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Review Article

Antibacterial activity in plants used as chewing sticks in Africa

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Abstract

In Africa, chewing sticks are the most common means of maintaining oral hygiene, and roots, stems and twigs of numerous plants are employed for this purpose. Chewing sticks are recommended for oral hygiene by the World Health Organization, and some of them, or their extracts, are also used in the ethnomedical treatment of oral infections. Primary screens have demonstrated that extracts from many chewing sticks have antimicrobial activity against a broad spectrum of microorganisms, including those commonly implicated in orofacial infections. Some chewing stick extracts have additional biological activities. Preparation, extraction and antimicrobial screening methodologies are largely unstandardized and bioactivity-guided fractionation has only been conducted on a few chewing stick extracts. It is therefore highly likely that many chewing sticks contain secondary metabolites with as yet unreported antimicrobial activity. Antimicrobial principles that have been identified include novel flavenoid compounds and alkaloids. Chewing sticks offer considerable and underexploited potential as sources of new antimicrobial backbones.

Introduction

The need for oral hygiene has been appreciated for a long time in virtually all cultures (1, 2). In modern Western cultures, oral hygiene is effected primarily through the use of toothbrushes of varying bristle hardness, most of them hand-held but an increasing percentage of which are electrically driven, with dentifrices. In the traditional societies of Asia and Africa, the mainstay of oral hygiene maintenance is the chewing stick made from the stem, roots or twigs of a plant. The preferred part or parts are cleaned with water to remove soil contaminants, cut to desired length and dried. They are then tied in bundles and sold in open market stalls (Fig. 1).

A typical chewing stick is hard but pliant, nonpoisonous and with a taste that is acceptable to the user. One end of the stick is held inside the mouth with a firm grip and chewed to give a fibrous fringe, which is used to brush the surfaces of the teeth and tongue. Complete devotion to this exercise for about 5 minutes will remove bacterial plaque, calculus, stains and food debris from the oral cavity. Avoidance of the habit of passively keeping a chewing stick in the mouth while neglecting to brush with it is important for the chewing stick to be of maximum benefit to the user (3).

The most important components of oral hygiene are the mechanical removal of plaque and the reduction of the number of oral bacteria through antimicrobial activity. Chewing sticks effect plaque removal mainly by a mechanical action (4-6), but many of the plants that are used as chewing sticks also demonstrate significant activity against oral bacteria, as well as fungi including *Candida albicans*. Results of clinical studies evaluating

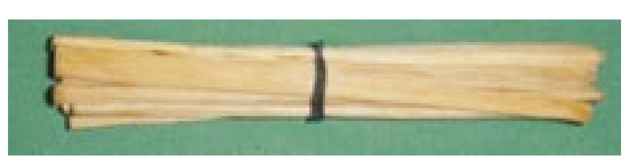


Fig. 1. A bundle of popular *Garcinia cola* chewing sticks as sold in a western Nigerian market.

the relative efficacy of chewing sticks compared to conventional toothbrushes in the control of plaque are somewhat inconclusive. Although many recent studies suggest that chewing sticks are more effective than the toothbrush in the maintenance of oral hygiene (7-10), Norton and Andy (11) reported more plaque formation and gingival bleeding in chewing stick users alone and Eid *et al.* (12) also recorded more gingival recession among chewing stick users than conventional toothbrush users. Despite these equivocal results, chewing sticks are still preferred to toothbrushes for oral hygiene maintenance in many African countries. They are traditional, inexpensive, available and simple to use, and the World Health Organization encourages their use (13).

