Poster Session 1 – Pharmacognosy

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Potential cytotoxic activity from some Venezuelan marine organisms

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Considerable research has been carried out concerned with marine organism products and their biological activity. Some organisms (algae, ascidians, bryozoans, soft-corals and sponges) have shown interesting properties as anti-tumour, anti-bacterial, anti-parasite, etc., the main compounds isolated being sterols, phenols, peptides, lactones and terpenes (Simon *et al* 1999). Examples of the therapeutic potential of marine organisms are the discovery of citarabine, a synthetic derivative of a family of nucleotide analogues identified in the Caribbean sponge Cryptothethya; Ecteinascidin-743 (ET-743), an alkaloid discovered in the Caribbean tunicate Ecteinascidia turbinate, which shows evidence of activity against solid tumours (Jimeno *et al* 2002). In consequence, this research proposed to evaluate the potential of some Venezuelan marine species as sources of new cytotoxic compounds, using in-vitro assay systems designed to assess the growth of human cancer cell lines. A total of 24 extracts were analysed. Five marine invertebrates were collected from Playa Tocuchare, on the northeast coast of Venezuela. Every group of organisms was divided into two equivalent portions (A and B) and immediately fixed; A was preserved in 100% methanol (MeOH) and B in dichloromethane (DCM). The samples were continuously macerated, filtrated and evaporated at reduced pressure (45°C). The water on samples extracted with DCM, was freeze-dried. The Pharmacognosy Group of Universidad de Oriente, Núcleo de Anzoátegui, Venezuela, provided twelve additional samples. The cytotoxic activity was analysed in-vitro by the protein-staining sulphorhodamine B (SRB) assay for cell growth (Skehan *et al* 1990), using 3 human cancer cell lines (CORL-23; MCF7 and LS174T). A range of increasing dilutions was tested from a 20 mg mL^{-1} stock solution of each extract (n = 12). Plates were incubated at 37°C, 5% CO_2 atmosphere for a maximum exposure time of 72 h, and then left for three days recovery. Some outstanding growth inhibition was observed with IC50 values as low as 1.74 μg mL^{-1}. The findings suggest that the marine organisms covered could be very promising sources of novel compounds. However, to understand the mechanisms of action of the active extracts, it is necessary to isolate and purify the active constituents that may provide lead molecules for the development of synthetic drugs useful against cancer and also furnish new mechanisms of action in cancer chemotherapy.

Jimeno, *et al*. (2002) Marine derived anticancer compounds, a journey from the seaside to clinical trials. Memories of the 50th GA Annual Congress, Sep 8–12 2002, Barcelona-Spain, p. 360
Simon, *et al* (1999) *J. Nat. Prod.* **62**: 214–218
Skehan, *et al*. (1990) *J. Natl Cancer Inst.* **82**: 1107–1112

067
α-Amylase inhibition in Malaysian local plants

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Diabetes is the world's largest endocrine disease involving metabolic disorders of carbohydrate, fat and protein. According to WHO projections, the prevalence of diabetes is likely to increase by 35% (King *et al* 1998). Currently there is over 150 million diabetes world-wide and this is likely to increase to 300 million or more by the year 2025 (King *et al* 1998; Boyle *et al* 2001). Therefore, it is necessary to look for new solutions to manage this health problem. Although many drugs and interventions are available to manage diabetes, in most instances these are expensive for developing countries and have adverse effects. Therefore plants could provide useful aids in therapy if their activity could be validated.

In this study, an α-amylase inhibition in-vitro model was used to screen antidiabetic agent from Malaysian local plants. Six plants, namely *Anacardium occidentale*, *Lagerstroemia speciosa*, *Phyllanthus amarus*, *Averrhoea bilimbi*, *Pithecellobium jiringa* and *Parkia speciosa*, were separated into various parts, dried and powdered. The powdered materials were extracted in aqueous and organic solvents; hexane, dichloromethane and ethanol. Eighteen extracts in hexane and dichloromethane were tested in the bioassay. This enzyme is responsible for hydrolysing dietary starch into maltose which then breaks down to glucose prior to absorption. Inhibition of this enzyme should reduce the postprandial hyperglycaemia in diabetes.

The bioassay method was adopted and modified from Sigma Aldrich. Both control and plant extracts were added with starch solution and left to react with α-amylase solution in alkaline conditions at 25°C. The reaction was measured over 3 min. The generation of maltose was quantified by reaction of 3,5-dinitrosalicylic acid, which was reduced to 3-amino,5-nitrosalicylic acid. The colour changes from orangey yellow to orangey red and is detectable at λ = 540 nm.

Out of eighteen extracts, the hexane extract of one species (i.e. *Phyllanthus amarus* 1 mg mL^{-1}) gave a significant inhibitory effect with 21% inhibition. The extract was tested again preincubated with α-amylase solution for 5 min before being left to react with starch solution. The significant inhibitory dose was 0.01 mg mL^{-1} with 26% inhibition and 0.05 mg mL^{-1} gave 72.4% inhibition.

