

Poster Session 2 – Pharmacognosy

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Anti-inflammatory activity of *Vernonia anthelmintica* (l.) Willd. seed extracts

M. Pires, J. R. S. Hoult* and A. Raman

Pharmacognosy Research Laboratories, Department of Pharmacy and *Centre for Cardiovascular Biology and Medicine, King's College London, The Franklin-Wilkins Building, 150 Stamford Street, London SE1 8WA, UK

Vernonia anthelmintica (L.) Willd. (Asteraceae) seeds are used in traditional Ayurvedic medicine as an anthelmintic, antipyretic and anti-inflammatory agent, as well as to treat skin diseases such as psoriasis (Kirtikar et al 1935). One of the key pathological features of this common, chronic, skin disorder is inflammation.

This study focuses on the use of *V. anthelmintica* seeds (VA) as a potential anti-psoriatic agent, employing mixed rat peritoneal leucocytes as an in-vitro anti-inflammatory model. These cells were chosen as they express inflammatory pathways for both cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) (Moroney et al 1988). The ability of VA to inhibit either of these pathways in this physiologically relevant cell system was determined using radioimmunoassay to measure the generation of two pro-inflammatory mediators, thromboxane B_2 (TXB_2) and leukotriene B_4 (LTB_4).

Different VA extracts were prepared using Soxhlet extraction (6 h), with solvents of different polarity. Sequential extraction with light petroleum (LP), chloroform and methanol (MeOH) gave extracts A3 (LP), A3EX (LP extract exposed to direct sunlight) and A7 (MeOH). Direct MeOH and ethanol extraction gave MET and ETVA respectively and extraction of LP-defatted seeds with dichloromethane, resulted in extract DCM.

All extracts were tested at three different concentrations (5, 15 and 50 $\mu g\,mL^{-1}$) for their ability to inhibit the generation of TXB_2 and LTB_4. The results revealed the oil extracts A3 and A3EX to display differences between each other (A3EX was less active). This indicated that the potential anti-inflammatory compounds may be light sensitive. As the DCM extract was also found to display good activity against the generation of both TXB_2 and LTB_4, this indicated the anti-inflammatory compound(s) were not solely present in the oil extracts. This suggestion, in part, correlated with the results for the relatively polar A7, a sequential (defatted) methanolic extract that was only found to inhibit LTB_4 generation. This was interesting, as in comparison with the direct methanol extract MET, which was active against both TXB_2 and LTB_4, A7 had a selective inhibitory effect on LTB_4 suggesting that methanol soluble components other than lipids were responsible for the effect. Consequently, A7 could be said to selectively inhibit 5-lipoxygenase and exert its effect only on the 5-LOX pathway whereas extracts containing less polar compounds affected both 5-LOX and COX. The direct ethanol extract ETVA was not found to be as effective as MET in inhibiting the generation of TXB_2 and LTB_4.

These results clearly demonstrate the anti-inflammatory effect of VA substantiating its traditional use in the treatment of psoriasis.

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This work was funded by Phytopharm plc.

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An investigation of the inhibitory effects of plant extracts on a starch - α-amylase assay

K. Bawden, J. Quant and A. Raman

Pharmacognosy Research Laboratories, Pharmacy Department, King's College London, Franklin-Wilkins Building, 150 Stamford Street, Waterloo, London SE1 9NN, UK

Diabetes mellitus, a disorder resulting in a disruption of glucose homoeostasis, is becoming a serious health care challenge worldwide with an expected incidence of 239 million people by the year 2020 Patel (1999). A number of complications may arise, some fatal, caused primarily by abnormally high blood-glucose levels. Attempts to control diabetes have increased significantly over the last decade with the aim of extending life duration and simultaneously improving its quality.

