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Full Length Research Paper

A study of the antibacterial activities of selected Australian medicinal plants

T. Smyth[1], V. N. Ramachandran[1], P. Brooks[2]* and W. F. Smyth[1]

[1]School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, Northern Ireland.
[2]Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore, Queensland, Australia.

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The antibacterial activities of twenty six extracts from ten selected plants used in traditional Australian Aboriginal medicines have been investigated. The extracts were tested for growth inhibition of broth cultures of four gram-positive bacteria (*S. Aureus*, MRSA, *B. subtilis* and *M. luteus*), two gram-negative bacteria (*S. typhimurium* and *E. coli*) bacteria and yeast (*C. albicans*). Twenty three of the extracts displayed antibacterial activities against one or more bacterial strain. The majority of the extracts showed greater activity against gram-positive bacteria. In particular, the extracts of the fruit and bark of *Petalostigma pubescens* and the extract of the bark of *Euodia vitiflora* displayed bioactivity against all of the tested organisms. Traditional Australian medicinal plant extracts were shown to have antibacterial activity against mutli-drug resistant MRSA bacteria.

Key words: Antibacterial activity, traditional medicine, medicinal plants.

INTRODUCTION

Plants have been used for many thousands of years for their medicinal properties. Today, approximately 80% of the world's population, particularly in the developing countries, rely on medicines from plant materials for their healthcare (Gurib-Fakim, 2000). Over many centuries this considerable body of knowledge on medicinal plants has been built up through trial and error and then passed on verbally from one generation to the next. Tribal communities in both the developed and developing world still tend to use herbal medicines for their well-being. One such community is the Aborigines of Australia.

A number of attempts to document information regarding the use of plants by the Aborigines have been carried out. In 1973, the Northern Territory Department of Health started collecting information on some of these medicinal plants. By 1982 over 50 different medicinal plants had been reported with the help of Aboriginal health workers and tribal elders (Denvanesen and Henshall, 1982). Forty Aboriginal communities contributed to the first Aboriginal Pharmacopoeia for the Northern Territory, published in 1988 (Lassak and McCarthy, 2001). In this Pharmacopoeia 70 plants and 6 other substances used by the Aborigines were documented (Lassak and McCarthy, 2001). A further book detailing Australian medicinal plants and their uses has also been published (Barr et al., 1988). These medicinal plant parts or their extracts were applied either internally or externally to alleviate pain and to cure common ailments. Documentation of the use of plants for medicinal purposes by Aborigines in other parts of Australia is non-existent and much of this information has been lost permanently (Lassak and McCarthy, 2001). A notable example of a plant used by Aborigines is *Eremophila alternifolia*, the leaves of which are used to treat a variety of illnesses including fevers, influenza and headaches (Smith, 1991).

Despite the use of Australian medicinal plants by Aborigines for the treatment of a large number of infections, only a few of these plants have been investigated for their antibacterial effects. Considering that a large number of plants are used to heal wounds and other injuries, it can be assumed that these plants could possibly contain antibacterial agents to combat infections. The literature reports only one large study into the antibacterial activities of Australian plants (Palombo and Semple, 2001). They studied the antibacterial activities of traditional Australian medicinal plants against both gram negative and gram positive bacteria. The results showed that 12 out of the 39 different plants had antibacterial activity, particularly against gram-positive bacteria. Based on

*Corresponding author. E-mail: pbrooks@usc.edu.au. Tel: 61 7 54302828. Fax: 61 7 54302887.

this study, five of the antibacterial plants were tested for activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (Palombo and Semple, 2002). The results showed that the plant *Eremophia duttonii* from the *Myoporaceae* family had the greatest antibacterial activity. Further investigation by another study revealed a number of antibacterial activities in *Eremophia duttonii* (Smith et al., 2007). Another plant from the same family, *Eremophila neglecta,* has also been shown to possess antibacterial activity (Ndi et al., 2007).

This study therefore aims to determine whether selected Australian plants, used by Aborigines for medicinal purposes, possess antibacterial activity. The ten plants investigated in this study are listed in Table 1 along with the medicinal properties that have been reported. Different extracts from each of the 10 plants will also be tested for their antibacterial activity against multi-drug resistant MRSA bacteria.

MATERIALS AND METHODS

Plant collection and extraction

The plants investigated in this study were collected from South East Queensland, Australia. Dr Alison Shapcott, Senior Lecturer in Vegetation and Plant Ecology, University of the Sunshine Coast, Queensland, Australia, confirmed the sample specimens. Voucher specimens were retained at the University. The plant materials collected (leaves, bark or fruit) were left to dry for 3 days. The dried plant part was then blended to a fine powder and left soaking in methanol for 48 h. The methanol extract was then filtered from the plant residue which was extracted twice more with methanol and the combined methanol extracts were rotary evaporated to yield a thick gummy residue. A small sample of this crude residue was retained for bioassay (methanol extract). The remaining crude residue was treated with equal volumes of ethyl acetate and water and the two layers were separated. The ethyl acetate layer was dried over anhydrous magnesium sulphate, filtered and the solvent removed to yield an oily product (ethyl acetate extract). The water layer was concentrated to yield the water extract.