The hexane extract of *Phyllanthus amarus* was fractionated using bioassay guided techniques. The extract was applied on prep-TLC with mobile phase Hex–$CHCl_3$–MeOH (25:73:2) (full length of plate) and $CHCl_3$–MeOH (3:1) (half-length of the plate). Ten percent of the plate was sprayed with Anisaldehyde reagent and heated. Five fractions were collected (Rf ~0.82, 0.71, 0.35, 0.18, 0.06) and tested again with the bioassay. Fraction D (Rf ~ 0.18) showed the most activity with 79.9% inhibition. Work is currently in progress in an attempt to isolate novel α-amylase inhibitors from fraction D using Flash Chromatography.

Boyle, J. P., *et al*. (2001) *Diabetes Care* **24**: 1936–1940
King, H., *et al*. (1998) *Diabetes Care* **21**: 1414–1431

068
A study of Korean herbal medicines relevant to Alzheimer's disease

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This study was carried out as part of our search for new compounds against Alzheimer's disease. Seven herbal medicines used traditionally in Korea to treat memory-related disorders have been selected (Kim *et al* 1998) and examined. The results presented concern Korean medicinal plants: *Acorus calamus* root (AC), *Acorus gramineus* root (AG), *Bupleurum falcatum* root (BF), *Dioscorea batatas* root (DB), *Epimedium koreanum* herb (EK), *Pachyma hoelen* fungus (PH) and *Zizyphi jujuba* fruit (ZJ). The methanolic extracts of the plants were tested for inhibitory activity on human erythrocyte acetylcholineseterase (Perry *et al* 2000). The methanolic extracts of AC and EK showed relatively high anticholinesterase activity (Table 1) and were partitioned between water and dichloromethane (DCM). These fractions were also tested for enzyme inhibition activity and EK-DCM showed the most potent effect with an IC50 of 82 μg mL^{-1}. One active compound was isolated by solid phase elution using Silica gel, Sephadex LH20 and XAD′ as solid phase and MeOH:water of different proportions as eluant. The structure determination of the compound is in progress.

Table 1 Inhibition of human erythrocyte acetylcholinesterase by methanol extracts of Korean herbal medicines

Plants (200 μg mL^{-1})	Inhibition (%)
AC	53.7 ± 5.5
AG	7.2 ± 6.7
BF	24.8 ± 15.5
DB	7.6 ± 13.2
EK	47.5 ± 4.1
PH	27.8 ± 9.2
ZJ	2.4 ± 2.6

Data are means ± s.d., n = 3

This work was supported by postdoctoral fellowship program from Korea Science & Engineering Foundation (KOSEF).

Kim, C. M., *et al.* (1998) *Korean version of Chinese Materia Medica*. Jung Dam Press. Seoul, Korea, pp 2989, 3404, 2678, 4403, 2321, 1297
Perry, N. S. L., *et al.* (2000) *J. Pharm. Pharmacol.* **52**: 895–902

069
Novel inhibitors of acetylcholinesterase from *Salvia miltiorrhiza*

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Acetylcholinesterase (AChE, EC 3.1.1.7) inhibitors are the only drugs registered used to treat Alzheimer's disease. New AChE inhibitors may contribute to the development of new drugs and will also supply information to help understand the interaction between inhibitors and the enzyme, as well as the structure of enzyme. At present anticholinesterases are diverse in chemical structure with three major identifiable groups, alkaloids and derivatives, organophosphorus compounds and toxic peptides. The dried root of *Salvia miltiorrhiza* is called Danshen, and has been used in treatment of cerebrovascular disease for over one thousand years. Howes (2001) indicated that the methanol crude extract possessed anti-cholinesterase activity but no active chemical component was identified. The modified Ellman method (Ellman *et al* 1961) was used to guide the isolation of the active compounds. By using vacuum liquid chromatography with gradient elution (toluene 100%, toluene:dichloromethane (DCM) 1:1, DCM 100%, ethyl acetate100%, acetone 100% and methanol 100%), six fractions were obtained from the acetone extract. The first two fractions only indicated activity. The two active fractions were subjected to column chromatography on silica gel using DCM. Four sub-fractions were obtained, of which two showed activity. Preparative TLC and crystallisation further purified the two sub-fractions to yield four inhibitory compounds. Mass spectra and NMR identified the structures as the diterpenes dihydrotanshinone, cryptotanshinone, tanshinone I and tanshinone IIA (Mitsuko *et al* 1983), which are different from previously-identified acetylcholinesterase inhibitors. Using a range of concentrations in the Ellman test, the IC50 of dihydrotanshinone and cryptotanshinone were found to be 1.0×10^{-6} M and 1.4×10^{-5} M respectively, about 4 and 40 fold weaker than physostigmine (IC50 2.5×0^{-7} M). Using HPLC (ODS 2 5 μm/methanol:water 8:2; 1 mL min^{-1}; detection 254 nm; injection: 20 μL), dihydrotanshinone and cryptotanshinone were found to be the major inhibitory compounds in the extract; respectively 0.054% w/w and 0.23% w/w in the dried root, and 1.3% w/w and 4.5% w/w in the extracts. The clogP values of dihydrotanshinone, cryptotanshinone, tanshinone I and tanshinone IIA were calculated using Chemoffice 6.0 software as 2.433, 3.37, 4.804 and 5.781, respectively. This indicates that these compounds are able to cross the blood–brain barrier.

