HB, logP and logS correctly assigned 11 of the 12 'good' enhancers (92%). Twelve of the sixty-one 'poor' enhancers (20%) were incorrectly assigned but 3 could be considered marginal (ER > 8). It is recognised that the methodology has been applied to the enhancement effect on a single drug. The effectiveness of an enhancer may vary with the physico-chemical properties of the drug as measured by its logP value, and the state of thinking regarding the enhancement of percutaneous absorption has recently been reviewed (Williams & Barry 2004). We are currently examining how far our approach can be extended to provide a general prediction of activity. The success of this simple approach in identifying potent enhancers suggests that it is sufficiently reliable to identify potential transdermal enhancers for in vitro screening.

Magnusson, B. M. et al (2004) *Pharm. Res.* **21**: 1047–1054
Williams, A. C., Barry, B. W. (2004) *Adv. Drug Delivery Rev.* **56**: 603–618

Poster Session 2 – Pharmacognosy

139

Studies on the anti-psoriatic activity of gossypol and its derivatives followed by pre-formulation and formulation studies of gossypol into a topical dosage form

K. Dodou, R. J. Anderson, W. J. Lough, D. A. P. Small[1], and P. W. Groundwater

Sunderland Pharmacy School, University of Sunderland, Wharncliffe Street, Sunderland SR1 3SD and [1]Stiefel International R&D, Whitebrook Park, 68 Lower Cookham Road, Maidenhead, Berkshire SL6 8XY, UK.
E-mail: kalliopi.dodou@sunderland.ac.uk

Gossypol, a natural anti-inflammatory compound, has been studied extensively since the discovery of its in vivo male antifertility activity in the late 1960s and has since shown anti-viral, anti-parasitic and anti-tumour activity. Psoriasis is a multifactorial skin condition characterised by benign keratinocyte hyper-proliferation, skin inflammation, defective keratinisation, altered dermal vasculature and insufficient anti-oxidant activity. In this study the in vitro anti-psoriatic activity of gossypol and its derivatives was evaluated using an anti-proliferative assay and an anti-oxidant assay (Dodou et al 2005). In the anti-proliferative study, the sensitivity of an HPV-16 keratinocyte cell line to each compound was determined using an MTT viability assay. The compounds that showed increased inhibition against keratinocyte proliferation were subsequently tested for their anti-oxidant effect against iron/ascorbate dependent lipid peroxidation, using the thiobarbituric acid (TBA) test. Racemic gossypol ($GI_{50} = 5.4 \pm 0.03\ \mu M$) and its enantiomers were the most potent compounds against the proliferation of HPV-16 keratinocytes, followed by the half-Schiff's bases ($GI_{50} = 15$–$50\ \mu M$), racemic gossypolone ($GI_{50} = 47.3\ \mu M$) and the bis-Schiff's bases ($GI_{50} > 100\ \mu M$). A comparison was made with the data from the MTT assays on HPV-16 keratinocyte cell lines using methotrexate ($GI_{50} = 148\ \mu M$) and dithranol ($GI_{50} = 0.58\ \mu M$). All tested compounds showed similar anti-oxidant activity ($IC_{50} \approx 17\ \mu M$) and were more potent than the positive control propyl gallate ($IC_{70} = 100\ \mu M$). Pre-formulation and formulation studies were then conducted on racemic gossypol, which was the most active compound according to the biological assays. The pre-formulation studies included saturation solubility in hydrophilic and lipophilic vehicles, compatibility with excipients, partition co-efficient over pH range 2–8, and physicochemical stability in solution under extreme light, heat, acidic, basic and oxidising conditions (Dodou 2004). Gossypol showed better solubility in lipophilic vehicles ($> 3\ mg\ mL^{-1}$) than hydrophilic ones ($< 1.5\ mg\ mL^{-1}$) and its water solubility was $0.075\ mg\ mL^{-1}$. It was compatible on storage with commonly used excipients in tetrahydrofuran (THF) solution at 25°C for 6 days. It was found to be stable in acidic, basic and high temperature conditions but was prone to oxidative and photolytic degradation. Its logP value was around 5–6 at pH < 5. The oil in water (o/w) cream of racemic gossypol ($0.065 \pm 0.03\%$ w/w) was physically and chemically stable on storage at temperatures below 30°C for 9 days, had pH = 3.2 and a median oil droplet diameter of $20\ \mu m$. In vitro release studies using Franz diffusion cells and a regenerated cellulose membrane showed that the cumulative flux of gossypol was $6.3\ \mu g\ cm^{-2}$ after 9 h. Gossypol was shown to be the most potent inhibitor of keratinocyte proliferation in the anti-proliferative MTT assay, a potent anti-oxidant in the TBA assay, and its formulation into a topical dosage form was feasible. The above findings, in conjunction with its low human toxicity and lack of mutagenic effects, make gossypol a good candidate for the topical treatment of psoriasis.

Dodou, K. (2004) Ph.D. Thesis
Dodou, K. et al (2005) *Bioorg. Med. Chem.***13**: 4228–4237

140

Characteristics of traditional Chinese herbal medicine (TCHM) retail outlets in central London: preliminary results of a cross-sectional study

J. Barnes, L. Teng and D. Shaw[1]

Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX and [1]Medical Toxicology Unit, Guy's & St Thomas' Hospital Trust, Avonley Road, London SE14 5ER, UK.
E-mail: joanne.barnes@ulsop.ac.uk

In the UK, unlicensed traditional Chinese herbal medicines (TCHMs) are widely available for over-the-counter purchase from TCHM retail outlets without the involvement of a statutorily regulated health care professional. Pharmacovigilance (safety monitoring) for herbal medicines is in the early stages of development (Barnes 2003) yet, in recent years, safety concerns have emerged associated with Chinese herbal remedies prepared by TCHM practitioners and manufactured TCHMs available for purchase from TCHM retail outlets (Barnes et al 2004). These issues raise questions about aspects of TCHM outlets, yet there is a lack of formal study of practices of such shops in the UK. This study aimed at exploring the characteristics of TCHM retail outlets in central London, including types of products sold and medical uses/conditions for which TCHM is promoted. A semi-structured questionnaire, which included questions regarding information visible inside and outside TCHM outlets, was designed and developed, tested for face validity and piloted on five TCHM retail outlets outside the chosen study area. Potential TCHM outlets, including complementary medicine providers, health-food stores and pharmacies, in the study area (W1 postcode) were identified systematically by searching the Yellow Pages on-line directory. After a screening procedure (physically visiting every street in W1), 12/173 (7%) outlets were classified as TCHM retail outlets. A letter describing the study was posted to each outlet one week before the data collection period. Data were collected for the 'outside' of all 12 outlets, and detailed 'inside' observations were done for the four outlets consenting to this. Overall, 11/12 outlets displayed manufactured TCHM products, and nine used drawers or transparent jars to display Chinese crude herbs. Eight of the 12 outlets listed medical uses/conditions visible outside the shop; the median number was 25.5 ($Q_L = 16.25$, $Q_U = 59.5$). There were 274 occurrences of 137 different terms for uses/conditions; each term was counted once only for each shop. Similar terms were combined to produce 108 use/condition categories. Table 1 presents the three most frequently listed categories for the three most common therapeutic areas, after classification by BNF chapter (BMA and RPSGB 2005). Other uses/conditions listed of particular interest include cancer, diabetes, HIV infection and contraception. Also, 77 TCHM-related advertisements were identified within 11 shops; of these, 38 were associated with specific uses/conditions, most commonly skin problems, weight loss and hair loss. TCHM retail outlets in central London sell both crude herbs and manufactured TCHM products. These outlets readily display names of serious medical conditions on their premises, visible to passers-by, which at least implies that TCHMs can be used to prevent, treat or cure these conditions.

