaves are ground and the juice is taken for abdominal pain. For treatment of chest pain, two small cuts are made on the chest and the juice from ground leaves is squeezed into the cuts. The roots are boiled with <i>Clerodendron johnstonii</i> and <i>Cassia didymobotrya</i> for treating malaria and headaches (35)

Table II. Continued.

Family, Botanical name	Local names (Language)	PP	Use and method of preparation
Myrtaceae			
<i>Eucalyptus globulus</i> Lab.	Mubau, (Kikuyu)	Muringamu	The leaves are used to treat malaria and they also repel mosquitoes (48)
Rutaceae			
<i>Clausena anisata</i> (Willd.) Benth.	Mutathi (Kikuyu)	R	Roots are used for the treatment of after birth pains, headaches and colds (48)
Santalaceae			
<i>Osyris lanceolata</i> Steudel (Ea Afr. Sandalwood)	Ololesiyai, (Maasai)	Ololesiyai, SB	Fresh stem bark is used in tea for maintaining good health (37)
Verbenaceae			
<i>Lippia javanica</i> (Burm. f.) Spreng	Ang'we-Rao (Luo), Kyulu, Mutithi (Kamba), Ol Sinoi, Maasai, Orwo (Achoi), Sulasula (Kabras and Kakamega), Ifani (Iraqw), Muthiriti Mucohi (Kikuyu)	Ang'we-Rao (Luo), Kyulu, L, F	Leaves soaked in water and the extract drunk for indigestion and tapeworms. Leaf infusion taken for fevers. Leaf decoction taken orally and the body bathed in the same for treatment of malaria. Leaves and flowers rubbed between palms and sniffed for a stuffy nose. Pounded leaves applied to wounds. Leaves used as insect (termite) repellent (35, 48)
<i>Lippia ukambensis</i> Vatke	Muthiriti Mucohi (Kikuyu)	Muthiriti Mucohi (Kikuyu)	The plant is a termite repellent and so it is used for making traditional granaries (48)
Zingiberaceae			
<i>Zingiber officinalis</i> (Ginger)	Roscoe	Mutanga-uthi (Kikuyu)	Rh
NOTE: PP: plant parts, A: Aerial parts. E: exudate. L: leaves. S: stem. R.: roots. Rh.: rhizome			

Conclusions

Kenya is endowed with diverse range of aromatic essential oil bearing plants due to the countries wide climatic and environments. Research in Kenya has revealed many plants that contain essential oils of commercial and medicinal importance. Preliminary work has shown that most these plants, whether indigenous or exotic, can be cultivated in Kenya. Given the indigenous aromatic plants and the adaptability of introduced essential oil plants into Kenya, this country is poised to expand its commercial essential oil sector. More research on validating the traditional knowledge of aromatic and medicinal plants may yield new insights into applications and provide additional incentives to further conserve and protect the indigenous genetic resources while providing interesting new aromas and/or new products for the control of plant diseases and insect pests and to improve human health.

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Chapter 28

Comparison of Ginger extracts from Africa and Asia

Discovery of the Specificity of the Madagascar Ginger for the Cosmetic Industry

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Ginger (*Zingiber officinale*) is the most important member of the zingiberaceae family. Its edible rhizome has been used throughout the ages as a spice and also in traditional medicine to heal a wide range of diseases, from digestive pain to burns. The objective of this research was to characterize the essential oil and oleoresin of ginger rhizomes from Madagascar and to compare them with those coming from China, India and Indonesia. The Malagasy ginger showed a unique and characteristic organoleptic and chemical profile which makes them ideal for cosmetic applications. Because of these properties, the company Chanel, has selected the Malagasy ginger and their extractives for using as ingredients for their line of perfumes.

Ginger (*Zingiber Officinale*, Poaceae family) was originated from southern Asia, and nowadays is cultivated and commercialized around the world, particularly in China, India, Indonesia and Africa. Ginger was brought to Africa by Phoenicians around the first century B.C. and introduced in Madagascar by Indonesian merchants a few hundred years later (1). From the same original species, gingers have then evolved differently depending on the soil and weather conditions of their crops. The result of this evolution gives Malagasy ecotype its specific elements making it one of the finest quality gingers. This spice has become an important ingredient for the Malagasy traditional medicine, being used to treat different ailments (2).

The objective of this research was to characterize the essential oil and oleoresin composition of ginger rhizomes from Madagascar and to compare them with rhizomes coming from China, India and Indonesia. The work is part of a study conducted by the “Centre de Recherche Cosmétique” (CRC) of CHANEL to find new ginger varieties with unique characteristic for the cosmetic industry.

Material and Methods

Material

Dried and cut rhizomes coming from China, India and Indonesia were purchased from different confidential partners.