Acknowledgement: Royal Pharmaceutical Society of Great Britain, ORS Scheme and The Henry Lester Trust for financial support

Ellman, G. L., *et al.* (1961) *J. Biochem. Pharmacol.* **7**: 88–95
Howes, M. J. R. (2001) Ph.D. thesis. King's College, University of London
Mitsuko, O., *et al.* (1983) *Chem. Pharm. Bull.* **31**: 1670–1675

070
Continuous extraction of colchicine from plant cell cultures

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Plant cell suspension cultures are increasingly being seen as a new source of existing and novel pharmaceuticals (Tom *et al* 1991). Colchicine is a traditional drug used to relieve the symptoms of gout, and has been recognized for some time as an anti-tumour agent (Davis & Klein 1980). To optimise production and stabilise colchicine, we have examined its continuous extraction onto adsorbents (Nonionic polymeric Amberlite resins). The adsorbents (XAD4, XAD7 and XAD16) were added to liquid plant culture medium spiked with standard solutions of colchicine, and the amount of colchicine remaining in the medium after overnight incubation was measured by spectrophotometry. The extraction was highly efficient with >99% of medium colchicine taken up by the adsorbents. Extraction from the adsorbents by using 100% methanol for two times gave a high efficiency of recovery 90 ± 8.9% and 88 ± 7.1% (mean ± s.d., n = 5) for XAD4 and XAD16, respectively. The adsorption capacity of the beads was unaffected by the pH of the medium from pH5 to pH7. XAD4 and XAD16 were re-used up to 10 times with no loss in binding affinity or capacity, or extraction efficiency.

Binding affinity and binding capacity can be seen in the following table. All the adsorbents show high binding capacity. XAD4 and XAD16 show a much higher affinity for colchicine than XAD7. The results suggest that XAD4 and XAD16 may be used for the continuous in situ extraction and recovery of colchicine from suspension cultures of *Colchicum autumnale* and *Gloriosa superba*.

Table 1 The binding capacity and affinity of the adsorbents to colchicine

Adsorbent	Binding capacity (g g^{-1}) Qm	Affinity Kd
XAD-4	0.097	1.47E-05
XAD-7	0.078	3.85E-03
XAD-16	0.092	1.43E-05

Davis, P. J., Klein, A. E. (1980) *J. Chromatogr.* **188**: 280–284
Tom, R., *et al.* (1991) *J. Biotechnol.* **21**: 21–42

071
The antiprotozoal activity of some plants used traditionally in malaria-endemic areas

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In the search for new antimalarials from plant origin, this work investigates the antiplasmodial and antitrypanosomal properties of some plants selected because of their ethnopharmacological use in malaria-endemic areas.

Different solvent extracts of 7 plants collected from Nigeria, Zimbabwe and Argentina (Table 1) were screened for in-vitro antiplasmodial, antitrypanosomal and cytotoxic activity. Antiplasmodial activity was tested using 2 different strains of *Plasmodium falciparum* — 3D7 (chloroquine-sensitive) and K1 (chloroquine-resistant). The assay employed involved the use of erythrocytic stages of *P. falciparum* and radiolabelled nucleic acid precursor ^{3}H-hypoxanthine (Debenedetti *et al* 2002). The inhibition of the uptake of the ^{3}H-hypoxanthine by the parasite after exposure to the plant extracts gave a measure of antiplasmodial activity.

Antitrypanosomal activity was tested using blood stream trypomastigotes of *Trypanosoma brucei rhodesiense* STIB900 strain and the fluorimetric Alamar Blue Oxidation-Reduction assay (Raz *et al* 1997). The assay measured parasite viability and proliferation as a function of the reduction of the Alamar Blue dye in viable cells.

Cytotoxicity testing on mammalian KB cells was performed on all extracts in order to determine their selectivity of action. The Alamar Blue assay was also used (Page *et al* 1993).
In all the tests, a range of concentrations was used so that IC50 could be determined.
All tests were performed at least twice.

