Pharmacognosy

158
Salvestrols—natural anticancer prodrugs in the diet

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Objectives An intriguing strategy for treating cancer is the design of prodrugs for specific activation by cytochromes P450 (CYPs) that are highly overexpressed in cancer cells, such as CYP1B1 (Murray et al 1997; Potter et al 2002a). In our laboratories this work has led to the discovery that the well-known dietary anticancer phytochemical, resveratrol, is metabolised by CYP1B1 *in vitro* to cytotoxic derivatives (Potter et al 2002b). Recently we have discovered several further natural dietary phytochemicals that are specifically activated into cytotoxic metabolites by CYPs overexpressed in cancer cells. These phytochemicals comprise diverse chemical types and have been given the collective name, Salvestrols.

Methods The selectivity and cytotoxicity of Salvestrol Q40 were determined using MCF10A (absence of CYP1B1) and MDA-MB-468 (presence of CYP1B1) cell lines. Cells were treated with a range of Salvestrol Q40 concentrations and the resulting percentage survival was measured spectrophotometrically using the MTT cytotoxicity assay. Activation of Salvestrol Q40 by CYP1B1 was confirmed by co-incubation with a selective CYP1B1 inhibitor.

Results Based on the concentration killing 50% of treated cells (IC_{50}), Salvestrol Q40 was approximately 20-times more toxic to the cells containing CYP1B1 (MDA-MB-468; IC_{50} 1.5 μM) than to cells lacking CYP1B1 (MCF10A; IC_{50} 20 μM) (Figure 1). The activation of Salvestrol Q40 in MDA-MB-468 cells was abolished by our specific CYP1B1 inhibitor, DMU713.

Conclusions Cytochromes P450, best known for their ability to biotransform pharmaceutical drugs and to activate carcinogens, might have originally conferred an evolutionary advantage on man by detoxifying noxious natural chemicals in their prehistoric diet. If so, then the reason why modern man-made chemicals, such as drugs and environmental pollutants, are metabolised by P450s may be the close resemblance of many of these to molecules in nature. Particularly important among natural dietary substrates of human P450s are the plant-based phytochemicals. The dietary phytochemicals, Salvestrols, are phytoalexins and are produced by plants as a result of challenge by pathogenic organisms. We have demonstrated here that Salvestrol Q40 can be activated to cytotoxic species by cells that express CYP1B1. We suggest not only that Salvestrols exemplify a new mechanism of dietary anticancer action, but that the depletion of Salvestrols in the modern diet, as a result of modern agricultural and horticultural practices, may be a major contributory factor to the increase of cancer incidence in human populations even though consumption of fruits and vegetables is increasing year by year.

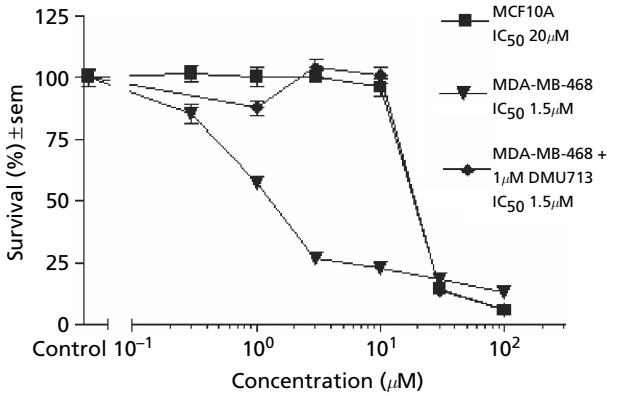

Figure 1 Bioactivation of Salvestrol Q40 in human cell lines. Results are average values of four determinations ± s.e.m.

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159
Distribution of ergosterol in different parts of *Lignosus rhinocerus*

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Objectives To examine the distribution of ergosterol in different parts of *Lignosus rhinocerus*. *L. rhinocerus* has been used in Malaysia as a traditional herbal medicine for the treatment of cough, cold, asthma and is also considered to have useful tonic properties (Burkill 1966). Ergosterol, which is a principal sterol of fungal cell membrane, may contribute to pharmacological activities such as reducing pain related to inflammation, antioxidant and antimicrobial activity (Yuan et al 2006). The quantitative determination of this compound is important