The fresh Malagasy rhizomes were cut and dry following the same industrial procedure conducted to the other Asian rhizomes. The Malagasy rhizomes were cut in slices and then dried at 50°C during five days.

Essential Oil and Oleoresin Extraction

The oleoresin was obtain by extracting the dried rhizomes (125-150g) with 700 ml of cyclohexane/2-propanol (80/20 v/v), then the solvent was removed by evaporation in a rotavapor (at 70°C and 100 mbar).

The essential oils were extracted by hydrodistillation. The dried rhizomes (270-360 g) were place in the distillation flask and filled with 5 L of water. The oleoresin and essential oil content was expressed as percent (g/100g of rhizome dry weight (Table I).

Table1. Essential oil and oleoresin yields of ginger (*Zingiber officinale*) rhizomes from China, India, Indonesia and Madagascar.

PROCESS		CHINA	INDIA	INDONESIA	MADAGASCAR
Oleoresin	Raw material (g)	167.0	150.0	125.0	138.0
	Extract (g)	9.5	11.0	11.5	6.5
	Yield (% w/w)	5.7	7.3	9.2	4.7
Essential oils	Raw material (g)	230.0	208.0	357.0	269.0
	Extract (g)	0.5	0.5	2.7	1.4
	Yield (% w/w)	0.2	0.3	0.8	0.5

Chemical Analysis

The essential oils were analyzed by gas chromatography/mass spectrometry (70°C to 250°C within 90 min and then 90 min at 250°C – J&W DB-1 column : 30 m x 0.25 mm x 0.25 μ). The samples have been diluted in ethanol before injection. Individual identifications were made by matching their spectra with those from mass spectral libraries (WILEY and NIST). The identity of each component was confirmed by comparison of its Kovats indices with those from known standards.

The oleoresins were analyzed by high performance liquid chromatography following the standard procedure of the Normalization French Association (AFNOR) (7). The quantification of each component was carried out by using the external standard vanillynonanamide.

Results and Discussion

According to an overview on various origins (3), the dried rhizomes contained around 2% of oil (from 1.4 to 2.6%). Our values were lower and showed the limits of our hydrodistillation conditions. In another study (4), the amount of oleoresin ranged from 3 to 11%, which met the observed yields. The Indonesian ginger showed the highest yields for both extracts. While, the Indian and the Malagasy exhibited lower values, and the Chinese rhizomes gave the lowest yields (Table I).

These values showed that there are significant variation in the essential oil and oleoresin content, showing that the Malagasy ginger contained the lowest levels of oleoresins, but higher amounts of essential oils as compared with the Chinese and Indian gingers.

African and Asian Gingers Essential Oils Analyses

The organoleptic evaluation of the essential oils can give the first impressions of the quality of a ginger. The aroma and the appearance of the oils are both important aspects to evaluate their quality. Buyers of essential oils usually rely on the organoleptic character to either accept or reject samples. The aroma and appearance of both the oil and the oleoresin were evaluated by an organoleptic test panel (Table II).

Table II. Viscosity, color and aroma of essential oil and oleoresin of ginger (<i>Zingiber officinale</i>) rhizomes from China, India, Indonesia and Madagascar.					
EXTRACT	CHARACTERISTIC	CHINA	INDIA	INDONESIA	MADAGASCAR
ESSENTIAL OILS	Viscosity	Liquid	Liquid	Liquid	Liquid
	Color	Light yellow	Light yellow	Yellow (cloudy)	Yellow
	Odor	Cocoa, citrus	Citru, fresh	Citrus	Citral
OLEORESINS	Viscosity	Thick liquid	Thick liquid	Thick liquid	Viscous liquid
	Color	Brown red	Brown red	Brown yellow	Brown yellow
	Odor	Cocoa	Ginger bread	Citrus	Strongly citrus

Good quality ginger oil is light yellow to yellow with a characteristic spicy and lemon aroma. All the oils were light yellow (Chinese and Indian) or yellow (Indonesian and Malagasy). All oils were low viscosity liquids at room temperature. Regarding the aroma, the Indonesian and Malagasy samples showed characteristic and strong citrus/lemon aroma notes, while the Chinese showed additional fresh notes and the Chinese off cocoa notes (Table II). The Malagasy oil showed the strongest citrus notes.

The oleoresins from the Chinese and Indian rhizomes were brown red, while the Indonesian and the Malagasy were brown yellow. From a cosmetic standpoint, these latter oleoresins are good candidates because of their lighter color.

The results showed that the Malagasy and the Indonesian rhizomes yielded high quality extractives with desired organoleptic characters for use as cosmetics.