Table 1 Mean IC50 values (μg mL^{-1})

Extracts	Country collected	3D7	K1	Tryp.	KB
Sclerocarya birrea bark DCM	Zimbabwe	>30	32.0	13.6	160
Buddleia *globosa* lvs Pet.et	Argentina	24.2	15.3	6.3	71.6
Scoparia dulcis plant DCM	Nigeria	7.7	5.9	7.9	13.8
Ipomea involu-crata lvs MeOH	Nigeria	24.0	16.8	>60	>300
Paullinia pinnata lvs MeOH	Nigeria	>30	33.6	14.2	>300
Chromolaena *odorata* lvs MeOH	Nigeria	24.1	27.9	7.8	238
Acacia albida bark MeOH	Nigeria	>30	49.6	39.5	>300
Standard drugs		CQ 0.003	CQ 0.28	Pent. 0.0005	Pod. 0.02

From the results obtained, all 7 plants showed some level of antiplasmodial and antitrypanosomal activity. The good antiplasmodial activity displayed by *Buddleia globosa* was in agreement with previous studies on *P. falciparum* K1 strain only (Debenedetti *et al* 2002). The good antiprotozoal activity reported here for *Sclerocarya birrea*, *Ipomea involucrata* and *Chromolaena odorata* and the anti-trypanosomal activity reported for *B. globosa* have not been previously described.
Four of the plants have been selected for further investigation involving bioactivity guided fractionation and structural elucidation because they displayed promising antiprotozoal activity and a certain level of selectivity. The plants chosen were *S. birrea*, *B. globosa*, *I. involucrata* and *C. odorata*.

Debenedetti, S., *et al.* (2002) *J. Ethnopharmacol.* **80**: 163–166
Page, B., *et al.* (1993) *Int. J. Oncol.* **3**: 473
Raz, B., *et al.* (1997) *Acta Tropica* **68**: 139–147

072
Antimutagenic activity of different fractions of *Salvia leriifolia* extract

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Salvia leriifolia (Lamiaceae) is indigenous to the east and north east of Iran. Several reports on antioxidant activity of *Salvia* species have been presented (Tabatabaei Yazdi 1997). Therefore the antimutagenic activity of *Salvia leriifolia* was investigated using the Ames test (Maron & Ames 1983).
The plant was collected in mid spring 1999, and dried in shade. It was ground and defatted by maceration using petroleum-ether. Extraction was completed using ethanol (85%). The crude extract was tested by TLC to confirm the absence of histidine and probable contamination with aflatoxin before proceeding with the extraction. Crude extract of *Salvia leriifolia* was divided into four different fractions using liquid-liquid extraction procedure applying four solvents (dichloromethane, n- hexane, isobutanol and water). The other steps were as follows:
Genotypes of three standard tester strains (TA98, TA100 and TA102 of *Salmonella typhimurium*) were confirmed with study of five important factors that were: histidine requirement, rfa mutation, uvrB mutation, presence of R factor and pAQ1 plasmid.
The MIC of fractions was determined.
The antimutagenic effects of four different concentrations of each fraction (less than 0.1 MIC) were studied in the absence and presence of S9. The sample, tester strains and mutagen specific for each tester strain were incorporated into the top agar of antimutagenicity assay plates and incubated for 48 h at 37°C. The revertant colonies were counted and compared with control plates.
For general screening, it is recommended to use liver homogenates S9 fraction from rats induced with phenobarbitone. However, the enzyme activity was measured in terms of histidine revertants.
Analysis of the results showed that the aqueous and isobutanol fractions exhibited antimutagenic activity.

Maron, D. M., Ames, B. N. (1983) *Mutat. Res.* **113**: 173–215
Tabatabei Yazdi, F. (1997) Phytochemical and antioxidant activity of *Salvia leriifolia*. M.Sc. Thesis, Ferdowsi University, Mashhad, Iran, pp 70–86

073
Medicinal plants as potential new treatments for psoriasis

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Psoriasis, an inflammatory skin disease affecting ~2% of the population, is a complex disease whose exact cause remains unknown but is thought to involve the interaction of multiple gene abnormalities and various environmental factors (Stern 1997). The epidermal growth cycle in psoriasis has been accelerated to 3–6 days from the normal 28–30 days, resulting in an epidermis and stratum corneum much thicker than usual (www.psoriasis.org). This increased cell proliferation leads to a build-up of cells at the skin surface that is then shed as scale. The two main types of psoriasis are pustular and non-pustular (Christophers 2001), incorporating three recognised degrees of severity that are measured physically and dependent upon extent of skin involvement, lesional activity and frequency of relapses.
Of the many treatments currently available for use in psoriasis, no one therapy is adequate. Traditional Chinese Medicine or Ayurvedic medicine in the treatment of atopic dermatitis and psoriasis has recently received renewed attention and has proved successful in individual cases where conventional treatments have failed or are not as effective as was thought. Initial investigations focused on six herbs (plants A, B, C, D E1 and E2)* used in alternative medicine all of which possess anti-inflammatory activity useful for skin disorders but have not yet been fully characterised. The aim of preliminary studies was to prepare and test crude extracts from these plants for antiproliferative activity in a cell system.
Ground plant material underwent sequential extraction in the soxhlet apparatus with hexane, dichloromethane (DCM) and methanol (MeOH) followed by evaporation of solvent to dryness. A separate water (Aq) reflux was produced and freeze-dried. These crude extracts were incubated for 72 h with a human keratinocyte cell line (SVK-14) and assayed with sulforhodamine B. Data was converted to percentage cell growth and analysed using the PRISM GraphPad by means of the Fit spline/Lowness in order to obtain IC50s (Table 1).