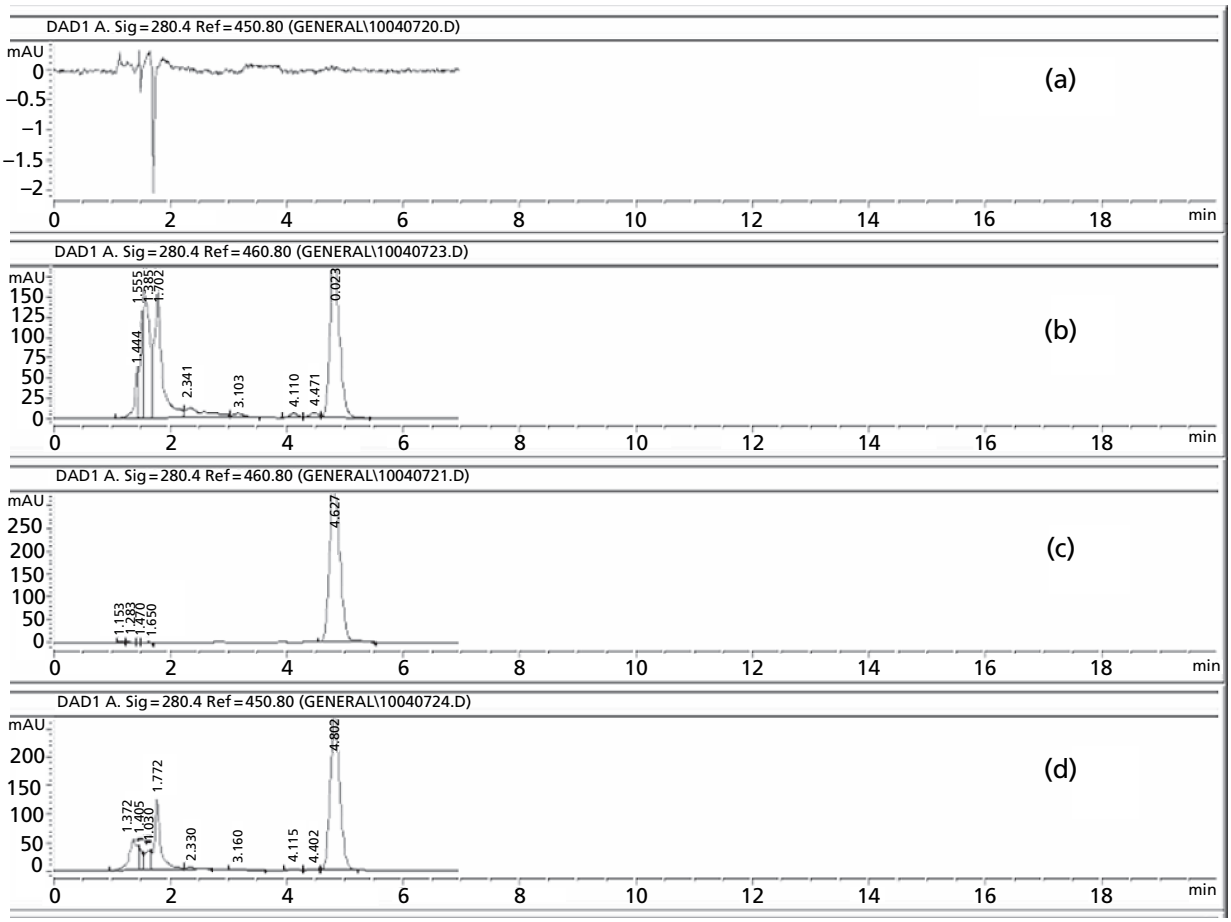

Figure 1 Chromatograms of (a) solvent solution, (b) sample solution, (c) standard solution and (d) spike solution for specificity result.

to ensure the safety and quality of traditional herbal medicines produced and used in Malaysia.

Methods A simple high-performance liquid chromatography method was developed for the determination of ergosterol in the caps, stalks and sclerotium of *L. rhinocerus*. The sample (1.0 g) was refluxed with a mixture of solution containing 0.5 M methanolic sodium hydroxide (2.0 mL), methanol (8.0 mL) and diethyl ether (6.0 mL) for 1 h. The resulting solution was transferred into evaporating dish and was evaporated to dryness. The residue was then reconstituted with methanol (2.0 mL), which was used as a sample solution. The analysis was performed on Hewlett Packard 1090 Chromatograph that connected with a Photo Diode Array detector and Chemstation software. A Column Symmetry Shield RP8 (Waters, 5.0 μm, 150 × 4.6 mm i.d) and 8%v/v water in methanol as mobile phase were used. The flow rate was 1.0 mL/min, the injection volume was 50.0 μL, the oven temperature was 40 °C and the wavelength was 280 nm. The run time of analysis was 8.0 min.