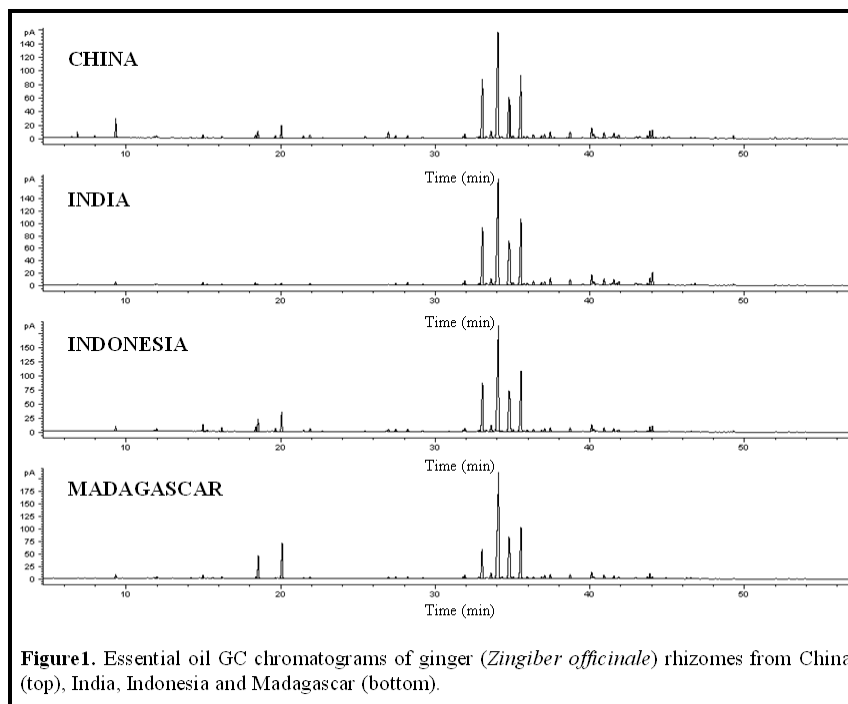


Figure 1. Essential oil GC chromatograms of ginger (*Zingiber officinale*) rhizomes from China (top), India, Indonesia and Madagascar (bottom).

Table III. Essential oil composition of ginger (*Zingiber officinale*) rhizomes from China, India, Indonesia and Madagascar

COMPOUNDS	CHINA	INDIA	INDONESIA	MADAGASCAR
Paracymene ¹	2.5 ²	0.5	0.7	0.6
Neral	1.0	0.2	2.0	4.3
Geranial	1.8	0.3	3.2	7.4
ar-Curcumene	11.3	11.9	10.8	6.7
Germacrene	1.5	1.5	1.5	1.4
α -Zingiberien	23.8	26.7	28.1	31.9
α -Farnesene	4.9	5.2	5.1	5.2
α -Bisabolene	6.8	7.8	7.6	7.8
β -Sesquiphellandrene	11.1	13.1	12.9	12.0
Nerolidol	1.1	1.3	0.8	0.9
β -Bisabolene	1.4	1.2	0.8	0.9
Zingerone	2.0	2.2	1.5	1.5

¹. components are listed in order of elution (PDMS column), 2. relative percentage

The essential oil composition showed variation among the different rhizomes (Figure 1, Table III). All essential oils were dominated by high levels of α -zingiberene (24-32%), with minor and varying amounts of β -sesquiphellandrene (11-12%), α -bisabolene (6.8-7.8%) and α -farnesene (4.9-5.2%) (Table III). The Malagasy oil and to lesser extent the Indonesian, were characterized by high levels of neral and geranial (also know as citral) and lower levels of ar-Curcumene (6.7%). The chemical analyses confirm the panel observations, in which the Malagasy essential oil has a strong citrus aroma caused by the high relative concentrations of neral and geranial.

African and Asian Gingers Oleoresins Analyses

In a spice like ginger, the volatile oils give ginger its characteristic aroma, while the nonvolatile pungent constituents are responsible for the spicyness and are extracted in the oleoresin which is a concentrated extract of the flavor of the spice.

The gas chromatography analysis of the oleoresin showed the presence of the same volatile components found in the essential oil (Fig 2), showing that the essential oils are also removed by the extraction solvent.