Antimicrobial properties of chewing sticks

Microbes that are resistant to clinical antimicrobials are becoming more prevalent due to widespread selective pressure from antimicrobial use and misuse (14). Agents with novel mechanisms of action will be required to stay ahead of this trend and potential new sources for antimicrobials must therefore be investigated (15-17). Known antimicrobials from higher plants are structurally distinct from those produced by antibiotic-producing microorganisms, making plants a promising source of future antimicrobials. The total diversity of potential active compounds in higher plants is believed to exceed that so far created synthetically, but logistical and financial limitations preclude systematic cataloging of this diversity. Ethnomedical use by humans and animals has led to folkloric identification of botanical species with selective activity and tolerable safety, presenting modern investigators with appreciably greater odds of success upon scientific evaluation than would occur if target plants were randomly selected (18). In this vein, since only a select proportion of roots, stems and twigs are used for dental cleaning, it is rational to infer that the selected species could contain medically useful constituents. Such a premise is supported by the application of preparations of some chewing sticks as traditional medicines for oral disease (19, 20). Thus, several investigators have hypothesized that antimicrobial constituents may play a physiological role in maintaining oral hygiene and/or be capable of preventing and treating oral infections (2, 19, 21).

Conservative estimates suggest that plant parts from at least 170 different plant species are used as chewing sticks (22), mainly in Africa and Asia, but also in other parts of the world. Predictably, there is considerable geographical and cultural variation in plant choice, parts used (including twigs, stems and roots, with or without bark) and extended ethnomedical applications. Botanical validation is a key requirement for chewing stick research since different species may bear the same local name. “Miswak”, or some variation of that name (*e.g.*, “Meswak”, “Mswaki”), is used in many parts as a generic name for chewing sticks, as well as in specific reference to *Salvadora persica* in Sudan and the Middle East (5), but refers to *Xerophyta suaveolens* in parts of Tanzania (22). Conversely and more commonly, the same plant is given a different name by each ethnic group. Iwu (23) lists almost 20 indigenous names for *Zanthoxylum zanthoxyloides* chewing sticks from only a limited number of Nigerian and Ghanaian ethnic groups.

An estimated 25-60% of all higher plant extracts will possess some antimicrobial activity that can be detected *in vitro*, often conferred by simple phenolic compounds with little therapeutic potential (15). Compounds with minimum inhibitory concentrations (MICs) above 100 μg/ml are unlikely to be useful chemotherapeutic agents since such high concentrations are almost impossible to achieve *in vivo* and could in many cases be toxic (24). One view presupposes, on this basis, that only crude extracts demonstrating very high activity should be pursued as leads (24). In screening chewing sticks, and higher plants in general, for antimicrobial activity, there is a compelling counterargument for investigating highly active as well as less potent extracts, since plants are more likely to yield a novel bioactive chemical backbone that could be chemically optimized for therapeutics than a directly applicable agent (15). The presence and activity of target constituents in an extract are highly dependent on collection and processing techniques, factors that can only be optimized after the constituent is known. Also, diffusion methods are popular for initial screening, but may not quantitatively reflect true activity since physicochemical properties that govern diffusion are also determinants of inhibition zone size. Thus, primary screens are more important for evaluating antimicrobial spectra than specific activity.

Extracts from a number of African chewing sticks have been evaluated for antimicrobial activity (Table I). There are no standard methods or microbial test strains, and consequently there is considerable interstudy variation in the detection of activity. Most studies have employed cold aqueous, or at least polar solvent, extracts to mimic the in-use extraction conditions (22, 25, 26). Aqueous extracts are, however, less stable and can be harder to work with, so that some groups have resorted to studying extracts prepared with nonpolar solvents. In a comparative study, Akpata and Akinrimisi (27) found that, in most cases, better activity was seen in isobutyl alcohol extracts than in aqueous extracts, while benzene extracts showed little or no activity. Activity varied considerably with solvent and has also been shown to vary with plant preparation techniques, drying temperature and test conditions, none of which are standardized (27-29).