Table 1 Most frequently use/condition categories listed

BNF chapter	Use/condition category	n (% of total N; N = 274)
Central nervous system	Stress/Anxiety/Relaxation	10 (3.6%)
	Obesity/Sliming/Weight loss	8 (2.9%)
	Insomnia/Sleeplessness	7 (2.6%)
	Total	53 (19.3%)
Obstetrics, gynaecology and urinary-tract disorders	Infertility	8 (2.9%)
	Menstrual problems	7 (2.6%)
	Impotence	6 (2.2%)
	Total	39 (14.2%)
Skin	Hair Loss	7 (2.6%)
	Eczema	6 (2.2%)
	Psoriasis	6 (2.2%)
	Total	37 (13.5%)

Barnes, J. (2003) *Drug Safety* **26**: 829–851
Barnes, J. et al (2004) *Pharm. J.* **273**: 342
BMA and RPSGB (2005) *British national formulary 49*. London: Pharmaceutical Press

141
Anti-proliferative extracts from a Welsh sponge

A. W. White, J. R. P. Lottin, D. Barrow[1] and R. I. Nicholson[1]

Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3NB and [1]Tenovus Centre for Cancer Research, Welsh School of Pharmacy, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff CF10 3NB, UK. E-mail: whiteaw@cf.ac.uk

Marine invertebrates are a rich source of natural products; the wide range of chemical diversity exhibited by secondary metabolites isolated from these organisms is particularly valuable to drug discovery. The anti-proliferative activity of marine natural products is well documented: 22 such compounds are currently undergoing clinical trials for a variety of conditions, 16 of these are under evaluation as new anti-cancer drugs (Newman & Cragg 2004; Simmons et al 2005). Marine Porifera (sponges) have yielded a wide range of interesting natural products. They are usually associated with warm or tropical seas but are also relatively common in colder waters. British sponges, and their medicinal properties, are largely unexplored and have untapped potential for discovery of novel drugs. A preliminary study of the potential for anti-cancer drug discovery in British Porifera has been undertaken. The growth inhibitory properties of a *Hymeniacidon sp.* sponge from southwest Wales has been evaluated in two human cancer lines, MCF7 breast carcinoma and A549 small cell lung cancer, using previously published methodology (Gee et al 2003). The dried, powdered sponge was extracted with solvents of increasing polarity (dichloromethane–ethyl acetate–methanol) yielding three fractions and an insoluble residue. To determine anti-proliferative activity, stock solutions of each fraction in ethanol were further diluted to suitable concentrations and added to the cell culture medium. All experiments were conducted against an ethanol control, growth inhibition is reported as a percentage of this control and no carrier effects were observed. The dichloromethane and methanol extracts displayed anti-proliferative activity in both cell lines. In particular, the dichloromethane extract demonstrated extremely potent activity against the A549 cells, inhibiting the growth of these cells by $>99\%$ at $45\,\mu g\,mL^{-1}$. Analysis of this fraction by thin layer chromatography (TLC) revealed it was a mixture of several distinct compounds. Preparative silica TLC employing chloroform (100%) as eluent was used to separate the mixture and a further five fractions were obtained for analysis in the MCF7 and A549 cell lines. The most polar extract from this separation displayed excellent anti-proliferative properties: 98% growth inhibition at $30\,\mu g\,mL^{-1}$ in A549 cells. In the MCF7 cell line, 88% inhibition at $30\,\mu g\,mL^{-1}$ and 20% inhibition at $3\,\mu g\,mL^{-1}$ was observed. Anti-proliferative effects were negligible at lower concentrations in the A549 cells (1% at $3\,\mu g\,mL^{-1}$). Interestingly, two fractions from the preparative TLC separation showed moderate mitogenic properties, one promoted cell growth by 20% (at $50\,\mu g\,mL^{-1}$) in the MCF7 cells and 16% at the same concentration in the A549 cell line. In conclusion, preliminary investigations have identified a number of bioactive extracts from a Welsh sponge, identifying a promising area for drug discovery. Mitogenic and anti-proliferative effects have been observed, but in particular, certain extracts have highly potent growth inhibition properties against human breast and lung cancer cell lines. Further studies are underway to characterise the natural products responsible for these properties and to identify lead compounds for further development.

Gee, J. M. W. et al (2003) *Endocrinology* **144**: 5105–5117
Newman, D. J., Cragg, G. M. (2004) *J. Nat. Prod.* **67**: 1216–1238
Simmons, T. L. et al (2005) *Mol. Cancer Ther.* **4**: 333–342

142
Structures of norditerpenoid alkaloids from *Delphinium* Pacific Giant seeds

P. Saensuk, M. G. Rowan, I. S. Blagbrough and M. F. Mahon[1]

Department of Pharmacy and Pharmacology, University of Bath, Bath BA2 7AY and [1]Department of Chemistry, University of Bath, Bath BA2 7AY, UK. E-mail: prsisb@bath.ac.uk

Plants in the genera *Delphinium* and *Aconitum* are a major source of norditerpenoid alkaloids. Some of these alkaloids are highly toxic to mammalian species and therefore their mode(s) of action are of interest to biological and medicinal chemists and pharmacologists. Pelletier & Joshi (1987) summarized the early X-ray crystallographic studies of norditerpenoid alkaloids. These norditerpenoid alkaloids have been categorized into three broad groups: the aconitine-type, which lacks an oxygen function at C-7; the lycoctonine-type, characterized by bearing an oxygen function at C-7; and finally three synthetic compounds where X-ray data have been published (Pelletier & Joshi 1987). These natural products, and their derivatives and analogues, are ligands with important biological activity at selected ion channels. The biological activity and aspects of the history and ethnopharmacy of *Delphinium* usage have been elegantly covered by Benn & Jacyno (1983). Ground *Delphinium* Pacific Giant seeds (500 g) were extracted in a soxhlet thimble sequentially with: hexane, dichloromethane and ethanol (5 cycles each, 2 L scale) (Goodson 1943). After concentration in-vacuo, the residues from each extract were extracted with 0.5 M sulfuric acid (4×100 mL). The combined acidic layers were basified with 5 M NaOH; each fraction was back-extracted with dichloromethane (4×150 mL). The combined organic extracts were washed with water (1 ± 50 mL), dried ($MgSO_4$) and concentrated in-vacuo. Total alkaloid yields were: the hexane extract, 4.0 g; the dichloromethane extract, 1.8 g; the ethanol extract, 5.9 g. TLC analysis (cyclohexane–chloroform–diethylamine 5:4:1) showed that MLA and delpheline were present in all three solvent extracts (Goodson 1943). MLA and delpheline were isolated by column chromatography and their structures established on the basis of ^1H, ^{13}C, DEPT, COSY, HMQC and HMBC NMR spectroscopic techniques. Furthermore, delpheline was recrystallized from ethanol–hexane (1:1) and studied for the first time by X-ray crystallography. As a result, the conformations of the six rings are: A and E, chair; D, half-chair (boat flattened at C-15); C and F, envelopes; and B, boat. Three other known alkaloids, namely aconitine, mesaconitine and lycoctonine, were also studied by X-ray crystallography for comparison. All showed similar conformations, with only slight differences in the shape of ring D. It is interesting to note that these similar alkaloids differ considerably in their biological activity, which seems to depend more upon the patterns and types of substitution than on the conformations of the norditerpenoid alkaloid framework.

We acknowledge the Collaborative Research Network (CRN) for a Royal Thai Government studentship (to P.S.).

Benn, M. H., Jacyno, J. M. (1983) In: Pelletier, S. W. (ed.) *Alkaloids: chemical and biological perspectives*. Vol. 1, New York: John Wiley & Sons, pp 153–210
Goodson, J. A. (1943) *J. Chem. Soc.* 139–141
Pelletier, S. W., Joshi, B. S. (1987) *Heterocycles* **26**: 2503–2518

143
Antiplasmodial activity of some Nigerian plants used traditionally in the treatment of malaria

C. S. Obeleagu and C. W. Wright

The School of Pharmacy, University of Bradford, Bradford BD7 1DP, UK. E-mail: c.s.obeleagu@bradford.ac.uk

Malaria as an endemic tropical disease continues to pose a major threat and causes 1–3 million deaths in Africa each year. The need for new antimalarial drugs has become increasingly urgent due to the increasing prevalence of malaria parasites resistant to standard antimalarial drugs (Addae-Kyereme et al 1997). This study reports on the preliminary investigation of the antiplasmodial activity of eight Nigerian medicinal plants used traditionally in the treatment of malaria, aiming at identifying the most effective plant(s) for further research (Table 1). In vitro antiplasmodial activity against *Plasmodium falciparum* (3D7 strain) was assessed using the parasite lactate dehydrogenase assay (Makler et al 1993). The methanolic extract of the leaves of *Tithonia diversifolia* was found to have the most potent antiplasmodial activity ($IC_{50} = 23.6\,\mu g\,mL^{-1}$). However, in a previous report (Goffin et al 2002), the IC_{50} of the ether extract of the aerial parts of this species from Sao Tome against *P. falciparum* (strain FCA) was found to be $0.75\,\mu g\,mL^{-1}$. This difference may be due to a variation in the parasite strains or the type of extract used or a variation in the constituents of the plant material. The plant material from Sao Tome was shown to contain the sesquiterpene lactone tagitinin

Table 1 Antiplasmodial effects of the plant crude extract and some standard drugs

Species/Drugs	Part used	IC_{50} ($\mu g\,mL^{-1}$) vs *P. falciparum* (strain 3D7)[a]
Cymbopogon citratus	Leaves	>100
Cnestis ferruginea	Leaves	>100
Cassia siamea	Stem bark	92.7
Nauclea latifolia	Root	84.1
Phyllanthus amarus	Whole plant	>100
Stachytarpheta angustifolia	Leaves	>100
Tithonia diversifolia	Leaves	23.6
Uvaria chamae	Leaves	>100
Artemether	—	0.04
Chloroquine diphosphate	—	0.07

[a]Mean of two determinations

C as the main active constituent and further work to determine whether this compound or others is present in Nigerian *T. diversifolia* would be worthwhile.