Results The identification of ergosterol was confirmed by comparing its retention time against a known standard and the quantification was determined by using a standard calibration curve. The HPLC method was validated for specificity (Figure 1), linearity (corr. coeff. = 0.9991), repeatability (% rsd = 2.53%), accuracy (range 95.95–104.15%), quantification limit (7.34 μg/mL) and detection limit (2.42 μg/mL). The content of ergosterol in caps, sclerotium, and stalks of *L. rhinocerus* was 0.43 mg/g, 0.30 mg/g and 0.12 mg/g, respectively. The results showed that the younger part of fungi (cap) tend to have higher content of ergosterol than the older parts (stalks and sclerotium).

Conclusions Ergosterol in *L. rhinocerus* may become a suitable marker compound for evaluating the quality of traditional herbal medicines and the HPLC method may form the basis of a routine programme of quality control testing.

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160

Stilbenes from *Cajanus cajan* engage apoptotic signals in acute human T-lymphoblastic leukaemia cell lines

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Objectives Development of multi-drug resistance remains a major barrier to successful cancer chemotherapy. Targeted induction of apoptosis via an extrinsic apoptotic pathway represents an unexploited therapeutic strategy to destroy cancer cells (Rowinsky 2005). Therefore, following our previous report about the *in-vitro* cytotoxicity of two prenylated stilbene compounds, longistylins A and C, from *Cajanus cajan* (L.) Millsp. leaves, in some solid tumour cell lines (Ashidi et al 2006), the present work reports the mechanism by which these compounds induce apoptosis in the human T-lymphoblastic leukaemia cell line CCRF-CEM and the multi-drug resistant subline CEM/ADR5000.

Methods For the anti-proliferative potential assay, the human T-lymphoblastic leukaemia cell line CCRF-CEM and the multi-drug resistant subline CEM/ADR5000 were cultured in RPMI 1640 with 10% FBS, 1% penicillin and streptomycin and 1% L-glutamine and maintained at 37 °C, 5% CO_2 and 95% relative humidity. They were subcultured every 2 days. Confluent cells were harvested, centrifuged, renewed with 10 ml of media and counted with a haemocytometer and seeded at a density of 1×10^4 cells/well in 96-well plates. The cells were incubated with or without longistylin A and C for 24 h at 37 °C and 5% CO_2. For the XTT assay, 50 μl of XTT and 1 μl of coupling reagents were added to each well and were further incubated for another 18 h. The absorbance was determined on a Bio-Rad multiplate reader at 490–655 nm. Propidium-iodide was used to detect the extent of apoptosis. In this case the cells were harvested after 24 h exposure to the compounds,

centrifuged and the supernatant discarded. The pellets were washed thrice with 1xPBS and were resuspended in 200 μl of Nicoletti reagent. The samples were incubated overnight at 4 °C in the dark before measurement on the FACScan flow cytometry to assess the impact of the compounds on cell cycle after 24 h incubation. The expression of the surface antigens (Fas receptors), mitochondria membrane potential ($\Delta\psi_m$) and the production of reactive oxygen species (ROS) were measured with flow cytometry. The regulation of Ying Yan 1(YY1), a key transcription factor of the extrinsic pathway, and catalase were evaluated by Western blot assay to further unravel the mechanism of action of longistylin A. In all the experiments DMSO control was used alongside a blank control.

Results The IC_{50} of the compounds tested in the XTT assay were 10.0 μM (longistylin A) and 10.2 μM (longistylin C) for the CCRF-CEM line and 10.0 μM (longistylin A) and 11.2 μM (longistylin C) for the CEM/ADR5000 line. Like most stilbenoids, longistylins A and C induced apoptosis of the leukaemia cells by arresting the G_2/M phase of the cell cycle, generated significant reactive oxygen species (ROS) and upregulated Fas receptors which consequently led to significant apoptosis. A dose-dependent downregulation of YY1 was observed with longistylin A over the concentration range 5.0–10.0 μM. There was also significant upregulation of anti-catalase protein over the same concentration range while the mitochondria membrane potential ($\Delta\psi_m$) remained uncompromised.