Bacteria and antibacterial assay

Muller-Hinton broth (MHB) and Muller-Hinton agar (MHA) were supplied by Oxoid Ltd. Hampshire, UK. The bacteria used for this study were the gram positive bacteria, *Bacillus subtilis* (ATCC 6051), *Micrococcus luteus* (University of Ulster, Coleraine, culture collection), *S. aureus* (ATCC 12600), *Methicillin-resistant-Staphylococcus aureus*, MRSA (clinical isolate, Coleraine hospital). The gram negative bacteria were *Escherichia coli* (ATCC11775), *Salmonella typhimurium* (ATCC 14028) and the yeast was *Candida Albicans* (University of Ulster, Coleraine, culture collection). The indicator p-iodonitrotetrazolium chloride (p-INT) was purchased from Sigma-Aldrich, Dorset, UK. Each plant extract tested was dissolved in acetone at a concentration of approximately 10 mg/ml and assays carried out according to the method described below (Smyth, 2008).

In column 1 of the 96 well plates, 100 µl of MHB was added as a blank control. Column 2 contained 25 µl of both sterile water and MHB as positive control. The acetone control was in column 3 and consisted of 10 µl of sterile water, 15 µl of acetone and 25 µl of MHB that is it contained the concentration of acetone that was added to each well for the plant extracts. Column 4 consisted of 25 µl of an antibiotic and 25 µl of MHB as the negative control. In columns 5 - 12 samples (extracts) can be added in either replicates of 4 (16 extracts per plate) or 8 (8 extracts per plate) down each column. Using replicates of 4, extracts were placed from the outside lane to the middle lane with each well containing 10 µl of sterile water, 15 µl of plant extract dissolved in acetone and 25 µl of MHB.

All the microorganisms, which were grown overnight in MHB, were adjusted to 10^6 cells ml^{-1} before use, according to their optical density (OD) at wavelength 650nm in MHB. Apart from the wells in column 1 all the other wells of the microtitre plate were inoculated with 50µl of bacterial suspension, sealed with parafilm™ and incubated at 37°C for 24 h in a Stuart orbital incubator (SI 50) at 50 rev min^{-1}. After incubation, 40 µl of the 0.2 mg/ml stock solution of p-iodonitrotetrazolium chloride (INT) was added to each well and then resealed and incubated at 37°C for 1 h. The plates were then visualised to check the controls in order to validate the assay. For wells that lacked a pink colour or contained strongly coloured extracts (making it difficult to determine whether there was a pink colouration), half of the totals of these wells (70 µl) were plated onto a MHA Petri dish and incubated overnight. Following incubation the colonies were counted in each case.

RESULTS

A total of 26 extracts (methanol, water and ethyl acetate extracts) of 10 different plants belonging to 7 different families were examined for their antibacterial activities against 7 different bacteria and yeast. The results are displayed in Table 2. After incubation with the indicator p-INT, the plant extract assays were visualised and those extracts which did not inhibit the microbial growth (that is presence of pink colouration), were considered not active. Extracts that displayed a faint pink colouration (that is, less coloured than from that of the controls) were considered bacteriostatic. Extracts that had no visible pink colouration were plated onto agar plates and then were incubated overnight and the colonies counted. Extracts that displayed between a 1 and 3 log decrease of cell numbers were considered bacteriostatic and extracts that showed a greater than 3 log decrease in cell numbers or no cell numbers were considered active (NCCLS, 1997).

DISCUSSION

From this study (Table 2), it can be seen that the majority of the methanol extracts exhibited a broad spectrum of activity with a number of extracts exhibiting activity against all microorganisms tested. Extraction with a mixture of water and ethyl acetate of the methanol soluble compounds of the plants resulted in the separation of water-soluble and water insoluble compounds. Water insoluble compounds present in the ethyl acetate extracts of the plants were found to be more active. This indicates that the hydrophobic secondary metabolites present in these plants were biologically active. The fact that the majority of these plants exhibit antibacterial activity demonstrates that their aboriginal uses, particularly for wound healing and infections, are well founded. The result also

Table 1. Selected Australian plants and their reported medicinal properties.