Addae-Kyereme, J. et al (1997) *J. Pharm. Phamacol.* **49**: 113
Goffin, E. et al (2002) *Planta Med.* **68**: 543–545
Makler, M. T. et al (1993) *Am. J. Trop. Med.* **48**: 739–741

144
Antibacterial and cytotoxic principles of *Commiphora glandulosa* stem bark resin

D. M. Motlhanka, A. Miljkovic-Brake, P. J. Houghton, S. Habtemarium[2] and P. J. Hylands

Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, and [1]Pharmacognosy and Phytotherapy Research Laboratory, Medway School of Sciences, The University of Greenwich, Central Avenue, Chatham-Maritime, Kent ME4 4TB, UK. E-mail: daniel.motlhanka@kcl.ac.uk

Plant-derived medicines have been part of traditional healthcare in most parts of the world for thousands of years and there is an increasing interest in plants as sources of agents to fight microbial diseases and cancer (Farnsworth et al 1985). Given the alarming incidence of antibiotic resistance in bacteria of medical importance (Pezzuto 1997), there is a constant need for new and effective therapeutic agents. This study is part of an ongoing project to search for novel drugs from a vast array of medicinal plants from Botswana, which have not been studied extensively (Van Staden et al 2000). Chloroform and water extracts from *Commiphora glandulosa* resins (Burseraceae) were evaluated for their therapeutic potential as antimicrobial and anticancer agents using in vitro assays. Both chloroform and water extracts showed activity against Gram-positive bacterial test organisms such as *Bacillus subtilis* (NCTC 10073), *Staphylococcus aureus* (NCIMB 9518), *Clostridium perfringens* (NCTC8237) and multiple drug resistant strain *Staphylococcus aureus* (SA1199B), with MIC values ranging from 7.8 to 31.3 $\mu g\,mL^{-1}$. Both water and chloroform extracts were also cytotoxic against the murine cell line RAW264.7 macrophages and human leukaemia cell line U937 monocytes with IC_{50} values ranging from 15 to 20 $\mu g\,mL^{-1}$. The isolated active principle had an IC_{50} of 6.18 $\mu g\,mL^{-1}$ against cell line RAW 264.7 macrophages. Neither the crude extracts nor the pure compound showed activity against Gram-negative bacterial test strains ((*Esherichia coli* (NCTC9002), *Pseudomonas aerigunosa* (NCIMB10421), *Klebsiella aerogenes* (NCTC5055) and the fungal test organisms (*Candida albicans* (NCPF 3179) and *Aspergillus fumigatus* (NCPF 7097)). These findings support the use of *Commiphora glandulosa* resins by the rural communities in Botswana as antiseptic agent.

Funding is provided by Botswana College of Agriculture, Botswana.

Farnsworth, N. R. et al (1985) *Bull. World Health Org.* **63**: 965–981
Pezzuto, J. M. (1997) *Biochem. Pharmacol.* **53**: 121–133
Van Staden, J. et al (2000) *J. Ethnopharmacol.* **71**: 281–292

145
Antibacterial and antioxidant cassane diterpenoids from *Mezoneuron benthamianum*

R. A. Dickson, P. J. Houghton and P. J. Hylands

Pharmacognosy Research, Pharmaceutical Sciences Research Division, King's College London, 150 Stamford Street, London SE1 9NH, UK. E-mail: rita.dickson@kcl.ac.uk

Plants have been found useful in accelerating wound healing, a complex process involving the interplay of many biochemical and cellular mediators. Microbial infections and the presence of oxygyen free radicals are known impediments to wound healing. Notable among the microorganisms delaying or inhibiting wound healing are *Staphylococcus*, *Streptococcus* and *Pseudomonas* species (Hollinworth 1997). Any agent capable of eliminating or reducing the number of microorganisms present in a wound, as well as reducing the levels of ROS, may facilitate the wound healing process. A number of in vitro models are employed to assess relevant antimicrobial and antioxidant properties. *Mezoneuron benthamianum* Baill. (Caesalpinaceae) finds use in Ghanaian folk medicine for the treatment of skin diseases, wounds and dysentery. The petroleum extract of this plant shows antimicrobial and antioxidant activity. Investigation of this extract has resulted in the isolation of new cassane-type diterpenoids, R1, R2, R3, R5 and R9, from the air dried roots of *M. benthamianum*, along with ß-sitosterol and stigmastinone. The structures of these compounds were elucidated by spectroscopic studies using a combination of 1D and 2D NMR Spectroscopy and Mass Spectrometry (ESI). Assessment of minimum inhibitory concentrations using the micro dilution assay (Eloff 1998) was carried out for each compound in 96-well plates. Four of the compounds were active against several bacterial species (Table 1). The antioxidant activity was tested using the free radical DPPH (2, 2 diphenyl-1-picrylhydrazyl) (Cuendet et al 1997). Significant antioxidant activity was observed for two of the compounds. The results obtained suggest that the traditional use of the plant may be due to its antibacterial and antioxidant properties.

Table 1 Minimum inhibitory concentration (MIC) of compounds and standard antibiotics ($\mu g\,mL^{-1}$)

Bacterial sp.	R_1	R_2	R_3	R_5	R_9
SA 1199B (MRSA)	32.0	32.0	64.0	—	64.0
XU 212 (TetK)	64.0	32.0	128.0	—	128.0
RN 4220 (MSRA)	—	—	—	—	—
NCTC 4263 (S.a)	31.2	15.6	62.5	—	31.2
NCTC 10073 (B.s)	15.6	31.2	62.5	—	—
NCTC 775 (S.f)	—	125	250	—	125.0
NCIMB 1042 (P.a)	500	125	—	—	250.0
NCTC 7743 (M.f)	31.2	15.6	62.5	250	62.5

All MICs were determined in triplicate. —, MICs greater than 1000 $\mu g\,mL^{-1}$. M.f, *M. flavus*; B.s, *B. subtillis*; S.a, *S. aureus*; S.f, *S. feacalis; P.a, P. aeruginosa*; MRSA, Methicillin-resistant *S. aureus* and TetK, tetracycline-resistant *S. aureus* (Culture collections obtained from the Dept. of Pharmacy, King's College London and the School of Pharmacy, University of London). Tetracycline was used as positive control.

Cuendet, M. et al (1997) *Helv. Chim. Acta* **80**: 1144–1151
Eloff, J. N. (1998) *J. Ethnopharmacol.* **60**: 1–8
Hollinworth, H. (1997) *Professional Nurse Study* **12** (Suppl.): 8–11

146
Enhancement of colchicine production and recovery from *Gloriosa superba* root tissue culture in liquid medium by using solid phase extraction

G. Aroud and M. Parkinson

School of Biotechnology, Dublin City University, Dublin, Ireland.
E-mail: aroud.gamal2@mail.dcu.ie

Plant cell and tissue cultures are increasingly being seen as a new source of existing and novel pharmaceuticals (Tom et al 1991). Colchicine is a secondary metabolite of *Gloriosa superba*, which has anti-mitotic and anti-inflammatory properties and has been used for centuries in the treatment of gout and, more recently, for familial Mediterranean fever (Levy et al 1991), and has been recognized for some time as an anti-tumour agent (Davis & Klein 1980). Root tissues grow rapidly in a liquid medium, with doubling times comparable with suspension cell culture or other organ tissue. Root tissue of *Gloriosa superba* established from callus culture initiated from shoot tissue germinated from seeds. The in situ extraction of plant products from tissue culture can dramatically increase the total amounts of secondary metabolites formed in a typical batch culture cycle or in a continuous bioreactor culture. A significant fraction of colchicine produced by *Gloriosa superba* was observed to be released to the liquid medium. To optimize production and stabilise colchicine, we have developed a strategy for continuous in situ extraction and recovery of colchicine from *Gloriosa superba* root cultures. A solid phase extraction using non-ionic exchange resins, various XAD Amberlite resins, were evaluated as extraction phases for accumulating colchicine. (XAD-4) and (XAD-16) have been investigated and have shown a high adsorption capacity and binding affinity towards colchicine. Amberlite resins (XAD-4) and (XAD-16) were enclosed individually in a bag of nylon mesh and incubated with the root culture in liquid medium for four weeks. At the end of the batch culture the mesh bag was removed and extracted with methanol. The tissues were harvested and extracted, and the amount of colchicine in the liquid medium, tissue and the beads was determined by ELISA assay. The concentration of colchicine in the medium was considerably reduced to less than 10% in the culture medium containing Amberlite resins bags compared with the culture without Amberlite resins bags. Average total colchicine accumulation was increased to 0.98 ($\pm$ 0.12) and 0.85 ($\pm$ 0.13) $\mu g\,g^{-1}$ fresh weight with (XAD-4) and (XAD-16), respectively, compared with the control which was 0.33 ($\pm$ 0.04) $\mu g\,g^{-1}$ fresh weights ($n = 3$). The average colchicine released to the liquid medium (Medium + Resins) was significantly increased by 3.7 and 3.5 fold with (XAD-

4) and (XAD-16), respectively, over unextracted culture medium. Also, Amberlite resins (XAD-4) and (XAD-16) sequestered 90.2% and 92.6%, respectively, of total colchicine accumulated in the culture. Amberlite resins greatly enhanced the total colchicine production at the end of the batch culture by 3.1 and 2.9 fold with (XAD-4) and (XAD-16), respectively. This experiment illustrated that further improvements in colchicine production and recovery were possible by using a continuous in situ solid phase extraction system.