Interstudy variation may in some cases be due to differences in gross plant preparation prior to extraction. In many cases, the bark is stripped or scorched off the chewing stick, to provide a somewhat more aesthetic appearance (Fig. 1). However, some chewing sticks, notably those prepared from *Z. zanthoxyloides, Massularia acuminata* and *Vernonia amygdalina,* are used with the bark. Brown and Jacobs (7) and Khan *et al.* (22) found that the antimicrobial activity from several chewing sticks, including sticks from *Acacia, Vernonia,*

Table I: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Acacia senegal var. *senegal* (L.) Willd. (Mimosaceae)	Tanzania, Sudan	Gum arabic tree		Methanol extract of unpeeled twig	ND	Oral streptococci, *Actinomyces vicosus*, *Candida albicans*		7, 22
Anogeissus leiocarpus (DC.) Guill. et Perr. (Combretaceae)	Nigeria, Cote D'Ivoire		Black tongue, thrush	Aqueous, isobutyl and benzene extracts (root)	Heat-stable, molecular weight < 8,000	*Bacillus subtilis*, *Staphylococcus* spp., oral streptococci, other *Streptococcus* spp., *Enterococcus* spp., *Burkholderia cepacia*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium nucleatum*, *Peptostreptococcus prevotii*	Bactericidal; some toxicity following parenteral but not oral administration to mice	19, 25-27, 69
Azadirachta indica A. Juss. (Meliaceae)	Africa	Neem tree	Antimalarial, antihelminthic, skin infections		Flavenoids, terpenoids	Oral streptococci, *Actinomyces* spp., oral *Candida* spp.	Antimalarial	2, 7, 23
Bridelia ferruginea Benth. (Euphorbiaceae)	Nigeria	Shea butter tree	Mouth ulcers, poison antidote, diabetes	Aqueous stem and root extacts, aqueous, methanol, ethyl acetate and hexane extracts of leaf	Tannins, terpenoids, flavenoids	*Pseudomonas frutescens*, *B. subtilis*, *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*	Antidiabetic, uterotonic, astringent	23, 26, 42, 45
Butyrospermum paradoxum (Gaertn. f.) Hepper (Sapotaceae)	Nigeria			Aqueous and methanol extracts of stem and root bark	Heat-stable, molecular weight < 8,000	*Porphyromonas melaninogenicus* ATCC 33184, *Porphyromonas gingivalis* ATCC3377	Bactericidal, not toxic to mice	25, 70
Cassia sieberiana	West Africa	Senna	Infectious diseases, aphrodisiac, diuresis	Roots				2
Citrus aurantifolia	West Africa	Lime	Oral infections, dysentery	Twigs, root		Broad antibacterial spectrum against reference organisms		2, 71

Continuation

Table I Cont.: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Citrus sinensis	West Africa	Orange		Aqueous extract, branch twigs		*B. subtilis*, *Staphylococcus* spp., *Streptococcus* spp.		26
*Cnestis ferruginea***	Nigeria			Aqueous extract of fruit (stem showed no activity)	Afrormosin 7-*O*-β-D-galactoside (isoflavone galactoside)	*B. subtilis*, *Staphylococcus* spp., oral streptococci, *P. aeruginosa*, *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*, *Aspergillus niger*		2, 26, 72, 73
Combretum molle R. Br. ex G. Don. (Combretaceae)	Tanzania			Methanol extract of peeled twig	Punnicalagin (a tannin)	*Streptococcus mutans*, *A. vicosus*, *C. albicans*		22, 74
Diospyros lycioides Desf. (Ebenaceae)	Namibia			Aqueous extract, methanol extract	Binaphathalenone glycosides, diospyrosides, juglone derivatives (structures)	*S. mutans*, *Streptococcus sanguis* and *P. gingivalis*	ND	38, 39
Diospyros usambarensis F. White (Ebenaceae)	Tanzania			Methanol extract of peeled twig	ND	*A. vicosus*		22
Distemonanthus benthamianus	Nigeria			Aqueous extract	Heat-stable, molecular weight < 8,000	*P. melaninogenicus* ATCC 33184, *P. gingivalis* ATCC3377	Bactericidal, not toxic to mice	25
Eriosema psoraleoides (Lam.) G. Don (Papilionaceae)	Tanzania			Methanol extract of unpeeled twig	ND	*S. mutans*, *A. vicosus*, *C. albicans*		22
Eucalyptus camaldulensis Dehn. (Myrtaceae)	Tanzania			Methanol extract of unpeeled twig	ND	*S. mutans*, *Streptococcus* spp., *Staphylococcus aureus*, *A. vicosus*		22, 75
Euclea divinorum	Kenya					Inhibits the activity of virulence enzymes from *P. gingivalis*, *Prevotella intermedia* and *Treponema denticola*		50