Conclusion Our findings lend support to the local use of *C. cajan* in Nigerian traditional medicine for prevention and therapy of cancer.

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161
Phenylphenalenones in elicited *Musa acuminata* (Musaceae)

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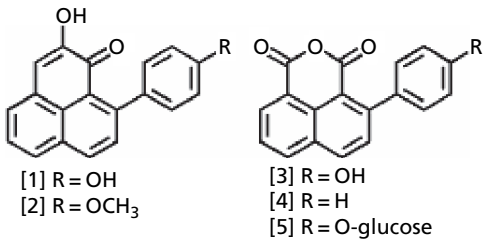
Objectives The overall objective was to study the ecological role of phenylphenalenones in banana plants *(Musa acuminata)*. Phenylphenalenones are natural products derived from the phenylpropanoid biosynthetic pathway. Literature review and our past works revealed the occurrence of these compounds in monocotyledon plant families such as Haemodoraceae, Pontederiaceae, Musaceae and Strelitziaceae. Interestingly in most of these plant families, phenylphenalenones were found in healthy plant, while in Musaceae the compounds were formed only after infection. According to a number of reports, phenylphenalenones may be involved in plant defence against fungal infection. However the real ecological roles of these compounds are still unclear. Treatment experiments of *M. acuminata* with bacteria, yeast and elicitation with chemical substances were conducted in order to establish the hypothesis of a more general role in plant defence.

Methods *In vitro-M. acuminata* plants were transferred to 100 ml MS liquid media 7 days before the experiments started. Treatments of *M. acuminata* were conducted by using bacteria (*Pseudomonas fluorescens*, 10^6 cell/ml, 3 weeks), yeast (*Sporobolomyces salmonicolor*, 10^6 cell/ml, 5 days) and jasmonic acid (100 μM, 7 days). Sterile water and 0.9% NaCl solution were used as control. The plants were harvested at increasing periods of time after treatment. Rhizomes and leaves were extracted separately with 96% EtOH. After evaporation, the EtOH extracts were partitioned with *n*-hexane, CH_2Cl_2 and EtOAc. Media were extracted with EtOAc. All the extracts were analysed by HPLC in comparison to control plants. Natural products were isolated and purified by means of chromatography. To identify chemical structures of the compounds, NMR and other spectroscopic techniques were used in comparison to authentic compounds.

Results HPLC analysis of CH_2Cl_2 fractions of rhizomes and EtOAc extracts from culture media of the treated plant and non-treated plants indicated quantitative differences in natural product patterns. Treated *M. acuminata* plants showed significantly higher levels of natural products. UV absorption suggested that the compounds are phenolics and very likely belong to phenylphenalenones group or related structures. Isolation, purification and spectroscopic analysis identified four known phenylphenalenones, hydroxyanigorufone [1], 2-hydroxy-9-(4′-methoxyphenyl)-phenalen-1-one [2], 2-(4′-hydroxyphenyl)-1,8-naphthalic anhydride [3] and 2-(phenyl)-1,8-naphthalic anhydride [4]. In addition, structure elucidation by NMR and mass spectrometry resulted in a new glucoside, 2-(4′–ß-glucosyloxyphenyl)-1,8-naphthalic anhydride [5], which is the first phenylphenalenone-type glucoside found in *M. acuminata*.

Conclusions Treatment of *Musa acuminata* with microorganisms and jasmonic acid resulted in a modified natural product pattern. The plants responded to elicitation by producing and accumulating high concentration of phenylphenalenones. Antifungal activity of these compounds indicated its function in *M. acuminate* as phytoalexin. The plant produced these compounds to defend themselves against



[1] R = OH
[2] R = OCH_3
[3] R = OH
[4] R = H
[5] R = O-glucose

fungal infection. These compounds have the potential to be used in pest control as antifungal compounds.

162
A critical assessment of some USP botanical monographs

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In the US, herbal remedies marketed under the guise of nutritional supplements escape the strict regulations mandated for conventional medicines. Setting up specifications for botanicals is problematic. Botanicals as raw materials are composite in nature. Moreover, the amount and quality of their active constituents can vary due to different cultivation and harvesting methods. Recently, USP took the initiative of setting up and publishing botanical monographs for popular plants used as nutritional supplements. The monographs could be used in the USP Dietary Supplements Verification Program (DSVP). However, inadequate compendial monographs could be misleading and confer doubtful legality on botanicals when used in the DSVP.