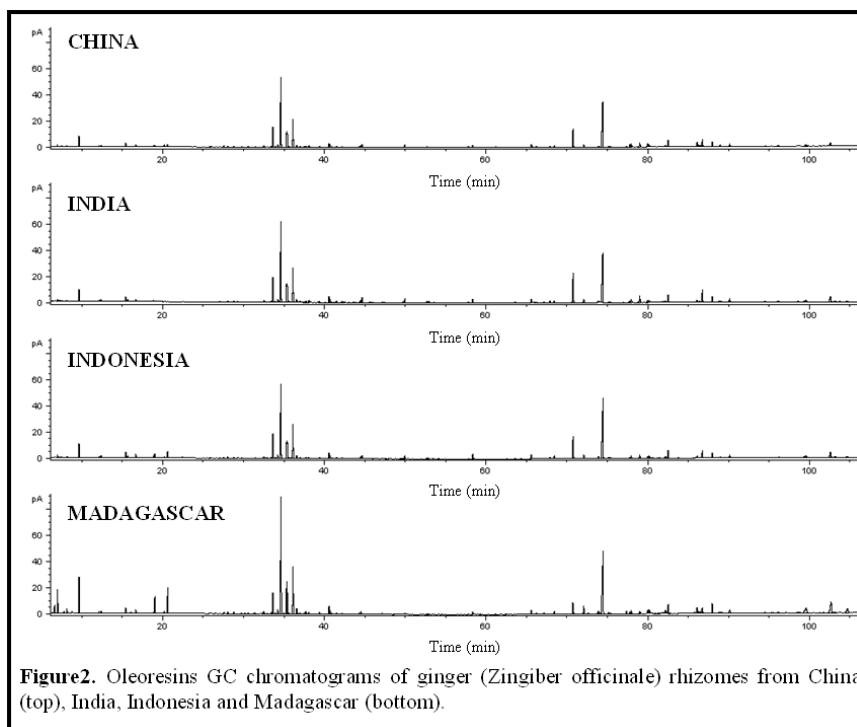
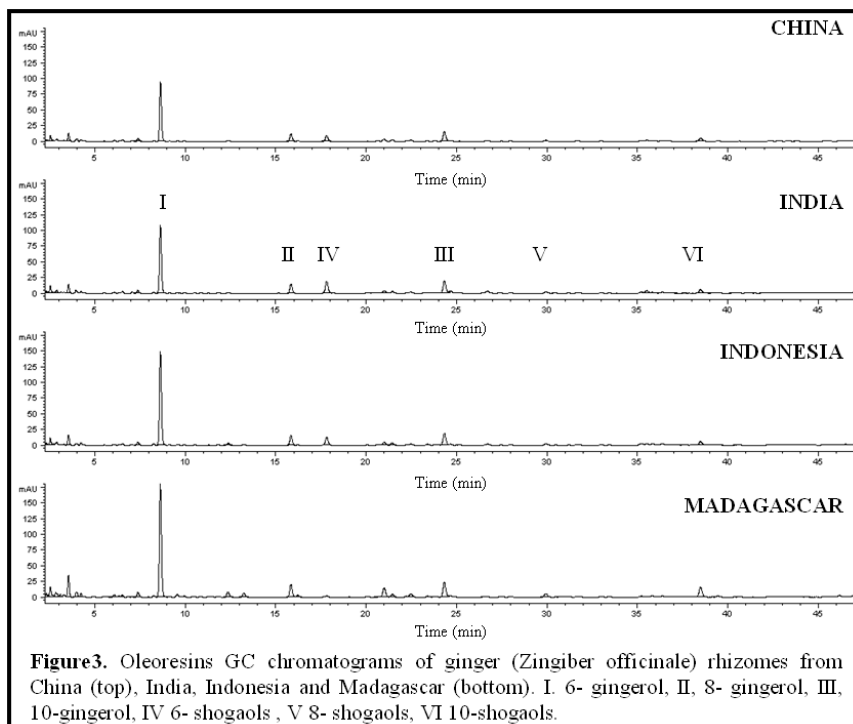


Figure 2. Oleoresins GC chromatograms of ginger (*Zingiber officinale*) rhizomes from China (top), India, Indonesia and Madagascar (bottom).

HPLC analyses were performed for the quantification of the non-volatile components of the oleoresin coming from the different rhizomes (Fig 3).

The targeted active compounds of these ginger extracts are divided in two families: gingerols and shogaols. They exhibit antioxidant activities (5) which is of current interest for the cosmetic industry. Shogaol is the dehydrated form of a gingerol. 6-Gingerol (I) is the main active component and its analogues are 8-Gingerol (II) and 10-Gingerol (III) (6), while 6-shogaol (IV) is the main active component and its analogues are 8-shogaol (V) and 10-shogaol (VI) (7).



The HPLC chromatograms clearly showed that the Malagasy Ginger contained higher levels of non-volatile components, particularly 6-gingerol (Figure 3).

Table IV. Gingerols and shogaols content of ginger (*Zingiber officinale*) rhizomes from China, India, Indonesia and Madagascar.

% of COMPOUNDS	CHINA	INDIA	INDONESIA	MADAGASCAR
6-Gingerol	8.6¹	9.2	12.4	14.2
8-Gingerol	1.5	1.7	1.7	2.1
10-Gingerol	2.6	3.4	2.8	3.6
6-Shogaol	1.2	2.1	1.6	0.4
8-Shogaol	0.3	0.5	0.3	0.1
10-Shogaol	0.6	1.0	0.7	0.9
TOTAL	14.8	17.9	19.5	21.3

¹ (g/100 g of oleoresin)

The quantification of individual gingerols and shogaols showed that the Malagasy samples exhibited the highest amounts of 6, 8, and 10-gingerols (Table IV). In relation to the total gingerols and shogaols, in the Malagasy oleoresin, 6-gingerol represented the 66% of this total, followed by the Indonesian (58%), Indian (43%) and Chinese (40%).