One approach in the discovery of new treatments is based on the reputed properties and uses of plants. Encouragement in this area comes from the fact that at one time traditional plant remedies were the only forms of treatment for diabetes, and their use is documented in many sources such as the Indian Materia Medica (Chopra et al 1956). As part of an ongoing research program for the discovery of novel anti-diabetic compounds a bioassay that would allow activity-guided fractionation of α-amylase inhibitors from plants was developed. This digestive enzyme is responsible for hydrolysing starch to maltose, which further breaks down to glucose prior to absorption in the small intestine. Inhibition of the enzyme should reduce the unfavourable high postprandial blood-glucose peaks in diabetics.

The Sigma-Aldrich method for measuring α-amylase activity was used as a basis for the assay. The recommendations were modified to provide optimal conditions for detection of enzyme activity, and to allow the evaluation of the inhibitory properties of plant extracts when dissolved in a low percentage of a suitable vehicle (DMSO). Starch substrate (0.25 % w/v) was incubated with α-amylase (1 $U\,mL^{-1}$) over a 3-min period at 25°C, pH 6.9. The generation of maltose was quantified using 3,5-dinitrosalicylic acid solution in alkaline conditions. The reduction of this compound to 3-amino-5-nitrosalicylic acid by maltose was detected at 540nm.

Thirty traditional anti-diabetic plant extracts were evaluated in this assay. Of these, cold hexane extracts of *Murraya koenigii* Spreng (Rutaceae) and *Cyperus rotundus* L. (Cyperaceae) were discovered to have significant inhibitory properties on the enzyme. Sequential soxhlet extraction using hexane, chloroform, methanol and water yielded further fractions and confirmed that the hexane extract of *Murraya koenigii* and the methanolic extract of *Cyperus rotundus* contained the highest levels of the active inhibitory compounds. Further activity-guided fractionation by vacuum liquid chromatography and flash chromatography is underway to isolate the active compounds.

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

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Antioxidant activity of some commonly used vegetables in Iranian diet

E. Souri

Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran (14155-6451), Iran

A large number of xenobiotics, including environmental agents and medically useful drugs, exert their toxic effects via free radical mechanisms. There is an interest in the use of antioxidants for treatment or prophylaxis of various disease states (Maxwell 1995; Halliwell 2000). Although an appreciable amount of daily carcinogens occurs in cooked meat, the cancer risk may be mitigated by having edible plants at the same meal or by addition of antioxidants before cooking. In recent years, considerable effort has been directed in search for safe antioxidants from natural sources.

In our laboratory, a program has been initiated to discover natural antioxidants from plant extracts. A simple fluorimetric assay for evaluating antioxidant activity was used which is based on inhibition of peroxidation of linoleic acid in aqueous media (Furuta et al 1997). Accordingly, methanolic extracts of 26 vegetables which are regularly used as components of the Iranian diet were evaluated for antioxidant activity at a range of 0.4 to 400 μg of extract in the reaction mixture using 40 μg of linoleic acid. The percent of inhibition was measured by comparing the results with a control without vegetable extract. α-Tocopherol and quercetin were used as

positive controls. IC50 values were determined using the percent of inhibition versus log concentration curve.

Among the vegetables tested, savory, radish leaf and garden-cress (IC50 = 0.01, 0.04 and 0.05, respectively) had exceptionally high activity — even higher than that of quercetin (IC50 = 0.10 μg). The IC50 values of some other vegetable extracts, including spearmint, leek, chive (aerial part), lettuce and dill, were lower than α-tocopherol (IC50 = 0.60 μg) but higher than quercetin (IC50 = 0.10 μg). According to the results of this study, daily consumption of small amounts of these vegetables may have antioxidant activity and a preventive role in diseases related to free-radical mechanisms.

Furuta, S., et al (1997) *J. Food Sci.* **62**: 526–528
Halliwell, B. (2000) *Lancet* **355**: 1179–1180
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Authentication of the Chinese drug Agarwood

M. W. Lee, P. J. Houghton, M. S. J. Simmonds*, C. Leon* and G. C. Kite*

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NN, UK and *Jodrell Laboratory, Royal Botanic Gardens Kew, Richmond TW9 3DS, UK

Agarwood is a prized drug in Chinese traditional medicine and so is expensive. It consists of the heartwood of *Aquilaria sinensis* or *A. agallocha* containing a resinous substance arising from infection with fungi such as *Penicillium* spp. (Yang 1997). Scarcity of good material due to overcollection from the wild has meant that many samples are adulterated so it is important that criteria exist to determine their authenticity and quality.