Table 1 IC50 determinations for plants A–E (n = 16)

Extract solvent	Average IC50 (μg mL^{-1})					
	A	B	C	D	E1	E2
Hexane	>200	14.5	71.3	>200	>200	>200
DCM	70.4	4.5	132.5	32.5	40.0	>200
MeOH	>200	>200	>400	69.6	108.0	132.6
Aq	>200	>200	>200	19.7	>200	>200

Plants B and D stood out as being of particular interest. The IC50s determined for the hexane and DCM extracts of plant B were 14.52 and 4.50 μg mL^{-1}. For plant D, the DCM, methanolic and aqueous extracts gave IC50s of 32.50, 69.60 and 19.74 μg mL^{-1}, respectively.
Good to moderate keratinocyte antiproliferative activity has been shown in plants B and D, which may be beneficial as natural based treatments for use in psoriasis. Further studies using these active crude extracts will aim to assess potential cytotoxicity and hopefully isolate the constituents responsible for this activity.
* Scientific names withheld under confidentiality agreement (Stiefel Laboratories International).

Christophers, E. (2001) *Clin. Exp. Dermatol.* **26**: 314–320
Stern, R. S. (1997) *Lancet* **350**: 349–353
http://www.psoriasis.org/facts/psoriasis/

074
Antioxidant activity of local food plants consumed in Southern Italy

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Studies of the Mediterranean diet have led to an in-depth understanding of biochemical mechanisms and health-benefiting effects associated with certain groups of natural products. However, little is known about the role of local food products which are consumed on a less regular basis and about their contribution to health (Pieroni *et al* 2002a). In 2002, a consortium of six research groups, coordinated by the School of Pharmacy, University of London, has obtained funding from the European Commission to address this issue in a project entitled "Local Food – Nutraceuticals". It studies plants traditionally used in selected rural communities of Southern Italy, Greece and Southern Spain as dietary by-products having potential anti-oxidant, anti-diabetic and memory mediating activity.

The research approach adopted for the project combines ethnobotanical methods with modern molecular biology and pharmacology. This includes the identification of wild food plant species in the selected communities and the documentation of the gathering, processing, cooking as well as the consumption of the different local food plant species. Furthermore, some health promoting properties of the collected wild food plants are evaluated, using different anti-oxidant assays, like the free radical scavenging activity (FRSA) in the 1,1-diphenyl-2-picrylhydrazil radical (DPPH) assay (Pieroni *et al* 2002b) and the evaluation of the inhibition of the Xanthine-Oxidase (XO) (Chimanga *et al* 2001).

More than one-hundred botanical species used as food have been identified in Galliciano (an Italian Greek community, Reggio Calabria) and in Castelmezzano (an Italian community, Lucania) in Southern Italy. Many of the plants consumed, mostly gathered during the spring season, are considered to be healthy by the local users because of their bitterness, as for example young leaves of *Cichorium intybus*, *Chondrilla juncea* and *Lactuca viminea*, and bulbs of *Leopoldia comosa*. The collected plant material was extracted in 90% ethanol for 30 min under reflux. Most extracts showed moderate or little activity in the evaluation of the Free Radical Scavenging Activity (FRSA) in the DPPH assay, but it could be shown, that some plant extracts showed a significant activity. Some extracts also showed promising activity in the XO-system. The poster will summarize the findings on the evaluation of the anti-oxidant potential of these little-studied resources. Other partners focus on additional cell-based antioxidant assays and on a variety of mechanistic in-vitro/in-vivo models focusing on the CNS and the cardiovasculac system (e.g. free radical generation, membrane fluidity, antioxidative enzymes; vasorelaxant NO, vascular endothelial function (CVS) and the inhibition of selecteted transcription factors like NF/KappaB).

Chimanga, K., *et al*. (2001) *J. Pharm. Pharmacol*. **53**:757–761
Pieroni, A., Nebel, S., Quave, C., *et al*. (2002a) *J. Ethnopharmacol*. **81**:165–185
Pieroni, A., *et al*. (2002b) *Phytother. Res*. **16**: 467–473

075
Fractionation and characterisation of compounds inhibiting α-amylase and their potential as anti-diabetic remedies

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Diabetes mellitus, defined by the World Health Organisation as "a metabolic disorder of multiple aetiology characterised by chronic hyperglycaemia with disturbances of carbohydrates, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both" (WHO 1999). It is a serious worldwide healthcare issue with a current conservative estimate of 150 million people suspected of having diabetes with approximately two thirds living in developing countries.

