A search for novel biologically active compounds in the phyllodes of *Acacia* species

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SUMMARY

For thousands of years, indigenous Australians have relied on native plants as a source of medicinal agents. With a wide distribution and abundance, *Acacia* species were used routinely for this purpose. Preparations from at least 30 species of *Acacia* were either applied externally for skin sores, cuts, scabies and burns or taken internally for colds, coughs, sore throats and headaches. Some were used in smoke treatments for mothers and their babies. We briefly review the traditional medicinal uses of *Acacia* species and report on some findings obtained through preliminary screening of extracts from more promising species such as *Acacia pruinocarpa* and *A. dictophleba*.

INTRODUCTION

Acacias (Mimosaceae) are well known to Australians as wattles. More than 950 recognised species occur in Australia (Maslin 2001). Many species have adapted to the harsh conditions of the semi-arid and arid zones but they are also common in tropical and subtropical regions. Being widely distributed and often attractive, they have become icons to the Australian people. Many species have appeared on stamps, coins, military insignia and even the national coat of arms (Pearn 1993). The Golden Wattle, *Acacia pycnantha*, is the national floral emblem.

Indigenous Australians would have tested many species for their medicinal potential. Preparations from those species considered useful were mostly applied externally to treat sores, cuts, burns and scabies (Table 1). Among the more favoured species were *Acacia ancistrocarpa*, *Acacia pruinocarpa* and *Acacia holosericia*. Others were taken internally to treat colds, sore throats and as cough suppressants (Table 1). Routinely prescribed for coughs, for example, were preparations of *A. bivenosa* subsp. *wayi* (Cribb and Cribb 1981), *A. dictophleba* (Bindon 1996), *A. ligulata*, *A. monticola* (Reid and Betts 1977) and *A. tetragonophylla* (Webb 1959). Interestingly, one of the better-known uses for *A. tetragonophylla* was in the removal of warts (Barr 1993). The needle-like phyllodes were used to puncture unwanted growths causing them to shrivel up and dry out. An infusion of the phyllodes of *A. tetragonophylla* was used as an antiseptic to treat dysentery (Reid and Betts 1977).

Similar illnesses were treated with the smoke from burning wattles. For example, *Acacia ancistrocarpa* was considered useful in treating diarrhoea in babies (Reid and Betts, 1977). Most smoke treatments, however, were used to strengthen the mother and her child following birth. As part of the sacred rituals that followed childbirth, mothers would lay with their babies near trenches in which *Acacia* and other plant species were being burnt. In some cases this was believed to also inhibit post-partum bleeding.

Despite their widespread distribution and broad spectrum of uses, it is somewhat surprising that there is little or no known use for wattles in some areas of Australia. Scarlett *et al.* (1982), for example, reported that they were not used for medicinal purposes in Arnhem Land, one of Australia's largest Aboriginal reserves. It seems unlikely that indigenous Australians would have ignored the wattles that occur in such areas, and it is possible that knowledge on the medicinal potential was lost before it could be recorded. Aboriginal people kept no written account of their knowledge, much of which was lost when European colonists and their western medicines and practices began arriving (Scarlett *et al.* 1982).

The traditional medicinal uses of Australian plants did not go unnoticed by the European colonists. Impressed with the 'healing' powers of wattles, colonial doctors soon added many to their own practices. Prescriptions for the treatment of dysentery, diarrhoea and sore eyes were common. Many of these were still being used as recently as 1940 and were later named in honour of those doctors who adopted them (Pearn 1993).

As part of a larger study aimed at identifying novel biologically active compounds from Australian native plants and animals, we collected plant material from a number of the more promising *Acacia* species (Table 1) and screened them on routine bioassays. Previous studies into the medicinal uses of other native plants have already yielded a number of interesting pharmacologically active compounds (Pennacchio *et al.* 1995, 1996a, 1996b, 1997). We hope that our work will substantiate further some medical claims made for Australian plants by indigenous Australians. The preliminary results of our study are presented here.