The Malagasy oleoresin also showed lower levels of shogaols as compared with the other oleoresins. Thus, the Malagasy oleoresin showed the highest levels of total gingerols and shogaols (21%), followed by the Indonesian and Indian oleoresins (18-19.5%, respectively), while the Chinese showed the lowest levels (14.8%) (Table IV).

Conclusions

This study confirms the variation of active principles of ginger rhizomes from various origins. The organoleptic profile of the essential oil and oleoresin, showed that the Malagasy samples showed strong citrus/lemon aroma, as compared with the samples from other origins. This strong citrus character, was confirmed by the higher levels of neral and geranial.

This study also showed that the Malagasy samples yielded oleoresin with the desired color, aroma and amount of non-volatiles components, suitable for the cosmetic industry. The Malagasy oleoresin also contained the highest levels of the antioxidant gingerols.

Considering the different varieties, the Malagasy ginger showed specific characteristic that make them ideal for the cosmetic applications. The company Chanel, has selected the Malagasy ginger because of its unique organoleptic and chemical profile, and their extractives are being used as ingredients in cosmetics manufactured by Chanel.

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Chapter 29

Models of Benefit-Sharing Policy: Opportunities and Challenges in Ensuring Equitable Natural Product Discovery in Africa

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Since the latter half of the 20th century, there have been a number of international and national agreements created for the purposes of distributing benefits derived from the commercialization of natural products and the protection of intellectual property rights. However, in Africa, there exist many challenges to the adoption, implementation and enforcement of the agreement at the national level. In this paper, I highlight different models that have been implemented across Africa. By identifying both the shortcomings and opportunities in current models, one can better seek to build more equitable partnerships in practice.

Historically, there have been vast array of interesting natural products developed from African plants. Once easily accessed and harvested the biogenetic resources and associated traditional knowledge from which many of these natural products derived are now subject to a host of international and national regulations governing their use.

Since the latter half of the 20th century, there have been a number of international and national agreements created for the purposes of distributing benefits derived from the commercialization of natural products and the protection of intellectual property rights. These agreements were formalized under the Convention on Biological Diversity (CBD) and were signed at the 1993 Earth Summit in Rio de Janeiro by many African countries, and as of 2002, up to 52 African countries have signed and ratified or agreed in principle to the terms of the treaty (1).

The CBD came a long way in addressing many of the ethical issues concerning natural product discovery and commercialization. However, in Africa, there exist many challenges to the adoption, implementation, and enforcement of the convention. This has resulted in a number of *ad hoc* access and benefit-sharing agreements at the national level. These subsequent agreements because many challenges to ensuring that fair distribution of benefits are delivered and that effective intellectual property rights are implemented (1, 4)

The primary purpose of this review is to provide an overview of the issues concerning access and benefit-sharing models that have been adopted across Africa. By identifying the shortcomings of the current models and highlighting opportunities that appear to exist, one may use that knowledge and experience to build more equitable partnerships among private and public institutions, host-governments and individuals involved in natural product discovery and commercialization.

Access and Benefit-Sharing and the CBD

Bioprospecting is defined as the systematic search and commercial development of natural products used in novel pharmaceuticals, nutraceuticals and industrial applications (1). Given their prior success, the champions of bioprospecting argue that natural products in the form of plants, marine organisms and micro-organisms remain the preeminent source for bioactivity and drug discovery (2, 3). For example, a survey revealed that over \$8 billion of U.S. prescription drugs in 1980 were plant-based, and found that out of the top one hundred and fifty brand names prescribed during a period of nine months in 1983, 57 percent of those drugs contained an active principle from a biological source (2). A more recent review concluded that from 1981 to 2002, over 60 percent of all new drugs introduced worldwide were based on a compound found in a natural product (3).

The early 1990s marked a watershed moment for bioprospecting. A time when advances in drug discovery science and genomics coincided with growing concerns for the environment, including mass species extinction (4). With the advent of high-throughput screening, automation and new information

technology, thousands of extracts of biological resources could be now tested at rates commercially attractive to large-scale laboratories and pharmaceutical companies (5). Those living in areas which supplied the source material, and provided the medicinal knowledge leading to the discovery, would be monetarily rewarded in a pre-determined compensation deal or access and benefit-sharing agreement (ABS). These natural product discovery schemes where founded on the logic that the commercialization of valuable genetic resources will foster social and economic development in areas that have marginal economic opportunities.