In such countries the economic costs of using new anti-diabetic treatments have limited their use so the majority of populations in Asia, South America and Africa still rely upon traditional remedies, mostly of plant origin, which appear to improve glycaemic control. These remedies may have the potential to provide new anti-diabetic drugs in the future but need scientific investigation.

In this context, traditional Indian plants and their mechanisms of anti-diabetic action has provided a basis for ongoing research. The development of an α-amylase assay (Bawden *et al* 2002) and the testing of traditional plants prepared as hexane extracts provided two plants *Murraya koenigii* Spreng. (Rutaceae) and *Cyperus rotundus* L. (Cyperaceae), which showed significant α-amylase inhibitory activity. A soxhlet extraction using hexane, chloroform, methanol and water confirmed that the hexane extract of *Murraya koenigii* ($1\,mg\,mL^{-1}$) and the methanolic extract of *Cyperus rotundus* ($1\,mg\,mL^{-1}$) contained the highest levels of the active inhibitory compounds (44.79, 61.95% inhibition, respectively).

Activity-guided fractionation of the *Murraya koenigii* hexane extract using vacuum liquid chromatography (VLC) flash chromatography and thin-layer chromatography (TLC) eventually provided an active fraction (33), unfortunately not a pure compound.

Preparative high-pressure liquid chromatography (prep-HPLC) using a C18 "prep" column and a methanol-water-tetrahydrofuran solvent system to fractionate was used to obtain more (33) and then a pure compound with amylase inhibition. Also liquid chromatography mass spectrometry (LC-MS) to characterise the compounds at significant peaks has commenced and results so far are very encouraging.

Fractionation of the methanolic extract of *Cyperus rotundus* using similar techniques has provided fractions showing α-amylase inhibition but further investigations are planned to discover the source of α-amylase inhibitory activity.

This work is supported by a grant from Merck Research Laboratories, Rahway NJ.

Bawden, K., Quant, J., Raman, A. (2002) *J. Pharm. Pharmacol.* **54** (Sept. Suppl.): 580
Report of a WHO Consultation, Part 1: Diagnosis and Classification of Diabetes Mellitus, Geneva; 59p., WHO/NCD/NCS/99.2 (1999) (http://whqlibdoc.who.int/hq/1999/WHO_NCD_NCS_99.2.pdf)

076
The AINP Project – identifying plant based modulators of nuclear factor-κB (NF-κB)

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NF-κB is a transcription factor that, once activated, transcribes a suite of proinflammatory cytokines and pro-apoptotic proteins (Silverman & Maniatis 2001). Therefore any means of intervention and modulation of this and related transcription factors, could lead to clinically significant treatments in chronic inflammatory conditions (Yamamoto & Gaynor 2001). The Anti-Inflammatory Natural Products from Plants (AINP) project is designed to utilize the potential of plant drugs and natural products to specifically inhibit activated NF-κB (Bremner & Heinrich 2002).

The AINP project has been in existence since 2000, funded under the framework 5 programme of the EU, and consists of experts in cell molecular biology,

ethnobotany and phytochemistry. The primary screening group have now screened > 1000 extracts representing 225 species from 75 families. The bulk of the plant supply was from the Southern Mediterranean and other sources included collaborations within Kenya and Panama. The screening data from a suite of specifically targeted assays (inhibition of NF-κB, IL-6, IL-8, cell cycle analysis, anti-apoptosis) has been collated to produce a list of extracts with potent activity. Overall, 3.1% of the tested extracts have activity to specifically inhibit NF-κB without any associated toxicity. In addition, 2.8% of the extracts were found to be inhibitory against other inflammatory cytokines, independent of any activity against NF-κB. *Bupleurum fruticosum* is an example of a Sardinian plant that displayed NF-κB inhibitory activity from an ethyl acetate extract. This extract later yielded two phenyl propanoids (PP1 and PP2), each having a minimum inhibitory concentration (20% of the positive control) of 50 μM. Phytochemical analysis of other active plants has now resulted in > 50 compounds being isolated and a small percentage (5–8%) have shown good inhibition against NF-κB. Indeed, one compound has an IC50 value of 2.8 μM. Confidentiality agreements currently preclude the identification of this compound and the source plant.

Bremner, P., Heinrich, M. (2002) *J. Pharm. Pharmacol.* **54**: 453–472
Silverman, N., Maniatis, T. (2001) *Genes Devel.* **15**: 2321–2342
Yamamoto, Y., Gaynor, R. B. (2001) *J. Clin. Invest.* **107**: 135–142

077
Chinese herbal patent medicines in the UK

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Medicinal Chinese herbs are generally available as raw herbs or formulated as Chinese herbal patent medicines, prepared from a mixture of herbs. Patent medicines are manufactured from traditional Chinese herbal formulas and are usually presented in tablet or pill form. Any number of different manufacturers can produce any patent medicine. While it is generally understood that for each traditional Chinese herbal formula the same ingredients will be adhered to, in practice different manufacturers might choose to add or remove ingredients, herbal, mineral or excipient, and then market the resulting product under the same generic name.