Continuation

Table I Cont.: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Euclea natalensis A. DC. (Ebenaceae)	Tanzania			Methanol extract of unpeeled twig and root	ND	*S. mutans*, *A. vicosus*, *C. albicans*		22
Garcinia kola Heckel (Clusiaceae)	Nigeria	Bitter cola	Toothache, periodontitis, gingivitis, mouth ulcers, black tongue, thrush, skin diseases	Aqueous extract (root, stem and twigs)	Benzophenone derivatives, biflavenoids	*B. subtilis*, *P. frutescens*, *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *B. cepacia*, *P. aeruginosa*, *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*	Antiinflamatory, antidiabetic, antihepatotoxic	19, 26, 27, 69, 76, 77
Hibiscus micranthus L. (Malvaceae)	Tanzania			Methanol extract of unpeeled twig	ND	*S. mutans*, *A. vicosus*		22
Jatropha curcas L. (Euphorbiaceae)	Nigeria	Physic nut, termite plant, Barbados nut, fig nut	Toothache, teething, dental caries, mouth ulcers				Coagulant, anticoagulant; some plant parts are toxic to humans in high doses	23, 78
Jatropha gossypiifolia L. (Euphorbiaceae)	Nigeria		Black tongue, thrush		Macrocyclic diterpene, a jatrophenone		Some plant parts are toxic to humans in high doses	23, 79
Juglans regia L.	North Africa, Middle East	Walnut			Flavonoid and xanthanolide	*S. aureus*, *S. mutans*, *E. coli*, *P. aeruginosa*, *Helicobacter pylori*, *C. albicans*	Causes gingival pigmentation	53, 80, 81
Khaya senegalensis (Desr.) A. Juss. (Meliacaee)	Nigeria	African mahogany	Thrush, malaria		Limonnoids, tannins, terpenes		Also contains antimalarial components	23, 82
Lonchocarpus cyanescens	Nigeria	Indigo vine	Toothache, fever		Lupeol (a triterpene			83

Continuation

Table I Cont.: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Massularia acuminata (G. Don) Bullock ex Hoyle	Nigeria	Chewing stick tree	Toothache, acute ulcerative gingivitis, black tongue, dysentery	Aqueous, isobutyl alcohol and benzene extracts (stem)	Heat-stable, molecular weight < 8,000	*Streptococcus pyogenes*, *S. mutans*, *S. aureus*, *P. melaninogenicus* ATCC 33184, *P. gingivalis* ATCC3377	Bactericidal, not toxic to mice	19, 25, 27, 69, 84
Morinda lucida Benth. (Rubiaceae)	Nigeria	Brimstone tree	Malaria, jaundice, laxative	Aqueous extract (root)	Anthraquinones and anthraquinone glycosides	*B. subtilis*, *Staphylococcus* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*	Antihypertensive, molluscicidal, antiparasitic	23, 26
Nauclea latifolia Smith (Rubiaceace)	Nigeria	African peach	Black tongue, toothache, caries, oral infections, malaria	Aqueous extract	Heat-stable, molecular weight < 8,000	*P. melaninogenicus* ATCC 33184, *P. gingivalis* ATC C3377	Bactericidal, not toxic to mice	25, 85
Ocimum suave Willd. (Labiatae)	Tanzania			Essential oil (methanol extract of peeled twig showed no activity [22])	Essential oil rich in eugenol***	*S. aureus*, *B. subtilis*, *E. coli*, *Trichophyton mentagrophytes*		23, 30
Opilia celtidifolia (Guill. et Perr.) Walp. (Opilaceae)	Tanzania			Methanol extract of peeled twig	ND	No activity against tested strains		22
Paullinia pinnata L. (Sapindaceae)	Nigeria			Aqueous extract (root)		*B. subtilis*, *Staphylococcus* spp., *Streptococcus* spp., *F. nucleatum*, *P. prevotii*		26
Phyllanthus muellerianthus (O.Ktze) (Euphorbiaceae)	Nigeria			Aqueous extract (stem)		*B. subtilis*, *Staphylococcus* spp., *Streptococcus* spp., *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*		26
Psidium guajava L. (Myrataceae)	Nigeria	Guava	Diarrhea	Aqueous and ethanol extracts of bark	Leukocyanidins, gallic acid	*B. subtilis*, *Staphylococcus* spp., *F. nucleatum*, *P. prevotii*, *E. coli*, *Salmonella typhi*, *Shigella*, *Proteus mirabilis*		23, 26, 77 86, 87