Davis P. J., Klein A. E. (1980) *J. Chromatogr*. **188**: 280–284
Levy, M. (1991) *Pharmacotherapy* **11**: 196–211
Tom, R. (1991) *J. Biotechnol*. **21**: 21–42

147
A novel extraction method for the active constituents of feverfew (*Tanacetum parthenium* L. Schultz Bip.)

M. C. Pasquali, S. Alizai, C. Rostron and M. I. Berry

School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: M.C.Pasquali@livjm.ac.uk

Herbal and other natural products represent an area of great growth among alternative medical practices. One of the most commonly purchased herbal medicinal products is feverfew, which is used in the treatment of a wide range of disorders, but perhaps more notably for its beneficial effects in the prophylaxis of migraine and treatment of inflammatory disorders, such as arthritis and rheumatism. The chemistry of the plant is very complex and poorly understood; however, the main constituents of feverfew are generally considered to be sesquiterpene lactones, namely parthenolide, and more recently flavonoids, namely santin (Williams et al 1999). To date, these two classes of compounds have been treated quite separately, although both are considered to contribute to the biological activity, and as such, there exists the possibility of synergistic action. A great wealth of literature exists for the isolation and characterisation of the sesquiterpene lactone content; however, similar information for the flavonoids is very sparse. The current lack of evidence, particularly the absence of a comprehensive knowledge of these active constituents together, formed the basis of this research. The research was directed towards developing a novel procedure to extract the active constituents of feverfew simultaneously, using simple solvent extraction, before isolation and characterisation by TLC and UV spectroscopy, utilising existing literature methods (Greenham et al 2003). Several extraction solvents were investigated and it was found that an acetone–ethanol–water (5:3:2; 10 mL) mixture not only extracted the greatest number of constituents (when compared with existing methods) from 1 g of dried, powdered *T. parthenium* leaf material, but more importantly extracted parthenolide and santin simultaneously. The active constituents were isolated using TLC in a toluene–acetic acid (4:1) solvent system and characterised by their R_f values when compared with reference data, (Greenham et al 2003). Pure parthenolide was available for comparative purposes. Further characterisation involved measurement of λ_{max} values from the methanolic UV spectrum; confirmed using reference data, (Greenham et al 2003). Parthenolide (R_f 0.45; λ_{max} MeOH 210 nm) and santin (R_f 0.58; λ_{max} MeOH 273 nm) were easily extracted and characterised, along with other major sesquiterpene lactone and flavonoid constituents of feverfew. The active components of feverfew are still the subject of much debate. Literature reports have found two main families of compounds associated with activity in bioassays and for the purposes of this research; these have been studied simultaneously using the novel extraction method developed. It is important that future standardisation work involving feverfew continues in this manner; currently, the identity and quality of most feverfew products only depend on the presence and concentration of parthenolide, wrongly ignoring the flavonoid content. An important prerequisite for establishing quality standards is the extraction, isolation and identification of all active components; the novel method developed here could prove a useful tool in minimising the current problems experienced.

Greenham, J. et al (2003) *Phytochem. Anal*. **14**: 100–118
Williams, C. A. et al (1999) *Phytochemistry* **51**: 417–423

148
Investigation of anti-bacterial, -fungal and -oxidant activities of *Solanum marginatum*, an Ethiopian medicinal plant used for topical wound healing

M. Lichtenstein and P. J. Houghton

Department of Pharmacy, Franklin-Wilkins Building, King's College, London, 150 Stamford St, London SE1 9NH, UK. E-mail: peter.houghton@kcl.ac.uk

Solanum marginatum L.f., commonly known by its Amharic name 'emboye' in Ethiopia, is one of many medicinal plants used in Ethiopian folk medicine. The fruits, resembling yellow tomatoes, are cut and the exposed surface rubbed on wounds to assist healing. Ripe fruits were harvested from Debre Zeit, Ethiopia and extracted with 96% ethanol, dichloromethane and butanol. Tests for activity related to improved healing of wounds were carried out (i.e. for antioxidant effects (Mensah et al 2001) and for antibacterial and antifungal activity). The major antioxidant test performed utilised liposomes challenged with an ascorbic acid/Fe^{2+} Fenton reagent and measured the amount of damage caused in terms of the levels of malondialdehyde produced, which was assessed by the colorimetric complex formed with thiobarbituric acid (TBA) (Galvez et al 2005). A range of concentrations of the extracts were tested to determine the IC_{50} value of the extracts and the butanol extract was found to be the most active with IC_{50} 1.73% w/v. TLC analysis (silica gel/chloroform:methanol 19:1) using 0.5% 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a spray reagent showed the presence of at least five major compounds which had oxygen free radical quenching effect. Micro-dilution and agar-well diffusion assays were performed for anti-bacterial and anti-fungal activity against Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*, Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* and the dermatophytic fungi, *Microsporum gypseum* and *Trichophyton interdigitale* (Mensah et al 2000). Minimum inhibitory concentrations (MIC) for the ethanolic extracts were 6 mg mL^{-1}, 1.5 mg mL^{-1}, 0.75 mg mL^{-1} and 0.375 mg mL^{-1}, respectively, for the four bacterial species and 0.375 mg mL^{-1} and 0.75 mg mL^{-1} against *M. gypseum* and *T. interdigitale*, respectively. The results suggest that *S. marginatum* berries may help wound healing by protecting damaged tissue from reactive oxygen species, which can cause tissue damage, and by preventing infection from pathogens such as bacteria and dermatophyte fungi. This may be assisted by the viscous juice from the fruits on an open wound which would reduce moisture loss.

Galvez, M. et al (2005) *J. Agric. Food Chem*. **53**: 1927–1933
Mensah, A. Y. et al (2000) *J. Nat. Prod*. **63**: 1210–1213
Mensah, A. Y. et al (2001) *J. Ethnopharmacol*. **77**: 219–226

149
Antifungal activity of saponins from *Medicago* species

N. Patel and P. J. Houghton

Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street London SE1 9NH, UK. E-mail: peter.houghton@kcl.ac.uk

Saponin extracts from *Medicago* species, commonly known as Alfalfa, have been known to possess a range of activity including inhibiting the growth of a number of fungal species and plant pathogens (Cheung et al 2004). This study was performed to test the antifungal activity of individual isolated saponins, particularly against dermatophytes involved in human skin infection. Antifungal assays were performed against two dermatophytes, *Microsporum gypseum* and *Trichophtyon interdigitale*, with a range of concentrations of nineteen saponins and five parent triterpenoids, medicagenic acid, hederagenin, zanhic acid, bayogenin and soyasapogenol. Eight glycosides of medicagenic acid, six of hederagenin, two of zanhic acid, two of bayogenin and one of soyasopogenol were tested. The agar dilution method was performed to determine activity expressed as minimum inhibitory concentration (MIC) using miconazole as a positive control (Mensah et al 2000). Microwell plates containing 200 μL of double-strength Saboraud dextrose agar were seeded with 20 μL of fungal spore suspension, 10^5 colony forming units. Two-hundred microlitres of saponin dissolved in 4% DMSO was added to the microwell plates over a range of concentrations from 2 mg mL^{-1} to 0.0625 mg mL^{-1}. Each microwell plate was incubated at 30°C and examined daily for fungal growth. Three replicates were performed for each extract. MIC was recorded as the lowest concentration showing the absence of fungal growth on visual examination after 7 days. *Trichophtyon interdigitale* appeared more sensitive to the saponins than *Microsporum gypseum*. Aglycones showed very little antifungal effect but the glycosides displayed a range of activity. Monodesmosidic glycosides of medicagenic acid were the most active compounds, especially the 3-O-β-D-glucopyranoside, which displayed MIC less than 0.0625 μg mL^{-1} against both fungi, although those of hederagenin and zanhic acid showed weak activity (MIC 0.5 μg mL^{-1} against both fungi). Bidesmosidic saponins had weaker activity than monodesmosidic ones (e.g. 3-O-β-D-glucopyranoside medicagenate had MIC < 0.0625 μg mL^{-1}, 3-O-β-D-glucopyranosyl-28-O-β-D-glucopyranoside medicagenate had MIC 1.0 μg mL^{-1} against *M. gypseum*). This corresponds to activity of these compounds against other fungi (Martyniuk et al 2004) but this is the first report of activity of these compounds against dermatophytes.