Raw herb prescriptions are usually supplied by Chinese herbal practitioners while Chinese herbal patent medicines are more easily available and can be obtained from high street herb shops, the internet, registered and non-registered practitioners, and from some supermarkets. Chinese herbal medicines are not subject to current medicines licensing legislation and consequently there is little control over the quality of these preparations. However, problems of nephrotoxicity resulting from Chinese medicines containing species of *Aristolochia* have highlighted the need for the effective quality control of Chinese herbal medicines (Barnes *et al* 2002).

In this study, a popular Chinese herbal patent medicine, Gui Pi, was selected. This remedy was chosen, following investigation, as it was shown that it is widely used throughout the UK and is available from most major UK manufacturers or distributors.

Samples of Gui Pi obtained from 8 different suppliers revealed considerable variations between products on visual inspection. Both the appearance of the medicines, and the information given on the product labels varied.

A total of 21 different plant species and 1 fungus were named on the labels of the 8 products but the number on any one product ranged between 2 and 13. The names of the herbs were stated on 7 of the 8 products but the relative amounts present were variable and were specified on only 5 of the sample medicines. The most commonly used herbs, each named on 7 of the preparations, were *Angelica sinensis*, *Atractylodes macrocephala*, *Euphoria longan* and *Glycyrhiza uralensis*. The fungus *Poria cocos* was also stated as a constituent of 7 of the products. Three herbs, *Astragalus membranacea*, *Polygalae tenuifolia* and *Zizyphus jujuba* were listed on the labels of 6 products while Ginseng (botanical name not stated) was found on 5. Information given on product indications and recommended dosage was also inconsistent.

This study has shown that preparations of Gui Pi are highly variable in important respects that could have implications for their efficacy and safety. Although only one herbal product is discussed here, this problem is common to many Chinese herbal patent medicines. Further work is clearly needed in order to assess the significance of these results with respect to the efficacy and safety of such products.

Barnes, J. *et al*. (2002) *Herbal medicines*. 2nd Edition, Pharmaceutical Press, London, p. 9

078
Microscopy of *Sanicula europea*

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Sanicula europea (Umbellifereae), commonly known as Sanicle is a perennial herb found throughout the British Isles, especially in woodlands. In medieval times Sanicle, (from the Latin Sano = I heal) was highly regarded for its value in wound healing (Dawson 1934), although its use for this purpose has declined in more recent times. Currently, we are investigating the phytochemistry and wound-healing properties of Sanicle but were unable to find a microscopical description needed to ensure the authenticity of the commercially available cut herb. To address this requirement we have determined the characteristic microscopical features of Sanicle using locally available plants.

In order to view the microscopical features of the dried herb, chloral hydrate was used as a clearing agent. There were several characteristic features. The leaf epidermal cells are polygonal in shape with wavy anticlinal walls (more wavy on lower epidermis) and have a striated cuticle; stomata are of the anisocytic type and are found mainly on the lower epidermis. The transverse section of the leaf is dorsiventral and a double palisade layer is present. Cluster crystals of calcium oxalate are found scattered throughout the mesophyl with occasional microsphenoids and rarely, acicular crystals are also found. Covering trichomes are present in the form of unicellular or multicellular (3–4 celled) trichomes with a smooth surface, and less commonly large, flat, ribbon-like trichomes which are often twisted are found. Sessile glandular trichomes with unicellular heads are also seen. An unusual feature of Sanicle is the midrib of the leaf which protrudes above the upper epidermis.

A preliminary examination of the flower parts revealed several characteristic features. The epidermal cells of the corolla are papillose. The fibrous layer of the anther shows a beaded appearance and the stamens contain tiny microrosettes of calcium oxalate. Pollen grains are relatively large, ovoid to oblong with a smooth exine and two pores. The fruit walls each contain 5 vittae and the seeds have hooked, lignified trichomes.

Dawson, W. R. (1934) *A leechbook or collection of medical recipes of the fifteenth century*. Macmillan and Co., London, p. 207

079
Synthesis, antiplasmodial and cytotoxic activities of some chlorinated cryptolepine analogues and 11-aminocryptolepine

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Cryptolepine is an indoloquinoline alkaloid found in the roots of the West African climbing shrub, *Cryptolepis sanguinolenta*, a species used traditionally for the treatment of malaria. Cryptolepine has potent in-vitro antiplasmodial activity and analogues of this alkaloid are of interest as leads towards new antimalarial drugs.

Several analogues have been prepared that have enhanced antiplasmodial activities as well as antimalarial activity in-vivo (Wright *et al* 2001). As part of our continuing work on cryptolepine we have synthesized a number of chlorinated analogues as well as 11-aminocryptolepine in order to study structure-activity relationships. The 1,2-dichloro-, 2-chloro-, 3-chloro- and 4-chloro-analogues were prepared from chloroisatin derivatives and *O*, *N*-diacetylindoxyl using methodology based on that of Holt & Petrow (1947) as previously reported (Wright et al 2001). Anthranilic acid was used as the starting point for the synthesis of 11-chlorocryptolepine, and the latter was converted into the 11-amino analogue by reacting with sodium amide at room temperature.