Continuation

Table I Cont.: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Rhus natalensis	Kenya					Inhibits the activity of virulence enzymes from *P. gingivalis* *P. intermedia* and *T. denticola*		56
Salvadora persica L.	North Africa	Arak tree, toothbrush tree, miswak	Ancyclostomiasis	Aqueous extract	Salvadourea, salvadorine, cyanogenic glycoside and essential oil comprised of benzyl isothio-cyanate, fatty acids and their esters, terpenoids	S. *mutans*, *Streptococcus faecalis*, *C. albicans*	Antiplaque	31, 32, 88-90
Serindeia werneckei	Nigeria			Aqueous extract	Heat-stable, molecular weight < 8,000	*P. melaninogenicus* ATCC 33184, *P. gingivalis* ATCC3377, *Streptococcus mitis*, *S. sanguis*, *Streptococcus salivarius*, *S. mutans*, *C. albicans*	Bactericidal, not toxic to mice	25, 91, 92
Sorindea warnecki	Nigeria		Toothache, black tongue	Aqueous extract		*Staphylococcus* spp., *Enterococcus* spp., *B. cepacia*, *P. aeruginosa*		69
Terminalia glaucescens Planch. ex Benth. (Combretaceae)	Nigeria, Ghana		Toothache, acute ulcerative gingivitis, black tongue, thrush	Aqueous extract (root)	Heat-stable, molecular weight < 8,000	*B. subtilis*, *Staphylococcus* spp., oral streptococci, *Enterococcus* spp., *B. cepacia*, *P. aeruginosa*, *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*	Bactericidal, not toxic to mice	19, 25, 26, 28, 69

Continuation

Table I Cont.: African chewing sticks with antimicrobial properties.

Chewing stick	Country/region	Common name(s)*	Ethnomedical use(s) of chewing stick, if any (20, 23)	Extract tested	Chemical nature of active principle (s)	Antimicrobial activity	Biological activity	Ref.
Vernonia amygdalina	Nigeria	Bitter leaf	Black tongue, tonsillitis	Aqueous and isobutyl extract (stem - with bark, root)	Heat-stable, molecular weight < 8,000; phenolics, including flavenoids	Oral streptococci, *S. pyogenes*, *S. aureus*, *P. aeruginosa*, *P. melaninogenicus* ATCC 33184, *P. gingivalis* ATCC3377	Bactericidal, not toxic to mice	7, 19, 25, 27, 69, 92
Vitex doniana Sweet (Verbenaceae)	Nigeria	Black plum				*S. aureus*, *Enterococcus* spp., *B. cepacia*, *P. aeruginosa*		69
Vitex trifolia L. var. *simplicifolia* Cham.	Nigeria		Skin infections, toothache, fever					2
Vitellaria paradoxa Gaertn. f. (Sapotaceae)	Nigeria			Root		*B. subtilis*, *Staphylococcus* spp., oral streptococci, *E. coli*, *Citrobacter* spp., *Enterobacter* spp., *Prevotella* spp., *Porphyromonas* spp., *F. nucleatum*, *P. prevotii*		26
Xerophyta suaveolens (Greves) N.L. Menezes	Tanzania			Methanol extract of unpeeled stem	ND	No activity against tested strains		22
Zanthoxylum zanthoxyloides	West Africa	Fagara, toothache bark, candlewood	Toothache, acute ulcerative gingivitis, black tongue	Aqueous and isobutyl extracts of root	Heat-stable, molecular weight < 8,000; benzoic acid derivatives, berberine, canthin-6-one, chelerythrine and other alkaloids	Oral streptococci, *S. pyogenes*, *S. aureus*, *P. aeruginosa*, *C. albicans*, *Cryptococcus neoformans* and 7 filamentous fungi	Antisickling activity	7, 19, 24, 25, 27, 28, 37, 69, 93, 94