Cheung, C. Y. et al (2004) *J. Pharm. Pharmacol*. **56** (Suppl.): S-77
Martyniuk, S. et al (2004) *Allelopathy J*. **13**: 75–81
Mensah, A. Y. et al (2000) *J. Nat. Prod*. **63**: 1210–1213

150
Antioxidant activity of plants from Cameroon used as dietary supplements

H. H. T. Tang and P. J. Houghton

Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK. E-mail: peter.houghton@kcl.ac.uk

There is an increasing interest in the antioxidant activity of plants used in the diet for health benefits. Methanol and water extracts of the leaves of six species of plants used as vegetables in the African country of Cameroon were tested for their antioxidant activity by screening using the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) (Guvenc et al 2005). The plants examined were *Celtis integrifolia* Lam. (Ulmaceae), *Hibiscus articulatus* Hochst. Ex A. Rich (Malvaceae), *Corchorus olitorius*L. (Tiliaceae), *Corchorus fascicularius* Lam. (Tiliaceae), *Adansonia digitata* L. (Bombacaceae) and *Ceratotheca sesamoides* Endl. (Pedaliaceae). Extracts of 1 mg mL^{-1} all showed free radical scavenging activity and TLC analysis (silica gel/ethyl acetate:formic acid:acetic acid:water 100:11:11:27 followed by spraying with 1% diphenylboric acid 2-aminoethyl ester then 5% polyethylene glycol and examination under UV light 365 nm) showed that this was due to the presence of flavonoids. Extracts of leaves of *Ceratotheca sesamoides*, *Corchorus fascicularis* and *Adansonia digitata* exhibited the strongest activity so were further investigated by DPPH spectrophotometric assay. Their methanol extracts were also examined for their effects on lipid peroxidation in bovine brain liposomes (Galvez et al 2005). Results showed that among the water extracts, *Adansonia digitata* displayed the strongest radical scavenging activity with 50% inhibitory concentration (IC_{50}) value of 0.11 mg mL^{-1}. *Ceratotheca sesamoides* was found to be the methanol extract having the strongest antioxidant activity with respect to radical scavenging activity (IC_{50} 0.11 mg mL^{-1}) and lipid peroxidation inhibition (IC_{50} 0.48 mg mL^{-1}). The compound contributing to most of the radical scavenging activity of *Ceratotheca sesamoides* methanol extract was identified using TLC and isolated. It was found to be a caffeic acid derivative by UV and visible light spectroscopy. The findings from this study supported the use of the plants investigated, in particular, *Ceratotheca sesamoides* and *Adansonia digitata* in the diet. These leafy vegetables may be useful in the prevention of aging-related diseases, cardiovascular diseases and cancer, due to their antioxidant effects.

Galvez, M. et al (2005) *J. Agric. Food Chem.* **53**: 1927–1933
Guvenc, A. et al (2005) *Pharm. Biol.* **43**: 173–177

151
Investigation of common vegetables for cholinesterase inhibitory activity

B. M. Chiu and P. J. Houghton

Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9 NH, UK. E-mail: peter.houghton@kcl.ac.uk

Acetylcholinesterase (AChE) inhibitors have been used as drugs for symptomatic treatment of Alzheimer's disease. There are reports in the literature that some common vegetables possess this activity, but no detailed investigation has been carried out (Liener 1980). Dichloromethane extracts of five common vegetables and fruit: orange, radish, apple, broccoli and potato, were tested for anti-acetylcholinesterase activity using the Ellman method (Perry et al 2000). All material used was bought as 'organic' from supermarkets to prevent effects that might be due to organophosphate insecticides, which are cholinesterase inhibitors. Preliminary screening using 2.5 μL of a 10 mg mL^{-1} solution of each extract showed that all five plants possessed acetylcholinesterase inhibitory activity, and the subsequent quantitative work showed that the broccoli extract possessed the lowest minimum inhibitory concentration (6.25 μg extract), so it was chosen to be investigated further. Extracts were examined on TLC (silica gel/dichloromethane:acetone 5:1) for the presence of AChE inhibitors using for detection the modification of the Ellman method (Rhee et al 2001). A parallel plate was used to test for possible false positives (Rhee et al 2003). Results showed that out of the four zones of inhibition, two zones were false positive effects. The remaining zones were further investigated by spraying plates developed in the same solvent system with a range of chromogenic reagents specific for major chemical types. Results showed that the compound responsible for the activity might be glucosinolates, such as are found in the Brassicaceae, since the zone giving AChE inhibition also gave a blue-green colour when sprayed with hexacyanoferrate reagent (Wagner & Blatt 1995). This study provided evidence that broccoli extract does possess anti-acetylcholinesterase activity, and this is due to its glucosinolate content. This is the first report that glucosinolates have AChE inhibitory properties. However, it is questionable that consumption of broccoli would have any beneficial effect on Alzheimer's disease, although the long-term effects of regularly consuming these compounds in vegetables belonging to the Brassicaceae (Cabbage family) might be beneficial in reducing a decline in ACh levels in the CNS.

Liener, I. E. (1980) Miscellaneous toxic factors. In: Liener, I. E. (ed.) *Toxic constituents of plant foodstuffs*. 2nd ed., New York: Academic Press, pp 449–451
Perry, N. S. L. et al (2000) *J. Pharm. Pharmacol.* **52**: 895–902
Rhee, I. K. et al (2001) *J. Chromatogr. A* **915**: 217–223
Rhee, I. K. et al (2003) *Phytochem. Anal.* **14**: 127–131
Wagner, H., Bladt, S. (1995) *Plant drug analysis*. 2nd edn, Berlin: Springer, p. 298

152
Investigating the wound healing activity of *Alocasia odora* (Roxb.) Koch

L. D. Viet, P. J. Hylands, P. J. Houghton, B. Forbes and O. Corcoran

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NH, UK. E-mail: peter.2.hylands@kcl.ac.uk

Stems of *Alocasia odora* and *Alocasia macrorrhizos* have been used in Vietnamese traditional medicine for the treatment of wounds (Chi 1999). While some experimental studies and clinical observations have shown positive effects of *A. macrorrhizos* on wound healing (Van Dong et al 1988), there is still no literature about this activity for *A. odora*. This study investigates some possible wound healing mechanisms of *A. odora*, and compounds responsible, by using bioassay guided fractionation and isolation. The dried stem of *A. odora* was ground and successively extracted with light petroleum, chloroform, methanol and water. These extracts were then tested for the ability to stimulate growth of human skin fibroblast cells (142BR) in vitro and for antioxidant activity in the same cells challenged with hydrogen peroxide. Neutral Red assay was used to assess cell growth and death. Active fractions were separated by HPLC. Isolated compounds were retested in the above bioassays to determine which ones contributed to the wound healing process of *A. odora*. Results obtained showed that the methanol extract was active in bioassays and therefore chosen for further study. Preparative HPLC was used to fractionate the methanol extract. A fraction that showed good dose-dependent activity in both bioassays was collected. This fraction was separated into four compounds by semi-preparative HPLC and retested in bioassays. Bioassay results of the fraction and its four compounds (P3-P6), which were tested in a range of 2-fold dilutions (3.13–100 μg mL^{-1}), are dose-dependent and shown in Table 1 and Table 2. Spectroscopic analysis is in progress to elucidate the structures of these compounds.

Table 1 Effect of an active fraction and its compounds from *Alocasia odora* on growth of human skin fibroblasts

Sample	Concn (μg mL^{-1})[a]	% Growth vs Neg.
Total fraction	50; 100	160–207
P3	25; 50; 100	119–157
P4	25; 50; 100	126–358
P5	50; 100	129–254
P6	100	114

[a]Concentration at which the effect of the fraction/compounds was significantly stronger than that of the negative control ($P < 0.05$); Neg, Negative control group with the identical conditions as those of the test groups but without test substances.

Table 2 Effect of an active fraction and its compounds from *Alocasia odora* on protecting fibroblasts against H_2O_2-induced oxidant injury

Sample	Concn (μg mL^{-1})[a]	% Protection vs Neg.
Total fraction	2.5; 25; 50; 100	43–100
P3	100	20
P4	3.13; 6.25; 12.5; 25; 50; 100	53–85
P5	3.13; 6.25; 12.5; 25; 50; 100	59–72
P6	50; 100	38–45

[a]Concentration at which the effect of the fraction/compounds was significantly stronger than that of the negative control ($P < 0.05$); Neg, Negative control group with the identical conditions as those of the test groups but without test substances.