Objectives 1. To help specify the criteria of compendial analytical methods suitable for the quality control of botanicals. 2. To critically assess current USP monographs on botanicals in relation to available scientific information.

Results Examination of USP monographs on powders and extracts of 7 botanicals commonly used as dietary supplements (*Ginkgo*, chamomile, *Echinacea* species, valerian, Asian ginseng, St John's wort, ginger) was made. The USP monographs adopt HPLC methods for the evaluation of active constituents and marker compounds in botanicals. However, the majority of methods do not provide information on stability and do not assess the constituents responsible for the claimed activity. According to USP, *Ginkgo* is assayed for flavonol glycosides and terpene lactones. The method involves extraction of the flavonoids followed by their hydrolysis by hydrochloric acid. Separation of the aglycones quercetin, isorhamnetin and kaempferol by gradient HPLC is followed by recalculation of the original total flavonol glycoside contents using a molecular mass of 756.7. *Ginkgo biloba* contains at least eight flavonol glycosides. (Tang et al 2001). The bioavailability of flavonol glycosides is different from their corresponding aglycones. It is claimed that the activity of *Ginkgo* can be attributed to the intact flavonol glycosides and terpene lactones. If the flavonol glycosides of a sample of ginkgo were hydrolysed due to poor storage conditions, the sample will pass according to the USP monograph. For valerian, the USP monograph determines the volatile oil and valerenic acid contents neglecting other constituents. The sedative activity of valerian has been attributed to the unstable valepotriates and to a lesser extent the sesquiterpene constituents of the volatile oils. Poor quality valerian samples can still comply with the USP monograph. USP botanical assays are mainly proximate. For example, different *Echinacea* species are assayed for total phenolic acids and for dodecatetraenoic acid isobutylamides. Batches of *Echinacea* having different amounts of individual phenolic acids and alkylamides can fulfil the USP requirements. Similarly, powdered Asian ginseng is assayed for total ginsenosides, chamomile for bisabolan derivatives and ginger for gingerols and gingerdiones. The USP monographs for the majority of botanicals include methods for determining the marker compounds rather than the active constituents. Estimation of the analytical marker compounds is suitable for the identification purposes and negative markers are useful for determination of toxic components. However, determination of bioactive compounds is necessary in order to reflect the activity of the products.

Conclusions The majority of analytical methods specified in the USP monographs for conventional drugs are sophisticated. They determine the drug in presence of decomposition products, related substances and are stability indicating. However, for botanicals more attention is needed for the design of analytical methods that selectively determine the active constituents and their possible decomposition products.

Tang, Y., et al (2001) *Phytochemistry* **58**: 1251–1256

163
Screening of some Indian plants for antiplasmodial activity

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Objectives Malaria, a leading cause of death, continues to challenge the world (WHO 2003). Resistance of *Plasmodium falciparum* to chloroquine and some other antimalarial drugs continues to increase. In the present investigation, antiplasmodial activity was carried out on 27 Indian medicinal plants collected from forest in the locality of Bhubaneswar, Orissa, against chloroquine sensitive (3D7) & resistant (K1) strains of *P. falciparum*, aimed at identifying the most effective plants for further research.

Methods Extracts were prepared by extracting dried plant material with methanol and concentrating the extract using a rotary evaporator. In some cases extracts were prepared from more than one part of a particular species. Antiplasmodial activities were assessed using the parasite lactate dehydrogenase assay (Makler et al 1993) and chloroquine and artemether were used as positive controls.

Results and Discussion Out of 43 methanolic extracts analysed from 27 species, 11 (25.6%) extracts from 10 (37%) species possessed significant activity ($IC_{50} \leq 100$ μg/ml) against strain 3D7; of these 10 extracts belonging to 9 plants were active against strain K1. Especially, the following five species exhibited activities with low IC_{50} values of 2–31 μg/ml against both strains: *Anogeissus acuminata, Glycosmis pentaphylla, Phyllanthus reticulatus, Sphaeranthus indicus* and *Tarenna zeylanica* (Table 1). A literature search revealed that no phytochemical studies have been reported on *T. zeylanica*. Hence, the phytochemical investigation was carried out on the leaf extract of *T. zeylanica* and seven constituents were isolated viz. ZAL1, ZAL3A, ZAL3B, ZAL7, ZAL15 and ZAL16. The high activity of ZAL3B and ZAL7 against strain K1 ($IC_{50} = 0.72$ and 0.67 μg/ml, respectively; Table 1) confirms the activity of *T. zeylanica* seen in this study. The identification of these constituents is in progress and preliminary spectral analysis suggests ZAL3B and ZAL7 compounds are triterpenes.