*Each African ethnic group has its own name(s) for chewing sticks and other medicinal plants used in that locality. As it would be impossible to list all these names, they have not been included in the table. Common names from larger ethnic groups can be found in the Handbook of African Medicinal Plants (23). **Technically not a chewing stick but employed ethnobotanically for dental hygiene. ***Eugenol is used as a temporary root canal filling in restorative dentistry. It can cause sensitization in some patients and could contribute to the development of aphthous stomatitis (95).

Zanthoxylum and *Azadirachta* spp., was entirely or predominantly localized in the bark, not an unexpected result since bark is often enriched in secondary metabolites. Almas and Al-Bagieh (29) reported that the combined bark and pulp of *S. persica* was more active than the bark or pulp alone.

The choice of test organism is principally influenced by the research objective, which could be to scientifically validate claims from traditional medicine or to identify novel antimicrobials for drug development. Clinical isolates such as *Streptococcus mutans*, or isolates from orofacial infections, including oral anaerobes (25, 26), are the rational choice in the former situation and well-characterized type cultures representative of different microbial phylogenies in the latter. Relatively few reports have screened for antifungal activity, an important area given the increased prevalence of oral candidiasis occasioned by the AIDS epidemic. Given the variation in methodologies employed in a small number of studies, it is possible that active compounds exist in plants which have not been detected.

Antimicrobial constituents from chewing sticks

Many chewing sticks owe their distinct flavors to essential oils, which often contain antimicrobial components (23, 30-32). Polyphenols, including flavenoids, are other commonly encountered antimicrobial constituents. These compounds possess direct antimicrobial activity, discourage bacterial overgrowth by chelating iron and other essential metal cations, and have also been shown to inhibit glucosyltransferase enzymes from *S. mutans*, an important cause of dental caries (33, 34). Several plant alkaloids also have antimicrobial activity and are likely sources of new molecular scaffolds (34). Other chewing sticks, for example *V. amygdalina* and *Opilia celtidifolia,* have high saponin contents, which may facilitate cleansing in addition to inhibiting or killing microbes.

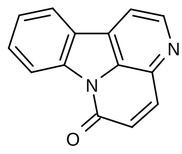
Bioactivity-guided fractionation represents the most rational means for identifying antimicrobial principles in chewing sticks and other higher plants. Although the technology for this approach and for chemical structure elucidation has improved tremendously in the last several decades, this work is being conducted in relatively few laboratories (as compared to chemical synthesis, for example). Thus, biochemical investigation of the many species known to demonstrate biological activity has been slow, particularly for African medicinal plants. Of the few chewing sticks for which biological activity has been reported in the literature, only about a third have been subjected to any bioactivity-guided fractionation and the proportion from which active chemical entities have been identified is minuscule.

Crude extract activity is often the result of an additive effect from many different antimicrobial components, or can be attributed to potentiation of one or more constituents by others. *S. persica* has been shown to contain the secondary metabolites salvadourea, salvadorine, cyanogenic glycoside and benzylisothiocyanate, in addition to miscellaneous tannins, saponins and inorganic chlorides (35, 36). A popular chewing stick used in western Nigeria is prepared from the roots of *Z. zanthoxyloides*, commonly known as Fagara or candlewood. This pungent chewing stick has a mild anesthetic property and has also been demonstrated to show broad-spectrum antimicrobial activity. Odebiyi and Sofowora (37) demonstrated that the activity was due to benzoic acid derivatives and the polycyclic alkaloids berberine (**1**), canthin-6-one (**2**) and chelerythrine (**3**). They reported that the benzoic acid derivatives are most active at pH 5, while the alkaloids showed best activity at pH 7.5, ensuring that some in-use activity will be present during application irrespective of oral pH (19).