Chi, V. V. (1999) *Vietnamese dictionary of medicinal plants* (in Vietnamese). Hanoi, Vietnam: Medicinal Publishing House
Van Dong, L. et al (1988) *Wound Rep. Reg.* **6**: A483

153
In vitro cytotoxicity of selected Nigerian medicinal plant extracts

J. S. Ashidi, P. J. Houghton and P. J. Hylands

Pharmacognosy Research Laboratories, Pharmaceutical Sciences Research Division, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NH, UK. E-mail: joseph.s.ashidi@kcl.ac.uk

There is only scant literature on the anticancer components of Nigerian medicinal plants (Sofowora 1993). This necessitated an ethnobotanical survey of plants commonly used by traditional healers in South-Western Nigeria. Thirty traditional medical practitioners were involved in the survey. Eight of the species identified (*Acanthospermum hispidum* (DC.) (shoot), *Cajanus cajan* (L.) Millsp (leaves), *Morinda lucida* Benth. (leaves and stem bark), *Nymphaea lotus* L. (whole plant), *Pycnanthus angolensis* (Welw.) Warb (leaves), *Diospyros canaliculata* De Wild (leaves) *Croton penduliflorus* Hutch. (seed) and *Annona senegalensis* Pers (leaves) were extracted with methanol and the extract tested for cytotoxicity using the SRB assay (Itharat et al 2004). The extracts were tested at concentrations of 100, 40, 20 and 5 μg in triplicate. Three cancer cell lines (human breast adenocarcinoma cell line MCF-7, ECACC no. 86012803, human large cell lung carcinoma cell line CORL-23, ECACC no. 92031919 and human amelanotic melanoma C32, ECACC no. 87090201) and one normal cell line (normal human keratinocytes SVK-14) were used. The optical density readings were taken at 492 nm on SpectraMax-190 (Molecular Devices, Sunnydale, USA) at exposure period of 48 h and recovery period of 48 h. Cytotoxicity was observed in 5 species – *A. hispidum* (AH), *C. cajan* (CC), *M. lucida* (MLL), *N. lotus* (NL) and *P. angolensis* (PA) – with IC_{50} values shown in Table 1. There was only a moderate range of selectivity shown by the extracts among the cell lines. The extract of *C. cajan* was further partitioned with hexane, CH_2Cl_2, $CHCl_3$ ethyl acetate and acetone. The resulting fractions were tested against the four cell lines. The CH_2Cl_2 fraction was found to be most active, with IC_{50} values of 10, 10, 12 and 7 $\mu g\,mL^{-1}$, respectively. Work is in progress to identify the active compounds.

Thanks are due to the traditional medical practitioners of the study area for information. JSA thanks the Commonwealth Scholarship Commission, UK for financial support.

Table 1 IC_{50} values (mean ± std $\mu g\,mL^{-1}$) for methanolic extracts in 96 h treatment (recovery)

Plant	MCF7	CORL23	C32	SVK14
AH	13.50 ± 1.27	8.99 ± 1.05	13.54 ± 0.81	10.25 ± 3.18
CC	14.55 ± 1.20	11.60 ± 2.52	33.07 ± 0.69	25.00 ± 8.48
MLL	41.00 ± 4.38	30.10 ± 3.37	43.82 ± 0.52	37.75 ± 10.9
NL	30.10 ± 0.71	47.31 ± 1.88	36.26 ± 2.98	28.50 ± 2.12
PA	48.45 ± 2.05	28.64 ± 0.57	66.88 ± 0.55	66.00 ± 9.90

Itharat, A. et al (2004) *J. Ethnopharmacol*. **90**: 33–38
Sofowora, A. (1993) *J. Ethnopharmacol*. **38**: 209–214

154
Antioxidant activity of the methanolic leaf extract of *Tetractomia roxburghiana (Family Rutaceae)*

C. Guntupalli, A. Worsley, P. W. Groundwater, R. J. Anderson, W. J. Lough and N. D. Satyanarayan

Sunderland Pharmacy School, University of Sunderland, Sunderland SR1 3SD, UK. E-mail: saraschakra2000@yahoo.com

Free radical reactions are involved in the pathogenesis of many diseases, including cancer, diabetes, rheumatoid arthritis, psoriasis, liver disorders, etc. Arising from these, a large number of antioxidant compounds are being successfully used in therapy (Utas et al 2002). Plants are known to produce phenolic metabolites to protect themselves from the damage by free radicals and based on these facts an attempt has been made to evaluate the antioxidant property of *Tetractomia roxburghiana*. The components of this plant were separated and fully characterized and found to be phenolics, tannins and furanocoumarins. *T. roxburghiana* (Rutaceae) is a perennial herb, with compressed branches and numerous simple leaves. It is widely distributed in tropical and subtropical regions. The leaves are used in the treatment of topical infections, itching, boils and skin inflammation and as a diuretic, toxic and astringent (Su et al 1996). The major factors in the selection of the plant for studies of therapeutical usefulness are traditional healer's claims and the type of active constituents present in the plant (Bowen & Leuis 1978). In this context, *T. roxburghiana* was selected for systematic biological screening to exploit and identify compounds that may serve as subsequent leads for the design of anti-psoriatic drugs. The fresh plant was collected from Malaya and a leaf extract was prepared by Soxhlet continuous extraction using a range of the solvents from non-polar to polar, so that all of the active constituents present in the plants would be extracted. The analytical reversed-phase high-performance liquid chromatography (HPLC) of the crude extracts was carried out to qualitatively assess the number of constituents present in each fraction. The separation was achieved by using ACE-5-C18 (250 × 4.6 mm) with a flow rate of 1.5 mL min^{-1}, with the UV trace measured at 215 nm and 254 nm. Semi-preparative and preparative HPLC were also carried out to isolate compounds from the analytical method using the same parameters scaled up. Mass spectroscopy of the proposed purified compounds was performed. Free radical induced lipid peroxidation model has been selected for evaluation of antioxidant activity of the extract (Utas et al 2002). The measurement of total lipid peroxidation product (thiobarbituric acid reacting substance, MDA) was made at λ_{max} 532 nm (Motta et al 1994). The results indicated that the methanolic leaf extract of *T. roxburghiana* showed marked antioxidant activity at a concentration of 18.7041 mM L^{-1}.The IC_{50} value of methanolic leaf extract of *T. roxburghiana* was found to be 4.023 μM. The methanolic leaf extract of *T. roxburghiana* at a concentration of 18.7041 mM significantly antagonized the induced increase in melondiadehyde (MDA) (92.50 ± 15.89%) in liposomes brain extract while in control group the percentage of lipid peroxidation was found to be 29.23 ± 0.024. The antioxidant activity of *T. roxburghiana* was found to be comparable with the propylgallate at a concentration of (0.1 mM L^{-1}), which was used as a standard drug thus confirming, as anticipated, that *T. roxburghiana* seems to be a good source of antioxidant drug.

Bowen, I. H., Leuis, J. R. (1978) *Planta Med*. **8**: 129–134
Motta, S. et al (1994) *Acta Derm. Venereol*. **186**: 131–132
Su, T. L. et al (1996) *Phytomedicine* **3**: 14–17
Utas, S. et al (2002) *Clin. Biochem*. **35**: 241–246

155
Antibacterial and resistant modifying activity of *Paullinia pinnata*

K. Annan, P. J. Houghton, P. J. Hylands and S. Gibbons[1]

Pharmacognosy Research Group, Department of Pharmacy, Kings' College London, FWB, 150 Stamford Street, London SE1 9NH and [1]Centre for Pharmacognosy and Phytotherapy, The School of Pharmacy, University of London, 29–39 Brunswick Square, London WC1N 1AX, UK. E-mail: kofi.annan@kcl.ac.uk

In traditional medicine in Ghana, *Paullinia pinnata* (Sapindaceae) is used for the treatment of wounds and other microbial infections, which is now a major problem not only in developing countries but the developed ones too, especially with the emergence of the 'superbug', which is resistant to all antibiotics except vancomycin. As part of the current study aimed at assessing the antimicrobial properties of the plant, the roots were exhaustively extracted using soxhlet, with methanol. Bioassay-guided fractionation of the methanol extract using an antibacterial assay determined that the petroleum ether and chloroform fractions were active, while acetone, methanol and water fractions were inactive. Column chromatography followed by preparative thin layer chromatography (TLC) led to the isolation of five compounds, K1, K2, K3, K9 and K11, from the active fractions, which were assessed for their antibacterial activity against different strains of *Staphylococcus aureus* (XU212 (TetK), RN4220 (MsrA) and SA1199B (NorA)) possessing efflux mechanism of resistance (Oluwatuyi et al 2004). All had an antibacterial effect, with minimum inhibitory concentrations (MIC) in the range 1–256 $\mu g\,mL^{-1}$. The most active compound, however, was K11 with an MIC in the range 1–4 $\mu g\,mL^{-1}$ (Table 1). Incorporation of K11 in the growth medium at 0.1 $\mu g\,mL^{-1}$ caused an 8-fold (norfloxacin), 256-fold (tetracycline) and 712-fold (erythromycin) potentiation of activity against SA1199B, XU212 and RN4220, respectively (Table 2). Identification of the active compounds using NMR and mass spectrometry experiments is underway in our laboratory.