Table 1 *In-vitro* activities of some extracts, pure constituents and control drugs against *P. falciparum*

S. No.	Species/Controls	Part	IC_{50} (μg/ml against *Pf* (mean ± SD))	
			Strain 3D7	Strain K1
1	*A. acuminata*	Bark	2.1 ± 0.8	3.2 ± 0.6
2	*G. pentaphylla*	Fruit	20.8 ± 1.7	23.8 ± 2.3
3	*P. reticulatus*	Leaf	12.4 ± 2.4	18.6 ± 1.9
4	*S. indicus*	Whole plant	25.2 ± 2.1	30.8 ± 2.9
5	*T. zeylanica*	Leaf	14.2 ± 2.7	31.4 ± 1.9
6	Constituents	ZAL3B	NT	0.72 ± 0.07
7		ZAL7	NT	0.67 ± 0.13
8	Positive controls	Chloroquine	0.02 ± 0.001	0.38 ± 0.03
		Artemether	0.008 ± 0.002	0.014 ± 0.01

Pf: P. falciparum; SD: standard deviation; NT: not tested; n = 3.

Makler, M. T., et al (1993) *Am. J. Trop. Med. Hyg*. **48**: 205–210
WHO (2003) World Health Organisation Fact Sheet No. 94: WHO information

164
Effect of cryptolepine and the analogue, 2,7 dibromocryptolepine on contractile activity of rat intestinal smooth muscle

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Objectives The indoloquinoline alkaloid, Cryptolepine, extracted from the West African climbing shrub *Cryptolepis sanguinolenta*, is reported to possess antibacterial and antiparastitic activity. In traditional African medicine decoctions of the plant are used to treat malaria, hypertension and intestinal disorders such as amoebiasis. An analogue of cryptolepine, 2,7 dibromocryptolepine, was found to have potent activity against chloroquine-resistant *Plasmodium falciparum* (Wright et al 2001); however, there is little information on the pharmacological actions of cryptolepine analogues. In these experiments our objective was to study possible antimuscarinic actions of cryptolepine on rat ileum and compared these with the analogue, 2,7 dibromocryptolepine, synthesised as described by Wright et al (2001).

Methods Segments of ileum derived from Hooded-Lister rats (250–350 g) were set up under 1 g tension in Krebs' solution (37°C, 95% O_2, 5% CO_2) containing 100 μM hexamethonium bromide to antagonise nicotinic effects of the agents tested. Contractions of longitudinal muscle to carbachol (0.1–100 μM) were recorded isometrically. Following incubation for 20 min with cryptolepine (10–30 μM) or 2,7 dibromocryptolepine (1–10 μM), concentration-response curves to carbachol were repeated. Cryptolepine and dibromocryptolepine were dissolved in distilled water: dilutions were freshly made daily.

Results Cryptolepine (10 μM) slightly potentiated the carbachol Emax, while cryptolepine (30 μM) reduced maximal contraction by 36 ± 9% ($P < 0.05$, N = 6). Increases in cryptolepine concentration reduced Emax further. 2,7 dibromocryptolepine caused greater reduction of contraction at lower concentrations: 2,7 dibromocryptolepine (3, 10 μM) reduced Emax by 20.0 ± 11% and 52 ± 12%, respectively ($P < 0.01$, N = 4). Both cryptolepine and 2,7 dibromocryptolepine shifted carbachol concentration–response curves rightwards. Since intestinal muscle possesses mainly M2 and M3 muscarinic receptors, actions of cryptolepine on the non-specific muscarinic agonist, oxotremorine (OXO, 0.01–100 μM), were compared with selective muscarinic antagonists, methoctramine (3 μM, an M2 antagonist) and 4-DAMP (N-(2-chloroethyl)-4-piperidinyl diphenylacetate) (0.01 μM, an M3 antagonist). Methoctramine shifted the OXO concentration-response curve rightwards, reducing Emax by 37 ± 7% (N = 4, $P < 0.05$): a combination of methoctramine (3 μM) and cryptolepine (30 mM) caused no further change in the concentration-response curve. 4-DAMP (0.01 μM) also caused a rightward shift in the OXO concentration-response curve and reduced Emax by 59 ± 11% (N = 4, $P < 0.05$): addition of cryptolepine (30 mM) caused no further effects on the concentration-response curve.