An authenticated sample of *A. sinensis* was compared microscopically with five commercial samples obtained in London and Hong Kong but no significant differences between them were noted. Ethanolic extracts were made and compared chromatographically using TLC (silica gel GF_{254}/chloroform–methanol 9:1 visualised under UV light 254 nm) and LC-MS (ODS/water–1% acetic acid in methanol in a 75:25 to 0:100 gradient).

The authenticated material and three of the samples showed a prominent zone or peak. This substance was isolated and found to be the chromone agarotetrol. This substance was considered to be a useful chemical marker for authentic Agarwood since it was only detected in fungus-infected samples and appeared to be chemically stable and could not be detected in a sample of the related species *A. beccarensis*.

Three traders' samples of powders claimed to be Agarwood and eight multi-ingredient pills claiming to contain the drug were examined by TLC and LCMS. Agartetrol could be detected in only two of the powders and none of the multi-ingredient pills. Thus it is doubtful whether the material used in many of these products had been authenticated as agarwood before being incorporated into the drug.

Yang, J .(1997) In: *Modern studies of Chinese herbal medicine*. Vol. 3, Chinese Academy of Medical Sciences, Beijing, pp 1–21

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Antioxidant activity of some common Chinese vegetables

L. Luk, G. Moi and P. J. Houghton

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NN, UK

There is great current interest in the potential role of antioxidants in foodstuffs as preventative agents against several diseases such as cancer and neurodegenerative conditions. Considerable attention has been paid to fruit and vegetables consumed in the Western diet but little work has been carried out on those used in other cultural contexts.

In this study, five common vegetables used in Hong Kong were studied: Chinese turnip roots (*Brassica rapa)*, lotus roots (*Nelumbo nucifera)*, chilli fruits (*Capsicum frutescens)*, loofah fruits (*Luffa cylindrica)* and ginger rhizome (*Zingiber officinale)*. The vegetables were purchased in London.

The vegetables were treated in the same way as they are usually cooked and extracts made with water (where the vegetable was boiled) or ethanol (where the vegetable was fried). The extracts were tested at 10 mg mL^{-1} and 20 mg mL^{-1} concentrations of vegetable using the thiobarbituric acid (TBA) test (Uchiyama & Mihara 1978) which measures, by colorimetry, the amount of malonaldehyde formed by oxygen free radical attack on the lipids in brain liposomes. The amount of malonaldehyde is directly proportional to the oxidation of the lipids in the liposomes. Propyl gallate (PG) 10^{-4} M was used as a positive control and the results (shown in Table 1) obtained for the extracts were expressed as a percentage of the value obtained with PG .

Table 1 Antioxidant activity of 5 Chinese vegetables

Vegetable	Anti-oxidant value	
	10 mg mL^{-1}	20 mg mL^{-1}
Brassica rapa (boiled)	5.6 ± 1.9	10.2 ± 1.1
Capsicum frutescens (fried)	54.4 ± 0.4	74.4 ± 0.6
Luffa cylindrica (fried)	19.7 ± 2.2	33.7 ± 0.8
Nelumbo nucifera (boiled)	98.9 ± 1.3	99.6 ± 1.4
Zingiber officinale (boiled)	45.9 ± 0.7	58.7 ± 0.8

Antioxidant activity is expressed as % absorbance of PG 10^{-4} M in TBA test; mean ± s.e.m.; n = 3. All activity showed a dose-related response and lotus root was shown to have the greatest antioxidant effect

The presence of antioxidants in the extracts was also examined by developing them using TLC and spraying plates with 0.2% DPPH in methanol (Cavin et al 1998). Duplicate plates were examined using a range of spray reagents for common phytochemical types. The antioxidant compounds present in lotus root did not give characteristic colours with any of the other detection reagents used. Phenolic compounds were shown to be responsible for the activities shown by ginger and chilli.