In June 1992, the CBD was open by the United Nations for signatures and was later entered into force on December 1993 (6). One of the preeminent protocols of the CBD, Article 15(1), describes the individual country's rights over its natural resources. In return for its control, national governments must facilitate access to their biodiversity for scientists and in return for fair and equitable sharing of the scientific and monetary benefits that derive from commercialization (6). The CBD also provides an overarching blueprint for a number of related research protocols and guidelines for national policy, most noteworthy, including prior informed consent, mutually agreed upon terms, and a specific code of conduct for collaborating scientists and researchers. The three main objectives of the CBD include:

- The conservation of biological diversity
- The promotion of sustainable use of its components.
- The equitable sharing of benefits that derives from the utilization of genetic resources (6).

Intellectual Property Rights, TRIPS and Traditional Knowledge

Internationally, the World Trade Organization's (WTO) Agreement on Trade Related Aspects of Intellectual Property Rights or TRIPS, in general, is the main force behind the implementation of intellectual property rights (IPRs). IPRs are goods that are derived from the mind or intellect, and are a feature of property that is reflected in copyrights, patents, trademarks, industrial design, trade secrets and domain names. In effect, they provide the "holder" the right to maintain exclusive control over the material (7). Overall, TRIPS, places emphasis on private property rights, especially concerning intellectual property to assist private companies and individuals the ability to patent discoveries made from nature based on scientific and/or traditional knowledge.

One of the most contentious issues facing IPRs has to do with the placing of novelty found with discovery involving biogenetic resources under IPR protection. Many critics of TRIPS remark that "traditional" knowledge was never formally accounted under the agreement, making it easier to privatize traditional knowledge under the framework of international patent rights (8). For some critics, the patenting traditional knowledge was an act of "biopiracy," or the misappropriation and downright theft of valuable biogenetic resources and associated traditional knowledge.

The TRIPS agreement was however, not the only international framework that addressed intellectual property rights. One can point to a number of

agreements that attempted to recognize traditional access rights and cultural knowledge in more formal means. One such agreement was the 1988 International Conference of *Belem*, Brazil, and in 1991 the second Code of Ethics of the International Society of Ethnobotany in Kunming, China (9). These two agreements were the first official recognition that traditional knowledge should be protected under formal property rights (10). Subsequently, both of these agreements were codified under the CBD in 1992. In the end, however, the use of both the CBD and TRIPPS as a framework for intellectual property rights only caused confusion about the correct way to move forward on the national level for many African nations (11).

Implementation of the CBD at the National Level in Africa

African governments for a while have been concerned about the condition of their natural resources. This concern is highlighted by the African Convention on the Conservation of Nature and Natural Resources held in Algiers in 1968. The convention addressed critical environmental issues facing Africa and put in place protocols concerning conservation and natural resource management. This meeting ultimately set the stage for subsequent international agreements on the environment, such as the CBD.

Developed in 2002, the Bonn Guidelines were created for the purposes of providing a framework for individual countries to implement the CBD at the national level. However, many African countries have been slow in adopting such measures. Examples of the different approaches selected African governments have taken to implement the CBD protocols at the national level are presented in Table I. Some African nations, such as Ethiopia, Kenya, Malawi and South Africa, have created new protocols for bioprospecting directly while others have adopted coverage that addresses access to genetic resources and protection of traditional knowledge within a country's existing environmental laws.

For example, a new comprehensive law developed in South Africa, requires anyone conducting any and all research and commercial trade involving medicinal plants to obtain research permits. This law, by far the most comprehensive in Africa may prove to be a new trend in the monitoring of natural product research and commercialization.

Table I. African Countries that have Adopted National Access and Benefit-Sharing Agreement (ABS) Measures

<i>Country</i>	<i>Measure</i>	<i>ABS Coverage</i>	<i>Year of adoption</i>
Cameroon	Law No. 96/12 Relating to Environment Management	AGR, NRM & Bio	1996
Central African Republic	Stratégie nationale et plan d'action en matière de diversité biologique	AGR, NRM & Bio, PIC	2000
Ethiopia	(a) Proclamation No. 120/98 to Provide for the Establishment of the Institute of Biodiversity Conservation and Research	(a) AGR, ESB	(a) 1998
	(b) Proclamation No. 482/06 to Provide for Access to Genetic Resources and Community Knowledge and Community Rights	(b) AGR, CTU, ESB, IPR,	(b) 2006
The Gambia	National Environment Management Act 1994	AGS, ESB	1994
Kenya	(a) Environmental Management and Co-ordination Act, 1999	(a) AGR,	(a) 2000
	(b) Environmental Management and Co-ordination, 2006	(b) AGR, ESB, IPR, NRM & Bio, PIC	(b) 2006
Madagascar	Law No 96-025 September 30, 1996	AGR, CTU	1996

Table I. Continued.