K. Annan is supported by the Ghanaian Government.

Table 1 Minimum inhibitory concentrations ($\mu g\,mL^{-1}$) of isolated compounds on different strains of *Staphylococcus aureus*

Compound	XU212	RN4220	SA1199B	NCTC4163
K1	256	256	128	64
K2	256	256	128	64
K3	256	256	128	64
K9	256	256	64	64
K11	4	4	1	1

All MICs were determined in triplicate.

Table 2 Antimicrobial susceptibility of test strains in the absence and presence of $0.1\,\mu g\,mL^{-1}$ of K11 and $10\,\mu g\,mL^{-1}$ of reserpine (a standard resistance modulator)

Antimicrobial agent	MIC of resistant strains of *Staph. aureus*		
	XU212 (TetK)	RN4220 (MSRA)	SA1199B (NorA)
Tetracycline	128		
+ K11	0.5		
+ Reserpine	32		
Erythromycin		256	
+ K11		0.5	
+ Reserpine		256	
Norfloxacin			32
+ K11			4
+ Reserpine			8

All MICs were determined in triplicate.

Oluwatuyi, M. et al (2004) *Phytochemistry* **65**: 3249–3254

156
Antioxidant activity of essential oils from Thai herbs

P. Lertsatitthanakorn, A. Priprem, S. Taweechaisupapong[1], C. Aromdee, C. Arunyanart[2] and W. Khunkitti

Faculty of Pharmaceutical Sciences, [1]Faculty of Dentistry and [2] Faculty of Medicine, Khon Kaen University, Khon Kaen, Thailand 40002. E-mail: pilan_s@yahoo.com

Several essential oils from Thai herbs have been used to treat various medical conditions. In this study, antioxidant activity of seven Thai herbal essential oils, namely *Zingiber cassumunar* Roxb. (plai), *Z. officinalis* Roscoe (ginger), *Citrus hystrix* DC. (kaffir lime), *Cymbopogon citratus* Stapf. (lemon grass), *C. nardus* Rendle (citronella grass), *Ocimum sanctum* Linn. (holy basil) and *O. basilicum* Linn. (sweet basil), were investigated by DPPH free radical scavenging assay. An attempt to compare the efficacy of the oils with positive controls, namely butylated hydroxytoluene, ascorbic acid and alpha-tocopherol, was also carried out. The DPPH assay was measured at different concentrations of each essential oil and positive control in ethanol solution. The decrease of absorbance resulting from hydrogen donor ability of the tested samples was read at 517 nm (Mambro et al 2003). Percentage inhibition was determined by comparison with an ethanol treated control group. The concentration of the tested samples required to scavenge 50% DPPH free radical (IC_{50}) was investigated. Of the essential oils tested, all of them possessed marked activity as hydrogen donor in a concentration-dependent manner. Holy basil oil exhibited the lowest IC_{50} value of $0.030\,\mu L\,mL^{-1}$. The IC_{50} values of citronella grass and plai oil were 1.959 and $6.916\,\mu L\,mL^{-1}$, respectively. The IC_{50} values of other essential oils ranged between 23 and $42\,\mu L\,mL^{-1}$, which quite differed from the previous works. The previously published IC_{50} value of sweet basil oil was approximate $0.263\,\mu L\,mL^{-1}$ (Tomaino et al 2005). Ginger and lemongrass oils had IC_{50} values about 10 and 1ess than $10\,\mu L\,mL^{-1}$, respectively (Sacchetti et al 2005). The different results might be due to various sources of essential oils suppliers. When the positive controls were investigated, the IC_{50} values of butylated hydroxytoluene, ascorbic acid and alpha-tocopherol were 31.435, 7.944 and $16.667\,\mu g\,mL^{-1}$, respectively. Therefore, the constituents of holy basil oil, the most active essential oil, were investigated by gas chromatography/mass spectrometry (GC/MS) method. The components of holy basil oil were identified by comparing their mass spectra and retention indices using commercial libraries of natural products. It was found that eugenol was the major composition (42.42%) in this essential oil. When the DPPH assay of eugenol was performed, the IC_{50} value of this compound was $0.012\,\mu L\,mL^{-1}$, which indicated approximately two-fold effectiveness of holy basil oil. The presence of phenolic compound in holy basil oil would account for the strong radical scavenging activity. In conclusion, all of the selected essential oils possessed antioxidant activity and holy basil oil was the most active one. Holy basil oil also revealed a stronger antioxidant activity than butylated hydroxytoluene, ascorbic acid and alpha-tocopherol.

Mambro, V. M. D. et al (2003) *Int. J. Pharm.* **262**: 93–99
Sacchetti, G. et al (2005) *Food Chem.* In press
Tomaino, A. et al (2005) *Food Chem.* **89**: 549–554

157
Isolation of less polar compounds from unripe rind of *Citrus reticulata*

V. Van Leo, T. Ngoc N. Chau, T. Hung and N. Van Thu

Faculty of Pharmacy, University of Medicine and Pharmacy Ho Chi Minh City, Vietnam. E-mail: leo.vo_van@kcl.ac.uk

Citrus reticulata is a cultivar plant that has been used as a source of food. Its main compounds include essential oil and flavonoid compounds. Flavonoids are widely used in medicine due to their antioxidant properties, including anticancer, antiviral and anti-inflammatory activity and inhibition of human platelet aggregation (Benavente et al 1997; Tanaka et al 1997). As a continuation of *Citrus* research, the aim of the study is to extract and isolate less polar compounds, but not essential oil, from the unripe rind of *C. reticulata* for use as standard substances in quality control of traditional medicines. The peel of *C. reticulata* was collected in Ho Chi Minh City, Vietnam. A voucher specimen was deposited in the Herbarium of Department of Pharmacognosy, University of Medicine and Pharmacy at Ho Chi Minh City. Solvents used for chromatography and extraction were of analytical grade. TLC analyses were performed on pre-coated silica gel plates (Merck). UV light was used for visualization. The ^1H NMR, ^{13}C NMR and DEPT spectra were recorded in $CDCl_3$ with TMS on a Bruker/XWIN_NMR. Chemical shifts were reported in ppm downfield from TMS. The powder of unripe rind of *C. reticulata* (1 kg) was percolated with 95% alcohol. The combined percolates (20 L) were concentrated under reduced pressure to a syrupy residue. This residue was diluted with water at a ratio of 1:1 and distributed with benzene several times. The combined benzene extracts were treated with active charcoal and concentrated to a syrupy extract (8 g). The benzene extract was chromatographed on silica gel (250 g, 0.015–0.040 μm), using benzene–butylacetate (1:1) as an eluent solvent. After checking fractions by TLC on 2 solvent systems, benzene–butylacetate (1:1) and toluene–acetone (7:2) fractions, having the same chromatographic patterns, were combined, evaporated and crystallized in methanol solvent. The seven following compounds were isolated: M1 (59 mg), M2 (181 mg), A (24 mg), B (72 mg), C (398 mg), D (322 mg) and E (43 mg). Two of them, C and D, were determined to be tangeretin and nobiletin, known polymethoxylated flavones found in *C. reticulata* previously (Stewart et al 2005), by comparing their MS and NMR spectrum data with those of standard substances. The structure elucidation of the remaining compounds is in progress.

Benavente, O. et al (1997) J. *Agric. Food Chem.* **45**: 4506–4515
Stewart, C. et al (2005) *Atherosclerosis* **178**: 25–32
Tanaka, T. et al (1997) *Carcinogenesis* **18**: 957

158
An investigation to determine the optimum harvest time of German chamomile (*Matricaria recutita* L.)

M. C. Pasquali, M. Ahmad, M. I. Berry, A. M. Dyas and C. Rostron

School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK. E-mail: M.C.Pasquali@livjm.ac.uk

German chamomile preparations are frequently purchased for use internally and externally. Extracts of the drug are often standardised to essential oil or apigenin-7-glucoside content (Willuhn 2004). *Matricaria recutita* L. flower heads are currently harvested at full flower for inclusion in phytomedicines as these are considered to yield the highest level of active constituents. There is, however, little documented evidence to suggest that this is the optimum harvest time for German chamomile, and it is well known generally, that levels of active constituents change throughout the growth cycle of a plant. This research was directed towards investigating the profile of chemical constituents throughout the growth cycle of *M. recutita* L., to recommend or confirm the optimum harvest time, to yield the

greatest number of active constituents. A simple methanolic extraction (10 mL) of dried *M. recutita* L. flower heads (3 g; harvested at bud, early flower, full flower and late flower) was carried out according to Pasquali et al (2003), before isolation using TLC in toluene–acetic acid (4:1). Characterisation was based upon R_f values of plant extract constituents and pure standards before final confirmation using λ_{max} (MeOH) values. HPLC retention times were also recorded using a reverse phase, gradient system of acetic acid–water according to Greenham et al (2003). All measured data were compared with literature values to confirm the presence of active constituents. Constituents identified include apigenin-7-*O*-glucoside, (-)-α-bisabolol, bisabolol oxides A and B, caffeic acid, chamazulene, herniarin, luteolin, cis-en-in-dicycloether and trans-en-in-dicycloether as expected (Willuhn 2004), showing the *M. recutita* L. samples analysed to be of good pharmaceutical quality. Indications are that harvesting could take place earlier in the growth cycle and still contain the major constituents required for biological activity and standardisation. A key finding to support this was the presence of apigenin-7-glucoside only in the budding stage of growth and not in full flower as expected. As commercial products of the plant are often standardised to apigenin-7-glucoside content this warrants further detailed investigation to include samples of *M. recutita* L. from a variety of sources and quantification of the active constituents. The chromatographic profiles generated at each stage of growth could also be used as a quality control tool to detect possible adulteration of German chamomile samples with related plant species (e.g. feverfew), for which profiles have already been developed (Pasquali et al 2003).