Cai *et al*. (38) and Li *et al*. (39) conducted bioactivity-guided fractionation of the extract of the Namibian chewing stick *Diospyros lycioides*. They identified 8 antimicrobial compounds with MICs against oral *Streptococcus* and *Prevotella* spp. ranging between 19 and 1250 µg/ml. One of these, juglone (**4**) is a broad-spectrum naphthoquinone that has also been found in other chewing sticks (40). Four disopyroside compounds (*e.g.*, **5, 6**) were novel. Although none of the compounds was active enough for clinical use (MICs against target organisms generally above 1 µg/ml), it is easy to perceive how the combined activities of these agents could account for potent antimicrobial activity of the extract. As with many other bioactive compounds from the same plant, all the compounds are likely to be synthesized via the same biochemical pathway and structure-activity relationship studies could lead to the identification of more potent derivatives.

Other types of biological activity associated with chewing sticks

Mechanisms other than specific antimicrobial activity could be responsible for positive effects of chewing stick use. It has been postulated that the high fluoride content of some chewing sticks could account for their protective activity against dental caries (2, 41). *Bridelia ferruginea* stem and root are used as chewing sticks and extracts of the plant are also used in traditional medicine for treating oral infections. The bark extract has been shown to have broad-spectrum antimicrobial activity (42, 43), at least in part due to flavenoids, other polyphenols and tannins commonly found in other plants (33, 44). In addition, Orafidiya *et al*. (45) have demonstrated that a *B. ferruginea* gargle formulation, "Ogun-efu", traditionally employed in the treatment of oral infections in western Nigeria, is highly astringent and coagulates milk proteins *in vitro,* likely as a result of astringency conferred by tannins (46). The *B. ferruginea* extract also has radical-scavenging activity, inhibits xanthine oxidase and shows antiinflammatory activity, potentially through interference with TNF-α induction (44, 47).



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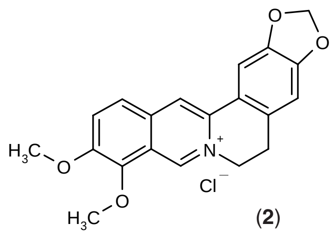

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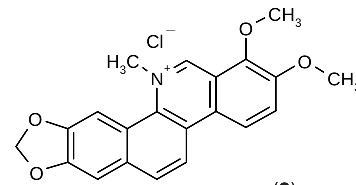

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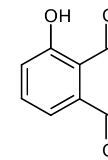

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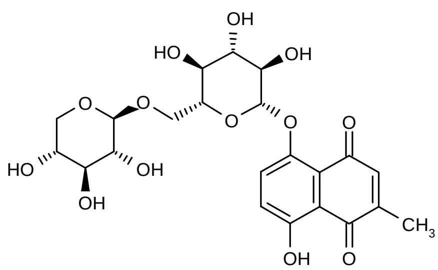

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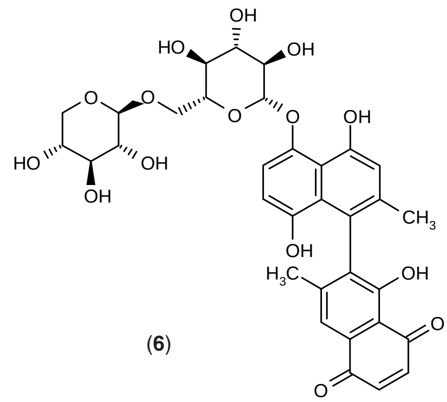

(6)

Almas has demonstrated that extracts of *S. persica* showed *in vitro* antiplaque activity on premolar discs that was comparable to chlorhexidine (48). Chewing stick constituents can also act by enhancing the activity of antimicrobial components in saliva, or by altering salivary pH (9, 49). Finally, chewing sticks and their constituents could act by interfering with specific microbial virulence factors or with coaggregation in oral microbial biofilms (2). Extracts of the Kenyan chewing sticks *Rhus natalensis* and *Euclea divinorum* were shown, to varying degrees, to be capable of inhibiting proteases from *Porphyromonas gingivalis*, *Prevotella intermedia* (formerly *Bacteroides intermedius*) and *Treponema denticola*. Tannic acid, gallic acid and its methyl ester, compounds that have been found in chewing sticks and other antimicrobial plant extracts, were demonstrated to be capable of producing this effect (50).