<i>Country</i>	<i>Measure</i>	<i>ABS Coverage</i>	<i>Year of adoption</i>
Malawi	(a) Environment Management Act No. 23/96	(a) AGR, ESB	(b) 1996
	(b) Procedures and Guidelines for Access and Collection of Genetic Resources in Malawi	(b) AGR, ESB,	(b) 1996
South Africa	(a) National Environmental Management: Biodiversity Act	(a) AGR, NRM & Bio.	(a) 2004
	(b) Regulations on Bio-Pro Prospecting and Access and Benefit-Sharing	(b) AGR, CTU ESB, IPR, PIC	(b) 2008
Uganda	(a) National Environment Statute	(a) AGR	(a) 1995
	(b) National Environment Regulations, 2005	(b) AGR, CTU, ESB, IPR,	(b) 2005
Zimbabwe	Environmental Management Act, Chapter 20:27	AGR, ESB	2002

SOURCE: Convention on Biological Diversity- Database on Access and Benefit Sharing Measures <http://www.cbd.int/abs/measures.shtml>; Accessed on: Dec. 5, 2008.

NOTE: AGR - Access to Genetic Resources; ESB - Equitable Sharing of Benefits arising out of the utilization of genetic resources; CTU - Customary or Traditional Use of genetic resources; IPR - Intellectual Property Rights; NRM & Bio - Natural Resource Management and Biodiversity Conservation; PIC - Prior Informed Consent

Bioprospecting Models in Africa

There are some commonly known examples of natural products derived from African plants. For example, the Devil's claw (*Harpagophytum procumbens*), is a plant used in a phytomedicinal remedies as an anti-inflammatory and analgesic (12). The southern African succulent Hoodia (*Hoodia gordonii*), which is commercially traded worldwide as an appetite suppressant (13). The bark of Pygeum (*Prunus africana*) is used as a phytomedicine for urinary complications and as an herbal remedy worldwide for benign prostatic hyperplasia and other urinary complications (12). There have also been a wide variety of African aloes have been used as a natural product in skin creams, shampoos and soaps. Furthermore, the fatty acids and oils form the fruits of a host of African plants have been harnessed to aid in cosmetic and fragrances, such as coconut oils, and jojoba, shea and almond butter (15). All of these products derive from advanced bioprospecting research and discovery.

In general, there are three main types of bioprospecting observed in Africa, including collaborative contracts, mass bioprospecting collaborative agreements,

and private-public partnerships (Table II). Each of these is noted as having different methods of delivering benefits and protection of intellectual property rights.

A collaborative contract, for example, is a project that involves agreements made between public research institutions each sharing similar goals of research and discovery of natural products. Institutions involved in this type of agreements usually see a mixture of short-term and long term benefits, including technology transfer, trainings and access to some monetary benefits in the form of milestone payments or payouts for significant breakthroughs in research leading to a discovery. The IPRs are scientific in scope and are determined through the national host-coordinating institution. One such example of a collaborative contract is the National Cancer Institutes' acquisitions and collection programs (Phase I 1955-1982 and Phase II 1986-1997).

The second type of bioprospecting in Africa is called a mass bioprospecting collaborative agreement (11). It is a collaborative arrangement between public and private research institutions and life science companies and environmental NGOs. A prime example of this type is the US federally funded International Collaborative Biodiversity Groups (ICBG) (16). The ICBG is funded by the National Institutes of Health, the Foreign Agriculture Service of the USDA, and holds current projects in both Nigeria and Madagascar. As second example of mass bioprospecting is the Gibex, a multi-partner project centered out of Rutgers University in New Jersey, USA. Gibex was originally patterned on both the ICBG and NCI models in organizational structure and project goals and objectives.

Organizations and institutions involved in mass bioprospecting usually see a number of benefits returned including technology transfer, trainings on high-tech equipment, milestone payments and monetary payments of royalties derived from the commercialization of a natural product. Because of the structural capacity and wide scope of mass bioprospecting projects they are most likely to be involved in long term public benefits, such as biodiversity conservation projects in the countries they operate. Intellectual property rights, under the ICBG and Gibex, are determined by the host-country research institution; however, scientific discoveries are protected by U.S. patent laws.

The third type of bioprospecting is that of the private-public agreement. Groups involved in this type of bioprospecting include public research institutions and private-for-profit companies. An example in Africa includes the agreement in South Africa between Phyto-pharm/ Unilever and the South African Council for Scientific and Industrial Research (CSIR). In this type of agreement, public institutions and individual groups are compensated in cash payments for their cooperation in supplying the natural product and associated traditional knowledge. Ideally, the terms of the intellectual property rights are determined by the agreement and signed by all parties prior to the research phase.