Conclusions The results show that both cryptolepine and its analogue cause concentration-related reduction in carbachol-induced contraction of ileal smooth muscle. No evidence of selective M2 or M3 muscarinic antagonism was observed since cryptolepine caused no further effect when either type of receptor was selectively antagonised. We conclude that cryptolepine and 2,7 dibromocryptolepine both relax intestinal smooth muscle but that this is unlikely to be due to any selective action at M2 or M3 muscarinic receptors.

Wright, C. W., et al (2001) *J. Med. Chem*. **44**: 3187

165
Post-marketing surveillance of some herbal remedies marketed in Egypt

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Objectives In Egypt, the majority of herbal products are registered as nutritional supplements. However, they carry medical claims and are used as medicines. The quality of such products is always questionable since they escape the strict control measures mandated for conventional medicines. This presentation examines the safety of some herbal products marketed in Egypt as a part of an ongoing project.

Methods 1. Categorization of selected herbal products according to their registration as nutritional supplements or medicines. 2. The safety of the herb ingredients declared on the label is assessed through literature survey. 3. Determination of levels of microbial and lead contaminants. 4. Assessment of the product inserts in relation to available evidence for any declared safety or efficacy.

Results Out of the 150 products examined, 95% are registered as nutritional supplements. The majority of these products include multiple ingredients and have claims of absolute safety and or efficacy with no scientific justification or evidence base. Two imported products, registered as medicines, contained 18 different plants and plant extracts. The label claimed to correct liver dysfunction in adults and children. Popular marketed products include ginseng, *Ginkgo biloba*, Echinacea, St John's wort (*Hypericum perforatum*), liquorice and chamomile. Of the 80 ginseng-containing products examined, three products are promoted for use in infants and children although ginseng preparations are usually recommended for adults above 40 years of age. Microbiological examinations of 19 products (3 batches each) were made. Seventy percent of products marketed as teas (ten products) showed bacterial counts above 10^7 CFU/g. Twenty two percent of herbal products marketed as dosage forms (nine products) showed bacterial and mould counts above accepted international limits. Lead contents of different batches of the products examined (17 products) were determined using atomic absorption. Seven of the products had lead contents above the accepted international limit. The highest contamination was found in two products imported from India and were registered as medicines. Assessment of the product inserts revealed the following: some products declared no quantities of ingredients; the plant species is omitted in most products; of 16 *Echinacea* products examined, only two products mentioned the species although constituents vary according to species used; exaggerated claims of safety and efficacy and no watch groups are specified in the majority of products; the majority of the inserts ignore well-known side effects and drug-herb interactions.

Conclusions Herbal remedies registered as nutritional supplements can pose a real heath threat. Herbal products carrying medical claims should apply evidence-based concepts for their claims of safety and efficacy.

166
Antioxidant activity of two medicinal plants from Botswana

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Objectives To assess and evaluate the antioxidant capacity of *Ozoroa paniculosa* Sond and *Colophospermum mopane* Kirk ex Benth. *Ozoroa paniculosa* is used by traditional healers in Eastern Botswana to treat gout and period pain whilst *Colophospermum mopane* is used for treatment of asthma. On the basis of this folkloric use, the two plants were assessed for their potential antioxidant activity. Antioxidants are radical scavengers, which protect the body against free radicals that cause pathological conditions such as ischaemia, anaemia, asthma, arthritis, inflammation, neurodegeneration and ageing process (Polterat 1997). Oxygen radicals induce oxidative stress that is believed to be a primary factor in various diseases as well as normal process of ageing (Aust et al 1993). Several studies have described the antioxidant properties of medicinal plants. Botswana hosts a rich diversity of plant species with therapeutic reputation. There is however, very little information regarding their phytochemical composition.