TABLE 1
Acacia species which are known to be used for medicinal purposes by Indigenous Australians. Those species marked with an asterisk **(*)** were screened for biologically-active compounds.

SPECIES	PART USED	PREPARATION	TREATMENT	REFERENCES
A. adsurgens **(*)**	Leaves and twigs	Smoke treatment	To treat babies for diarrhoea	Low (1990)
A. ancitrocarpa	Leaves and twigs Leaves and twigs	Bashed and soaked Heated Smoke treatment	Skin sores & headaches Swellings and pain To treat babies for diarrhoea	Reid and Betts (1977) Bindon (1996) Low (1990)
A. aneura **(*)**	Leaves and twigs	Smoke treatment	Strengthens mothers and newborn babies	Barr (1993)
A. auriculoformis **(*)**	Leaves Leaves and pods. Leaves and pods.	Decoction Lather Lather	Antiseptic cleanser Pruritis & allergy rash Pain in legs and body	Barr (1993) Levitt (1981)
A. bivenosa subsp *wayi* **(*)**	Bark and roots	Infusion	Cough and cold medicine	Cribb and Cribb (1981)
A. coriacea **(*)**	Trunk and lateral roots	Ash	Chewed with pituri	Cleland and Tindale (1958)
A. cuthbertsonii	Bark Bark Bark	Unknown Stripped Moistened	Toothache, pain and rheumatism Splint and bandages Headache	Reid and Betts (1977) Barr (1988)
A. dictophleba **(*)**	Leaves Leaves and branches	Infusion Smoke treatment	Cold, coughs and headaches Strengthens mothers and newborn babies	Bindon (1996) Meggitt (1962) Low (1990)
A. difficilis	Bark	Stripped	Bandages and splints	Barr (1998)
A. estrophiolata	Bark from branches Bark Root bark	Decoction Moistened Infusion	Antiseptic for boils, scabies and sore eyes Bandage for sores and burn wounds Headache, sore throat, colds and alimentary discomfort	Barr (1993)
A. holosericea	Roots Leaves Roots Pods and seeds Pliable bark	Infusion Decoction Infusion Lather Moistened	Laryngitis Cleanser, deodorant and mild antiseptic for skin sores Sore throats Relieves itchiness Headaches	Reid and Betts (1977) Barr (1993)
A. inaequilatera **(*)**	Bast under cork bark	Infusion	For skin sores, cuts, chicken pox rashes.	Reid and Betts (1977)
A. kempeana	Leaves Leaves and twigs	Decoction Smoke treatment	Wash for severe colds Strengthens mother and newborn babies and help s stop post partum bleeding.	Barr (1993)
A. leptocarpa	Green leaves	Infusion	Sore eyes	Reid and Betts (1977)
A. ligulata	Leaves and branches Bark	Smoke treatment Decoction	Diaphoretic for several complaints including those of the nervous system and after childbirth Cough medicine	Barr (1993)
A. lysophloia	Leaves and twigs Leaves and branchlets Young leaves and twigs Phyllodes	Infusion Scorched on hot embers Decoction Smoke treatment	Body wash for colds and flu Relieves pain and aches when applied externally Anodyne of post-natal therapy Stregthens mothers and newborn babies	Barr (1993)

TABLE 1 (continued)

SPECIES	PART USED	PREPARATION	TREATMENT	REFERENCES
A. melanoxylon	Bark	Infusion	Skin wash	Low (1990)
A. monticola	Branches Roots	Decoction Infusion	Coughs and colds Cures coughing	Webb (1969) Reid and Betts (1977)
A. multisiliqua	Leaves	Boiled or crushed	Vapour inhaled for nasal congestion	Barr (1993)
A. oncinocarpa	Leaves	Decoction	Respiratory illnesses	Barr (1993)
A. pellita	Pods	Lather	Pruritic skin	Barr (1993)
A. pruinocarpa **(*)**	Leaves and branches	Smoke treatment	Strengthens mothers and newborn babies	Low (1990)
A. pyrifolia	Bark	Decoction	Skin sores (applied around the sore)	Webb (1969)
A. salicina	Leaves	Unknown	Considered to have medicinal properties	Latz (1995)
A. spondylophylla	Unknown	Unknown	Skin wash	O'Connell et al. (1983)
A. tetragonophylla	Inner bark Wood Leaves	Infusion Burnt to ashes Chewed	Cough medicine Antiseptic Dysentery	Webb (1969) Reid and Betts (1977)