When assessed for antiplasmodial activities against *Plasmodium falciparum* (multi-drug resistant strain K1) using the parasite lactate dehydrogenase assay, the most potent compound was 1,2-dichlorocryptolepine (IC50 = 0.09 μM), while cryptolepine was about 4-fold less potent (IC50 = 0.44 μM). Activity progressively decreased with substitution in the 2, 3 and 4 positions (IC50 = 0.17, 0.45 and 4.7 μM, respectively). Although 11-chlorocryptolepine has antiplasmodial activity comparable with that of cryptolepine (IC50 = 0.24 μM), the 11-amino- analogue was at least 4-fold less active (IC50 > 1 μM). However, the use of sodium amide to substitute a halogen for an amino group may afford a useful route for the synthesis of other aminocryptolepine analogues.

Overall, the results show that the position of chloro-substitution greatly affects antiplasmodial activity. When assessed for in-vitro cytotoxicity using MAC15A (mouse adenocarcinoma) cells the 1,2-dichloro, 2-, 3- and 4-chloro-analogues displayed moderate toxicities (IC50 = 1.91, 2.24, 1.75 and 3.54 μM, respectively). The position of substitution did not greatly affect cytotoxic activity showing that antiplasmodial activity does not parallel cytotoxicity in this series of compounds. The best selectivity was shown by 1,2-dichlocryptolepine (cytotoxic/antiplasmodial ratio = 22) and this compound is worthy of assessment for in-vivo antimalarial activity.

Acknowledgement: Chlorinated isatin derivatives were generously provided by Dr Brian Goodwin and Professor Vivette Glover, Imperial College, London.

Holt, S. J., Petrow, V. (1947) *J. Chem. Soc.* 607–611
Wright, C. W., *et al.* (2001) *J. Med. Chem.* **44**: 3187–3194

080
The AINP project: screening of medicinal plants for activity against the transcription factor: NF-κB

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Natural products which modulate NF-κB and related transcription factors could lead to clinically significant treatments in chronic inflammatory conditions (Bremner *et al* this meeting; Bremner & Heinrich 2002).

The AINP consortium has now screened 255 species from 76 families. Each species may also yield various botanical drugs and this resulted in 331 plant drugs tested. Each drug was extracted with at least three solvents resulting in 1103 extracts screened so far. The species belonging to fifteen families ($\geqslant$ 4 species tested) were selected for analysis to determine trends in activity to inhibit NF-κB. The families selected were (number of species investigated in parenthesis): Asteraceae (28), Lamiaceae (24), Fabaceae, s.l. (20), Apiaceae (10), Euphorbiaceae (10), Rosaceae (10), Scrophulariaceae (8), Ranunculaceae (6), Brassicaceae (5), Chenopodiaceae (5), Solanaceae (5), Verbenaceae (5), Rutaceae (4), Liliaceae (4) and Caryophyllaceae (4).

The family with the highest percentage of species providing extracts that inhibited NF-κB are the Chenopodiaceae (4 out of 5), the Asteraceae (68%) and Verbenaceae (3 out of 5). The Asteraceae provide the highest overall number of inhibitory plants. However, of the compound classes currently known to inhibit NF-κB, many occur within the Asteraceae and include sesquiterpene lactones (parthenolide) and flavonoids. Therefore, the presence of such compounds would be an exclusion criterion to avoid the replication of identifying known inhibitory compounds. The only family under discussion here not to produce any inhibition of NF-κB were the Solanaceae, an interesting result in view of the inhibitor we had identified from a species of *Solanum* (Heinrich *et al* 2001). It is also noteworthy, that the Chenopodiaceae have not yet been evaluated in detail for novel NF-κB inhibitors and this family may thus be of particular interest for further studies.

Helichrysum stoechas (Asteraceae), for example, was collected from Southern Spain and an ethyl acetate extract (100 μg mL^{-1}) displayed potent inhibitory activity (treatment value was 0.84% of the positive control) in the IL-6/luciferase firefly assay (Bork *et al* 1997). In this case an acetophenone was isolated following bioassay-guided fractionation and was found to be an active inhibitor of NF-κB in the luciferase assay (366 μM, gave a value $<$ 20% of the positive control). Recently, this compound has also been shown to have effective anti-inflammatory activity using an in-vivo model (Sala *et al* 2001).

Bork, P. M., *et al.* (1997) *FEBS Lett.* **402**: 85–90
Bremner, P., Heinrich, M. (2002) *J. Pharm. Pharmacol.* **54**: 453–472
Heinrich, M., *et al.* (2001) *Planta Med.* **67**: 156–157
Sala, A., *et al.* (2001) *J. Nat. Prod.* **64**: 1360–1362