Methods Water and methanol extracts from roots of *Ozoroa paniculosa* (Anacardiaceae) and seeds of *Colophospermum mopane* (Caesalpiniaceae) were assessed for *in vitro* antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging assay adopted for spectrophotometry. Extracts that exhibited strong antioxidant capacity were further pursued to quantify their antioxidant capacity using the DPPH method proposed by Brand-Williams et al (1995). Briefly, a 0.1 mM solution of DPPH in methanol was prepared and 1.0 ml of this solution was added to 0.5 ml of samples in different concentrations. After 20 min, the absorbance was measured at 525 nm. The DPPH radical scavenging activity was calculated according to the following equation:

$$\text{DPPH radical Scavenging activity } (\%) = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 was the absorbance of the blank i.e no sample (DPPH solution only), and A_1 was the absorbance in the presence of the test extract.

Results Water and methanol extracts of *Ozoroa paniculosa* exhibited higher free radical scavenging effect than extracts of *Colophospermum mopane* at all tested concentrations. Above 50 μg/ml both extracts of *Ozoroa paniculosa* exhibited 91% scavenging activity, similar to control compounds epicatechin (92%) and L-ascorbic acid (91%). A 100–200 μg/ml methanol extract of *Colophospermum mopane* showed 80% scavenging activity whilst water extract exhibited 70% activity.

Conclusions Extracts from the two studied plants have strong antioxidant activity. These findings support the ethnomedical use of these two plants to promote good health.

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167
Echinacea liquid formulations – NMR spectroscopy and multivariate data analysis of fractions with CYP3A4 inhibitory activity

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Objectives Previously we have shown that commercially available Echinacea liquid formulations (ELFs) vary widely in their inhibitory activity on CYP3A4 (median inhibitory concentrations (IC_{50}) values of 12.71–1812 μg/ml) using our adapted supersome assay (Modarai et al 2007). Nuclear magnetic resonance (NMR) spectroscopy and principal component analysis (PCA) allows for the evaluation of differences between complex mixtures (Holmes et al 2006). To correlate such differences to CYP3A4 inhibition, we examined six commercially available ELFs including the least (1812 μg/ml) and most (12.71 μg/ml) active extracts.

Method Each extract was separated into ethanolic and water fractions, which were assayed for CYP3A4 inhibition. In tandem, 400 MHz ^{1}H NMR spectra were obtained in deuterated ethanol or deuterium oxide with an internal standard. Principal component analysis was conducted on the data using the Unscrambler software.

Results For all extracts the inhibitory activity resided predominantly in the ethanolic fraction. The IC_{50} value of the ethanolic fraction was five to ten-folds lower than the original extract (e.g. IC_{50} of Echinaforce 27 μg/ml, vs ethanolic fraction 2 μg/ml). ^{1}H NMR spectra of the ethanolic fractions showed a clear difference between the most and least active extracts. Greater inhibition was associated with the presence of peaks at 1–3 ppm and 6–8 ppm (visual inspection). Principal component analysis revealed good correlation between differences in ^{1}H spectra and IC_{50} values. Key contributors were identified at 0.875, 0.925, 1.275 and 1.325 ppm. 0.875 ppm and 0.925 ppm possibly belong to protons from CH3- while 1.275 ppm and 1.325 ppm may belong to -CH2-; both groups are present in alkylamides. The ethanolic fraction of Echinaforce was further fractionated by solid phase extraction (SPE) (C-18, water: ethanol, 10% step gradient). Two potent fractions were identified (IC_{50} values: 0.43–0.58 μg/ml). ^{1}H NMR analysis revealed peaks at ~7 ppm, which were unique to these fractions, and maybe indicative of protons attached to a phenol group – a potential inhibitory compound.

Conclusions Previously we had shown that alkylamides are associated with CYP3A4 inhibitory action (Modarai et al 2007). Whilst these effects are unlikely to be of clinical concern, it is essential to identify the core compounds responsible for these effects in order to further increase the safety window. The differences between the most and least inhibitory ELFs could be clearly observed using NMR spectroscopy, and can be partially attributed to alkylamides. Amongst other compounds, alkylamides (potent CYP3A4 inhibitors) are likely to be found in the two most active SPE fractions. This technique coupled to principal component analysis has great potential in identifying differences in composition between ELFs and as a tool for quality assessment.

Holmes, E., et al (2006) *Planta Med*. **72**: 771–785
Modarai, M., et al (2007) *J. Pharm. Pharmacol*. **59**: 567–573