MATERIALS AND METHODS

The phyllodes and stems of eight species of *Acacia* (asterisked in Table 1) were collected in early 2001 from the Ophthalama Dam area of Newman, Western Australia, by researchers working for the Mulga Research Centre (Curtin University of Technology). Additional material was collected from the Department of Environmental Biology's field trial area at the Bentley campus, Curtin University of Technology. All specimens were identified by Bruce Maslin. Voucher specimens (KW 1-8) were deposited at the Department of Environmental Biology's Herbarium.

All material was dried in an oven at 50°C for 48 hours and then ground to a fine powder using a vegetative grinder (Dietz-Motoren KG, Eleckromotorenfragnik, West Germany, 220V, type WRB 80C12QSIL). The powdered material was then put through a series of chemical extractions using hexane, dichloromethane, methanol and deionised water. Only the aqueous extracts were screened for biological activity. The other extracts were set aside for future testing.

Brine Shrimp Lethality Test

The aqueous extracts of the leaves of the eight species were screened for chronic toxicity (24-hour exposure) with the Brine Shrimp Lethality Test (BSLT). The median lethal concentrations were derived graphically after the data were treated according to the Reed-Muench procedure.

Dried eggs of brine shrimp (*Artemia salina*) were placed into 20% seawater, which was constantly aerated at 25°C, for one hour. They were then immersed in 40% bleach for seven minutes and rinsed in water until the smell of bleach had disappeared. The eggs were then returned to the 20% seawater and left for 48 hours, during which time most of them had hatched. One-day-old larvae were used in the tests.

Four concentrations of the aqueous extracts were prepared with 20% seawater and then transferred to vials (5 ml), with six replicates. Ten brine shrimp were transferred to each vial and left at a constant temperature for 24 hours. A fifth group, the control, comprised brine shrimp placed in vials containing 20% seawater only. After 24 hours the dead brine shrimp were counted to establish the median lethal concentration (LC_{50}).

Allelopathy

Allelopathy is the inhibition of germination and growth in plants caused by the secondary metabolites of other plants (Mueller-Dombois and Ellenberg 1974). These substances are usually phenolics, but tannins have also been shown to inhibit germination and growth in plants. Lettuce seeds were used in germination trials aimed at identifying allelopathic substances from *Acacia* species. As with the previous test, four different concentrations of the aqueous extracts were used in each trial. The control group was treated with deionised water only. Lettuce seeds were germinated in petri dishes (25 in each) lined with filter paper, with four replicates. The petri dishes were kept in the dark in an incubator set at 25°C and were checked every 24 hours for 72 hours.

Antimicrobial bioassays

The aqueous extracts were also tested in a concentration-dependent manner for antimicrobial activity. The test

organisms were comprised of two species of bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes*) and one fungus (*Candida albicans*). Each extract was tested via small, sterilised paper disks saturated with a known concentration of the extracts. The disks were similar in size and shape to the commercial Oxoid Susceptibility Disks used in the controls (see below).

All three test organisms were grown on Mueller-Hinton blood agar plates obtained commercially from Royal Perth Hospital. All tests were performed in sterile conditions. Known antibiotics were used as controls. Penicillin G (Oxoid Susceptibility Disks) was used in all tests involving *S. aureus*. Erythromycin (Oxoid Susceptibility Disks) was used as a control for *Streptococcus pyogenes* and Nystatin was used in tests with *C. albicans* (Oxoid Susceptibility Disks).