Routine use of chewing sticks may provide systemic as well as local effects. It has been postulated that dental plaque could serve as a reservoir of *Helicobacter pylori*, the etiological agent of many chronic gastric infections, particularly in African populations (51, 52). *Juglas regia*, a North African and Middle Eastern tooth cleaner, has been shown to have constituents active against *H. pylori* (53). While very little work has been done in the area, it is possible that chewing stick use could perturb this reservoir and prevent person-to-person transfer or individual reinfection. A number of the chewing sticks listed in Table I are also components of traditional medicines for malaria. *Anogeissus leiocarpus*, *Azadirachta indica*, *Cassia* spp., *Garcinia cola*, *Khaya* spp., *Nauclea latifolia* and *Terminalia glaucescens* have undergone bioassay-guided fractionation and subsequent identification of antiplasmodial components (54-60). It is feasible that chewing stick use by individuals in endemic areas could provide some prophylaxis against malaria and other infections. Leaves of *V. amygdalina* are used to prevent/treat gastrointestinal disorders, while the stem and root are the source of a popular chewing stick in Nigeria. The plant is also an ethnomedicine deliberately selected by chimpanzees in the wild. It has been shown to obtain its bitter taste from steroid glucosides, which also contribute antiamebic activity (61), another protective effect that could potentially be conferred during routine chewing stick use.

Potential for development as drugs or agents for dental hygiene

In a recent clinical trial comparing the immediate antimicrobial effect of a toothbrush and Miswak on cariogenic bacteria, Almas and Al-Zeid (62) reported a statistically significant reduction in *S. mutans* count with a 50% solution of Miswak. Although further research is needed, these results suggest potential for a mouth rinse that may serve as an adjunct in the control of smooth surface caries. Chewing stick extracts have already been incorporated into proprietary herbal formulations such as *A. indica* toothpaste and toothpowder (23, 63).

There may be as many as 500,000 plant species on earth and through the diversity of biochemical processes, an inestimable number of primary plant derivatives that could potentially be useful pharmaceutically. High standards for safety and efficacy must be met, and bioavailability and production must be optimized. Ethnomedical evaluation of safety, and to a lesser extent efficacy, of chewing sticks, and potentially their cogeneric species, offers a greater likelihood for the discovery of clinically useful compounds with acceptable therapeutic indices than chance alone (7).

Clues from ethnomedicine and preliminary data in the literature suggest that prospecting could be initiated with collection of plants for which activity has already been shown but constituents have not yet been isolated. Admittedly, there are distinct logistical challenges in seeking antimicrobials from chewing sticks and other plants. Chemical constituents and biological activity from plants are often not reproducible or depend on multiple external conditions, including season, climate and growth conditions. The technology for bioactivity-guided fractionation has advanced significantly but is still labor-intensive, often time-consuming and requires considerable skill and instrumentation (64). Other essential considerations include bioconservation and the need to ensure that, should drug development be successful, indigenous people whose historical use alerts science receive just compensation (65-67).

Purely synthetic strategies have heretofore been less successful in generating novel biologically active molecular scaffolds (17, 68). In antiinfective drug discovery from natural sources, there has been a tendency to follow serendipitous routes with concerted screens from similar sources until the specific source fails to provide more leads (15). Thus, most clinically applied antimicrobials have been isolated from microorganisms, specifically *Penicillium* and *Streptomyces* species. Chewing sticks could represent an underexplored potential source of bioactive agents with novel chemical structures and biological mechanisms. The potential for antimicrobial discovery justifies the challenge.

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