Cavin, A., et al. (1998) *Planta Med.* **64**: 393–396
Uchiyama, M., Mihara, M. (1978) *Anal. Biochem.* **86**: 271–278

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There is little correlation between 1,8-cineole content and choline-esterase inhibitory activity of different samples of sage

R. Jones, P. J. Houghton and Y.-H. Ren

Pharmacognosy Research Laboratories, Department of Pharmacy, King's College London, 150 Stamford Street, London SE1 9NN, UK

A study to investigate any scientific basis for the reputation of sage (*Salvia officinalis*, *S. lavandulaefolia*) as being good for the memory showed that the volatile oil possessed choline-esterase inhibitory properties and that 1,8-cineole was the compound present in the greatest concentration which showed a major inhibitory effect (Perry et al 2000). Inhibition of choline esterase results in elevated levels of acetylcholine (ACh) in the CNS and this approach is used clinically in the symptomatic relief of early stages of Alzheimer's disease, where loss of cognitive function is associated with low levels of ACh.

The relative amounts of the constituents of volatile oils are known to be very variable due to genetic and environmental factors and so oils of different composition may exhibit different degrees of biological activity. It was therefore considered of interest to correlate cineole content with choline-esterase inhibition for a variety of samples of sage.

Samples (25 g) of sage from four different origins were extracted with dichloromethane and, after removal of the solvent under low temperature and pressure, the residues were dissolve to a standard 10 mL. The cineole content of each extract was determined by capillary gas chromatography (Phase DB5 30 m × 0.32 mm i.d. with nitrogen; 70°C rising by 6°C per minute up to 200°C, maintained for 30 min detected by FID) using methyl salicylate as an internal standard.

The choline-esterase activity of each extract was determined using human erythrocytes and thiocholine as a substrate with physostigmine being used as a positive control (Perry et al 2000). Results are shown in Table 1.

Table 1 Content of 1,8-cineole in extracts of sage samples

Sage sample	Origin	1,8-Cineole content (% w/w)	IC50 value ($\mu g\,mL^{-1}$)
S. lavandulaefolia (A)	Crete	0.0136	0.08
S. lavandulaefolia (B)	UK	0.0947	0.43
S. officinalis (C)	UK	0.0299	0.12
S. lavandulaefolia (D)	Spain	0.0061	0.09
1,8-Cineole			0.018

There appears to be little correlation between 1,8-cineole content and choline-esterase activity since sample B, which contained the highest concentration of 1,8-cineole, had the weakest inhibitory effect.

These results indicate the presence of choline-esterase inhibitory compounds other than 1,8-cineole and may also de due to synergistic effects.

Perry, N. S. L., et al (2000). *J. Pharm. Pharmacol.* **52:** 895–902

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Methoxylated flavones from *Artemisia rupestris*

S. Halike and P. J. Houghton*

Xinjiang Uyghur Autonomous Region Institute of Drug Control, 9 South Xinhua Road, Urumchi,830002, China and *Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 8WA, UK

Artemisia rupestris L. (Compositae) is used by the Uighur people of Xinjiang Uighur Autonomous Region of China, for a variety of anti-inflammatory conditions including influenza, dermatitis, measles, burns, hepatitis and snakebite (Xinjiang Public Health Bureau 1975). Previous research on this plant have resulted in isolation of seven known compounds, including flavonoids and sesquiterpenes such as rupestric acid (Liu & Yu 1985*)*, rupestonic acid (Xu & Chen 1988) and iso rupestonic acid (Xu et al 1991). Methanolic extracts of the aerial parts of air-dried, powdered plant material followed by preparative chromatography using a polyamide column yielded three compounds. The most abundant compound was identified as 5,3'-dihydroxy-6,7,8,4'-tetramethoxyflavone, also known as gardenin D, by UV, MS and NMR spectroscopic methods. I t is likely that these compounds contribute to the anti-inflammatory effect. The anti-inflammatory and anti-oxidant screening of extracts and other constituents is in progress.