Issues and Challenges to Effective Access and Benefit-Sharing Agreements

Currently, there exist many challenges to the development and implementation of effective access and benefit-sharing in Africa. First, the majority of the biologically rich flora and fauna desired for natural products discovery reside in tropical and subtropical ecosystems, quite a distance from the research sites of Europe, Japan and the USA. This resource/research imbalance raises a number of issues concerning the proprietary use of natural resources and knowledge systems associated with their commercialization (17).

Second, sometimes the natural resources collected for bioprospecting are found growing across national boundaries. Furthermore, resources can be collected in one country and transported to another by those looking to circumvent the scope of access and benefit-sharing laws. These geographic challenges may result in misidentifying who is the rightful receipts of benefits and how to adequately address ownership of intellectual property rights (18).

Third, in some African countries there exist multiple documents that address access and benefits-sharing regulation. For example, a country may have a law regulating agricultural biogenetic resources while simultaneously have another separate law regulating biodiversity or research on animals or other mineral resources but not plants (see Table I) (19). These overlapping and sometimes conflicting laws may result particular delays for researchers and the return of benefits overall.

Fourth, laws directed for research on bioprospecting are at time over-restrictive and may cause unintentional consequences for associated researchers and businesses. For example, sometimes laws may cause problems for commercial growers as well as home gardeners, nursery managers, educators, researchers, food scientists and many others for which the law may not have been intended, but now may need to apply for “user” permits.

Table II. Examples of Different Types of Natural Products Discovery Partnerships Seen in Africa

<i>Structure of projects</i>	<i>Institutions involved</i>	<i>Example of project(s) in Africa</i>	<i>Example of countries involved</i>	<i>Potential benefits delivered</i>
Collaborative contract	Public research institutions	National Cancer Institute (NCI) - Natural products acquisitions and collection programs	Cameroon, the Central African Republic, Gabon, Ghana, Madagascar, and Tanzania, South Africa and Zimbabwe	Technology transfer Monetary payments
Mass bioprospecting collaborative agreement	Public research institutions Private research institutions Life science companies Environmental NGOs	International Cooperative Biodiversity Groups (ICBG) Global Institute for Bioexploration (Gibex)	Nigeria, Madagascar 17 countries across Africa	Biodiversity conservation Technology transfer Training Monetary payments
Private-public partnership	Public research institutions Life science companies	Phyto-pharm/ Unilever and the South African Council for Scientific and Industrial Research (CSIR)	South Africa	Monetary payments

Fifth, some countries in Africa lack the resources to adequately enforce access and benefit-sharing agreements. Although recent attempts have been made to ensure that research institutions adhere to the protocols set out in the access and benefit-sharing agreements, in many of these cases, governments lack the ability to ensure that benefits are being delivered to the rightful recipients, and moreover, if infractions are found, there is very little recourse for national governments to prosecute violators of the agreements.

Finally, laws governing biodiversity programs and associated benefit-sharing agreements are sometimes complex and difficult to understand. The potential for problems thus exists between parties who understand the terms of the agreements and those who do not. These misunderstandings may extend into

mistrust among institutions and governments and may development of over strict polices causing unnecessary restrictions even for host-country scientists to access material for basic research.

Conclusions: Opportunities for Benefit-sharing in the Landscape of Natural Products Discovery

The purpose of the CBD, one of the premier documents in environmental governance, was to lay the framework for the development of national strategies to negotiate access to genetic and biological resources in return for adequate benefit-sharing. These benefits were to be returned in a number of different forms including, technology transfer and financial returns from subsequent commercialization.

Advocates for the CBD maintain that in an ideal landscape, as long as consent is gained from host-country governments and agreements can be made so that participants receive sufficient benefits, then access should not only be granted, *but facilitated* (20). African countries that have since adopted access and benefit-sharing agreements at the national level seem to be receiving some beneficial returns (21). However, others note, that monetary benefits have been reportedly steady on the short-term; yet results of long-term benefits have been mixed (22).

Beyond economic benefits, the transfer of technology, trainings on new scientific techniques and shared use of equipment among research institutions also may also be incorporated into a benefit sharing or collaborative agreement (4, 20). Furthermore, techniques on quality control and assurance, which is a main feature of this American Chemical Society book, have shown to have indirect benefits by linking small-scale producers with larger regional and international markets (15).

In sum, the assurance of benefits back to host-countries must be in concert with the public and private research institutions and involved in the research and individuals who supplying both the natural product and associated traditional knowledge. Access and benefit-sharing agreements must be flexible enough to account for differences in social, political and cultural context of each research program and be comprehensive enough to cover the all interests of the groups participating. Property rights must be determined prior to research agreements and include prior consent by all parties active in the research.

Overall, it is vital that agreements include information sessions of the research so that consent is granted and accountability between partnering institutions is transparent. At this point, the best way to ensure that benefits are to return to host countries, and property rights are respected, is by following the Bonn Guidelines to adopt suitable and comprehensive ABS laws and practices at the national level.

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