RESULTS

Brine Shrimp Lethality Test

Following 24 hours of exposure to the extracts (chronic toxicity), the aqueous extract of the leaves of *A. pruinocarpa* resulted in an LC_{50} of 100 ppm when tested on the nauplii larvae of brine shrimp (Table 3). This was the most toxic extract. The second most toxic was *A. dictophleba*, which produced an LC_{50} of 158 ppm. These values were compared to those reported for potassium dichromate ($K_2Cr_2O_7$) by Sam (1993) to establish a relative toxicity for the extracts (Table 2). The least toxic extract was from the phyllodes of *A. inaequilatera*. Interestingly, the powdered foliage of this species gave one of the authors a skin rash.

TABLE 2
Chronic median lethal concentration for *Acacia* species using the BSLT. Relative toxicities are compared to $K_2Cr_2O_7$ (from Sam 1993).

PLANT SPECIES	LC_{50} (24 H) PPM	RELATIVE TOXICITY
A. pruinocarpa	100	0.37
A. dictyophleba	158	0.21
A. adsurgens	251	0.15
A. aneura	562	0.06
A. auriculiformis	1259	0.03
A. bivenosa	1778	0.02
A. coriacea	2113	0.01
A. inaequilatera	8912	3.8×10^{-3}

Allelopathy

Like the BSLT, the aqueous extract of phyllodes of *A. pruinocarpa* was the most potent of the species tested. A concentration of 0.19 g/L (Table 3) was sufficient to inhibit germination in all lettuce seeds. In contrast, all 25 control seeds in each petri dish germinated within 72 hours. The aqueous extract of *A. coriacea* phyllodes had no effect on the seeds at the concentrations tested, the highest of which was 20 g/L.

TABLE 3
Results of the Seed Germination Inhibition Assay on *Acacia* species. The lowest concentration, which completely inhibited germination in the lettuce seeds, is given.

PLANT SPECIES	CONC. (G/L)
A. pruinocarpa	0.19
A. dictyophleba	2.50
A. bivenosa	5.00
A. inaequilatera	7.00
A. aneura	10.00
A. auriculiformis	50.00
A. adsurgens	80.50
A. coriacea	No inhibition

Anti-Microbial bioassays

None of the aqueous extracts screened for antimicrobial activity inhibited the growth of the three test pathogens (Table 4). All three pathogens were, however, inhibited by known antibiotics. The average inhibition induced by Penicillin G on *S. aureus* was 42.60 ± 1.37 mm, and on *Streptococcus pyogenes* it was 38.63 ± 1.32 mm. Erythromycin was less potent. Nystatin is effective only on fungi. The inhibition mediated on *C. albicans* was 21.42 ± 1.19 mm. The average inhibition induced by these three controls was greater than that required before a pathogen could be considered susceptible to them.

TABLE 4
Results of the tests for antibiosis. Inhibition is reported in mm (mean ± SEM) for all extracts and for the controls. NA = not applicable.

SPECIES	*Staphylococcus aureus* (mm)	*Streptococcus pyogenes*(mm)	*Candida albicans* (mm)
A. adsurgens	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. aneura	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. auriculiformis	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. bivenosa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. coriacea	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. dictyophleba	*0.00 ± 0.00*	*0.00 ± 0.00*	*0.00 ± 0.00*
A. inaequilatera	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
A. pruinocarpa	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Penicillin G	42.60 ± 1.37	38.63 ± 1.32	NA
Erythromycin	27.00 ± 0.89	25.31 ± 1.75	NA
Nystatin	NA	NA	21.42 ± 1.19

Discussion

Few studies have attempted to substantiate the medical claims made by Aboriginal people for *Acacia* and other plants. Previous work by our research team has shown that some of the plants routinely used for medicinal purposes by indigenous Australians do, in fact, produce

beneficial natural products (Pennacchio *et al.* 1995, 1996a; Pennacchio and Ghisalberti 2000). The results of our study with *Acacia*, despite being preliminary, add to the growing evidence that native plants may be a source of biologically active compounds.