Greenham, J. et al (2003) *Phytochem. Anal.* **14**: 100–118
Pasquali, M. C. et al (2003) Unpublished PhD data, School of Pharmacy and Chemistry, Liverpool John Moores University
Willuhn, G. (2004) In: Wichtl, M. (ed.) *Herbal drugs and phytopharmaceuticals*. Stuttgart: Medpharm, pp 369–373

Poster Session 2 – Pharmacology

159
Effects of *Hypoxis hemerocallidea* (Fisch. & C. A. Mey) Corm ('African potato') aqueous extract on renal electrolyte and fluid handling in the rat

C. T. Musabayane[1] and J. A. O. Ojewole[2]

Department of Human Physiology & Nutrition[1] and Department of Pharmacology[2], Faculty of Health Sciences, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa. E-mail: ojewolej@ukzn.ac.za

Recent biomedical evidence suggests that the corm of *Hypoxis hemerocallidea* Fisch. & C. A. Mey (family: Hypoxidaceae) has antidiabetic potential and may be useful in the management of adult-onset, non-insulin-dependent, type-2 diabetes mellitus (Mahomed & Ojewole 2003). This 'miracle ' medicinal plant of southern Africa is a tuberous, perennial herb with long, strap-shaped leaves and yellow, star-shaped flowers (Van Wyk et al 2002). The traditional health practitioners of southern Africa have widely employed the tuberous rootstock (i.e., the corm – popularly known as 'African potato') of the herb as a muthi (the South African isiZulu word for medicine) for an array of human ailments. Currently, the humble African potato (AP) has been claimed to be an amazing and wonder plant medicine in the fight against various modern human disorders, including HIV/AIDS-related diseases, arthritis, hypertension, diabetes mellitus, cancer, psoriasis, gastric and duodenal ulcers, tuberculosis, and so on (Van Wyk et al 2002). However, biomedical literature reports have indicated that certain herbal extracts and plant products attenuate the deterioration of kidney function associated with diabetes mellitus. This study was, therefore, undertaken to examine the effect of short- (acute) and long-term (chronic) administration of *Hypoxis hemerocallidea* corm aqueous extract (APE) on renal electrolyte and fluid handling in male Wistar rats. To establish the acute effects of APE, separate groups of anaesthetized control and test rats were challenged with continuous jugular infusions of 0.077 M NaCl at 9 mL h^{-1}. After 3 ½ h equilibration period, consecutive 30-min urine collections were made over the subsequent 4 h of 1 h control, 1 ½ h treatment and 1 ½ h recovery periods for measurements of urine flow and Na^+ and K^+ excretion rates. In the test rats, APE was added to the infusate at doses of 90, 180 or 360 μg h^{-1} for 1 ½ h during the treatment period. For chronic studies, individually-caged rats were treated with APE (30 mg kg^{-1} p.o.) every third consecutive day at 0900 h, followed by the same APE dose (30 mg kg^{-1} p.o.) 8 h later in the day, for 5 weeks. Control rats were given distilled water (3 mL kg^{-1} p.o.). Urine volume and total urinary outputs of creatinine, Na^+ and K^+ were determined from 24-h samples. Acute administration of graded doses of APE provoked a dose-dependent, significant ($P < 0.05$–0.01) decreases in urine flow and urinary Na^+ and K^+ excretion rates. Chronic APE treatment significantly ($P < 0.05$) reduced urinary Na^+ output from the 2nd to the 5th week, without affecting urine flow or K^+ excretion rates. By comparison with control rats, APE significantly ($P < 0.05$) increased plasma creatinine concentration (68 ± 6 μmol L^{-1} vs 55 ± 3 μmol L^{-1}, n = 6 in all groups) with a concomitant reduction in glomerular filtration rate (GFR) by the end of the 5th week (1.52 ± 0.02 mL min^{-1} vs 2.54 ± 0.09 mL min^{-1}). The results of this experimental animal study appear to suggest that APE impairs some functions of the kidney.

Mahomed, I. M., Ojewole, J. A. O. (2003) *Methods Find. Exp. Clin. Pharmacol.* 25: 617–623
Van Wyk, B.-E. et al (2002) *Medicinal plants of South Africa*. 2nd Edn, Pretoria: Briza Publications, pp 156–157

160
Deregulated glucose transport as a target for anticancer drug development: use of a Glut-1 over-expressing colon carcinoma cell line to screen tyrosine kinase inhibitors for Glut-1-dependent toxicity

G. Hodgson, A. Evans and R. Airley

Tumour Metabolism and Therapeutics Group, School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool L3 3AF, UK. E-mail: R.Airley@livjm.ac.uk

Malignant cells show a significant increase in glucose uptake and metabolism, a phenomenon described as the Warburg Effect, which reflects a switch to anaerobic glycolysis. This change, which is mediated in part by the overexpression of the facilitative glucose transporter Glut-1, occurs in response to tumour hypoxia, oncogene expression and the production of certain growth factors. Glut-1 is an important tumour biomarker, being overexpressed in a wide range of tumours, and correlating with poor prognosis(Airley et al 2001). Therefore, Glut-1 may be an attractive target for novel anticancer agents. Glut-1 is made of two sub units each possessing an active site to which AMP or ATP can bind. Binding of ATP to the site induces a conformational change that reduces glucose uptake. ATP-binding tyrosine kinase inhibitors, such as quercetin, directly bind to and inhibit Glut-1 at its ATP-binding site (Vera et al 2001). Further, certain clinically used tyrosine kinase inhibitors are known to target the ATP-binding sites of tyrosine kinases, such as imatinib (Glivec), which targets the oncogenes C-kit or /bcr-abl. Phase 1 studies of ST1571 in gastrointestinal stromal tumours have shown a rapid decrease in fluorinated deoxyglucose (FDG) uptake by tumours, sometimes as early as 24 h after administration of drug. This may be due to a combination of a decreased tumour volume and a drug-induced, Glut-1-mediated reduction in FDG uptake by the tumour. It may be possible, therefore, that the level of Glut-1 expression in individual tumours may influence the activity of such tyrosine kinase inhibitors. To test this hypothesis, MTT and clonogenic cell survival assays were carried out following 24-h exposures to quercetin (0–1000 μM) and imatinib (0–100 μM) in the human colon carcinoma HT-29 cell line (designated GLAR-HT29), which we have genetically manipulated to constitutively overexpress Glut-1, relative to wild type cells. To evaluate the influence of hypoxia, cells were also exposed to drug in normoxic and anoxic conditions. IC50 data (Table 1) show that although cells were significantly less sensitive to quercetin after exposure to anoxia alone ($P = 0.017$), overexpression of Glut-1 increased sensitivity in both normoxic ($P = 0.03$) and anoxic ($P = 0.06$) conditions. Although imatinib showed greater toxicity, this was not influenced by the level of Glut-1 expression or the level of oxygenation. It has been observed previously that the HT29 cell line constitutively overexpresses c-kit, the major target of imatinib, which may override any Glut-1-dependent toxicity. However, Glut-1 clearly influences toxicity to quercetin. Structurally related ATP-binding TKI's, which may partially exert toxicity through glut-1 inhibition, may therefore be used in the future as possible lead compounds in the design of Glut-1 inhibiting novel anticancer agents.

Table 1 IC50 values under normoxic and anoxic conditions

Cell line	Drug	I	C50 (μM)
HT29 WT	Quercetin	Normoxia:	642.9
		Anoxia:	1200.1
HT29 WT	Imatinib	Normoxia:	181.9
		Anoxia:	274.8
GLAR-HT29	Quercetin	Normoxia:	492.9
		Anoxia:	631.1
GLAR-HT29	Imatinib	Normoxia:	265.1
		Anoxia:	259.9

Airley, R. et al (2001) *Clin. Cancer Res.* **7**: 928–934
Vera, J. C. et al (2001) *Biochemistry* **40**: 777–790