The project has been sponsored by The Chinese Scholarship Council

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An investigation of the role of protein kinase C in the melanocyte stimulant effects of piperine and analogues

C. M. T. Chronnell, R. Venkatasamy and A. Raman

Pharmacognosy Research Group, Department of Pharmacy, Kings College London, Franklin-Wilkins Building, 150 Stamford Street, London, SE1 9NN, UK

Vitiligo is a skin disorder characterised by loss of pigmentation and active melanocytes from lesional areas. Numerous theories exist regarding its aetiology but as yet there is no consensus. The hair follicle is believed to harbour a melanocyte reservoir. Plant-derived compounds called psoralens are currently used in conjunction with UVA light treatment (PUVA) to induce repigmentation in vitiligo by activation and proliferation of these hair follicle melanocytes.

A potential future therapy under investigation by our group is the use of piperine (PIP) from black pepper (*Piper nigrum* L.). This compound has the capacity to stimulate melanocyte proliferation and alter cell morphology in-vitro, an effect which is blocked by the protein kinase C (PKC) inhibitor RO-31-8220 (Lin et al 1999a). PKC is one of the major signal transduction pathways regulating growth and cellular metabolism and has been implicated in a number of melanocyte functions.

Two analogues of PIP, tetrahydropiperine (THP) and the cyclohexylamide analogue of piperine (CHP), were synthesised and tested in-vitro using a pigmented mouse melanocyte cell line to determine if they also possessed melanocyte stimulatory activity similar to the parent compound. To confirm the preliminary findings on mechanism of action, three inhibitors of PKC (RO-31-8220, staurosporine and calphostin C) were tested alone and in combination with PIP, THP or CHP to determine if cell stimulation by these compound(s) is affected and to outline a possible role for PKC.

Cells were seeded in 96-well plates (6×10^3 cells per well) and allowed to settle for 24 h before addition of compound alone (PIP/THP/CHP) at 1 μM final concentration or compound (1 μM) plus inhibitor (10^{-4} to 10^{-9} M). Cells were fixed on experimental day five and assayed using Sulphorhodamine B as described by Lin et al (1999b). Optical density was read at 550 nm (SpectraMax 190, Molecular Devices, USA) and converted to percentage growth compared to values obtained for cells alone (no compound, no inhibitor). Results are shown in Table 1.

Table 1 Effect of piperine and analogues on melanocyte proliferation

% Cell growth for each combination	Inhibitor concn (nM)				
	0	0.1	1	10	100
RO-31-8220	100.0	93.3	95.2	67.0	77.1
PIP + RO	159.4	132.7	133.0	141.6	144.6
THP + RO	158.7	106.1	106.5	112.7	108.7
CHP + RO	142.9	93.2	84.9	86.4	89.0
Staurosporine	100.0	63.2	80.2	102.1	Not tested
PIP + ST	127.5	76.5	75.1	88.3	
THP + ST	140.9	92.0	74.2	85.6	
CHP + ST	128.2	85.2	68.4	70.6	
Calphostin C	100.0	Not tested	59.6	61.2	100.3
PIP + CPC	194.9		96.9	103.8	90.3
THP + CPC	247.1		89.9	113.2	126.2
CHP + CPC	170.0		92.4	92.6	82.8

RO = RO-31-8220; ST = staurosporine; CPC = calphostin C

In conclusion, piperine and its analogues are able to stimulate melanocyte proliferation and inhibitors of PKC affect this activity. Therefore, the ability of these compounds to increase melanocyte cell numbers is likely to involve activation of PKC pathways.

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Lin et al (1999b) *J. Ethnopharm.* **66**: 141–150