One likely source for such compounds is the phyllodes of *Acacia pruinocarpa*. An aqueous extract of phyllodes grown at the field trial area at the Curtin University of Technology campus resulted in a significantly toxic effect on brine shrimp nauplii larvae and inhibited seed germination in a standard test for allelopathy. Equally promising are *A. dictyophleba* and *A. adsurgens*. Whether these three species are worth further investigation depends largely on the type of activity sought and the concentrations required to mediate the desired effect. The BSLT, for example, is a rapid, relatively simple toxicity test (Sam 1993). This preliminary bioassay, which may serve as a test for antitumour and pesticidal activity, does, however, have its limitations. There is currently no accepted standard for determining whether or not the level of toxicity exhibited by a compound or an extract is useful.

In our laboratory, we have adopted a practice similar to that of Sam (1993), comparing the median lethal concentration to that of potassium dichromate ($K_2Cr_2O_7$). Extracts that produce a similar toxicity to $K_2Cr_2O_7$ (in the 24-hour test) are considered worth additional testing. Work on extracts from *A. pruinocarpa*, *A. dictyophleba* and *A. adsurgens* will therefore continue. Before any bioassay-guided fractionations are attempted, however, the tannins will be removed from the extracts using a polyamide column.

Acacia pruinocarpa and *A. dictyophleba* also featured prominently in tests for allelopathy (the inhibition of germination and growth of plants caused by secondary metabolites of other plants), but the tannin content of these species is not known and it is therefore not clear whether those present in the phyllodes are likely to be responsible for inhibiting germination of lettuce seed. Both species will be rescreened once the tannins have been removed from the extracts.

To indigenous Australians, species such as *A. auriculiformis* were considered useful as antiseptics and for treating topical infections (Table 1). These were often prepared by crushing phyllodes and then mixing them with water to create a lather. Others were prepared as decoctions. This prompted our team to test for antimicrobial agents using similar aqueous extracts.

Surprisingly, none of the species we screened inhibited the growth of the two test bacteria, *Staphylococcus aureus* and *Streptococcus pyogenes*, or that of the thrush-inducing fungus, *Candida albicans* (Table 5). Barr (1993) performed similar tests with using *Klebsiella pneumoniae*, *Salmonella typhimurium* and *Escherichia coli*, none of which was inhibited by the extracts tested. The pods of *A. auriculiformis* did, however, inhibit *S. aureus* (Barr 1993). This was not considered a useful antimicrobial agent since it only inhibited that bacterium. A preliminary study we conducted earlier revealed that the hexane, dichloromethane and methanol extracts of our *Acacia* species were also ineffective in inhibiting the test pathogens. It is unlikely that the eight species screened produce substances with antimicrobial activity.

Our work, which is still at a preliminary stage, will soon focus on isolating and identifying the active constituents responsible for some of the activity we have observed. Previous phytochemical surveys have resulted in several simple compounds, but the number is disappointing (Collins *et al.* 1990). Thirteen of the 30 species listed in Table 1 were screened for alkaloids with only nine testing positive in standard tests. *A. aneura*, *A. estrophiolata*, *A. holosericea* and *A. tetragonophylla* all tested positive in the Mayers alkaloid test (Collins *et al.* 1990; Barr 1993). Along with *A. lysiphloia*, *A. multisiliqua* and *A. pellita*, these also tested positive for the Silicotungstic acid test and Dragendorff's test.

A number of *Acacia* species were also screened for the presence of saponins and tannins. *A. aneura* (phyllodes and twigs), *A. holosericea* (phyllodes, bark and pods), *A. ligulata* (phyllodes and twigs), *A. lysiphloia* (phyllodes and twigs), *A. multisiliqua* (phyllodes) and *A. pellita* (pods) have all been shown to produce saponins and tannins . None of these has any known pharmacological activity but some have been implicated in the death of fish in Aboriginal fishing practices. Bark from these species was pounded and dispersed in small waterholes, where it was left overnight (Barr, 1988). Dead and stunned fishes were collected the following day, most of them victims of asphyxiation.

In conclusion, there is some evidence to suggest that *Acacia* species do produce interesting and useful biologically active compounds that are worth investigating further.

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