Family	Genus (common name)	Medicinal application	Reference
Euphorbiaceae	*Euphorbia hirta* (Asthma weed)	Treatment of asthma, bronchitis, stomach disorders and sedative properties. Anthelmintic	Everist (1981) Lassak and McCarthy (2001)
Euphorbiaceae	*Omalanthus populifolius* (Bleeding heart)	Wound healing	Webb (1948)
Euphorbiaceae	*Petalostigma pubescens* (Quinine tree)	Treatment of malaria, sore eyes, Toothache. Antiseptic wash	Webb (1948) Reid and Betts (1977) Reid and Betts (1979)
Malvaceae	*Hibiscus tiliaceus* (Cottonwood)	Wound healing. Antiseptic	Lassak and McCarthy (2001) Barr et al. (1988)
Moraceae	*Ficus coronata* (Sandpaper fig)	Wound healing	Lassak and McCarthy (2001)
Rhamnaceae	*Alphitonia excelsa* (Soap bush/Red ash)	Treatment of sore eyes, toothache, inflammation, stomach disorders, headaches. Antiseptic	Lassak and McCarthy (2001) Webb (1969)
Rutaceae	*Euodia elleryana* (Corkwood)	Antimicrobial	Khan et al. (2000)
Rutaceae	*Euodia vitiflora* (Toothache tree)	Toothache and general body pains	Webb (1969) Lassak and McCarthy (2001)
Sapindaceae	*Dodonaea viscose* (Sticky hopbush)	Treatment of toothache, fever, wounds, diarrhoea, stings.	Lassak and McCarthy (2001)
Sterculiaceae	*Sterculia quadrifida* (Peanut tree)	Wound healing and treatment of sore eyes. Also used for bites and stings.	Webb (1969)

show that a large number of these plant extracts exhibited activity against the multi-drug resistant MRSA bacteria and therefore may contain potentially novel compounds that could be used to treat MRSA infections. Only 3 out of the 26 extracts exhibited no antibacterial activity against any of the microorganisms tested. They were the water extracts of *Euodia vitiflora* (bark and leaves) and the methanol extract of the leaves of *Sterculia quadrifida.* According to the aboriginal pharmacopeia (Barr et al., 1988) *Sterculia* has been used for wound healing and the treatment for sore-eyes. This non activity could be because the leaves of the plant do not contain the necessary active substances and even if the Aborigines use the leaves they could have harvested them at different times of the year. A number of extracts were active against all microorganisms tested showing an excellent broad spectrum of activity. For example, the ethyl acetate extract of the fruit of *P. pubescens* and the methanol, ethyl acetate and water extracts of the bark of *P. pubescens* were active against all microorganisms tested. The ethyl acetate extract of the bark of *Euodia vitiflora* was also active against all microorganisms tested.

Other extracts showed varied bioactivities against the selected microorganisms. Nine extracts were either bacteriostatic or active (bactericidal) against all the microorganisms tested. For example, the water extract of the leaves of *O. populifolius* was active against six of the microorganisms but only bacteriostatic against the gram-negative bacteria *E. coli.* The water extract of the leaves of *S. quadrifida* and the ethyl acetate extract of the bark of *E. elleryana* were bacteriostatic against *B. subtilis* and the gram-negative bacteria but were active against the rest of the microorganisms tested. The methanol extract of

Table 2. The antibacterial activities of the various Australian plant extracts against 6 different microorganisms and a yeast.

Plant species (extract)	Solvent extract	*M. luteus*	*C. albicans*	*B. subtilis*	*S. aureus*	*E. coli*	*S. typhimurium*	MRSA
A. Excelsa (leaves)	Methanol	BS	NA	NA	NA	NA	BS	BS
D. viscosa (leaves)	Methanol	A	A	NA	BS	NA	BS	A
Euodia. elleryana (Bark)	Methanol	A	A	NA	NA	NA	NA	BS
	Water	BS	BS	BS	BS	NA	BS	BS
	Ethyl acetate	A	A	BS	A	BS	BS	A
E. elleryana (leaves)	Methanol	A	A	BS	BS	BS	A	A
E. vitiflora (Bark)	Methanol	A	A	NA	BS	NA	BS	A
	Water	NA	NA	NA	NA	NA	NA	NA
	Ethyl acetate	A	A	A	A	A	A	A
E. vitiflora (leaves)	Methanol	A	A	BS	BS	BS	BS	A
	Water	NA	NA	NA	NA	NA	NA	NA
	Ethyl acetate	A	A	A	BS	BS	BS	A
Euphorbia hirta (leaves)	Methanol	BS	A	NA	NA	NA	BS	BS
F. coronata (Bark)	Methanol	BS	BS	NA	BS	NA	NA	BS
F. coronata (Leaves)	Methanol	A	BS	BS	BS	BS	BS	BS
H. tiliaceus (Bark)	Methanol	A	A	A	A	NA	A	A
O. populifolius (leaves)	Methanol	BS	BS	NA	BS	NA	BS	A
	Water	A	A	A	A	BS	A	A
P. pubescens (fruit)	Methanol	A	A	BS	BS	BS	BS	A
	Water	A	A	A	BS	BS	BS	A
	Ethyl acetate	A	A	A	A	A	A	A
P. pubescens (Bark)	Methanol	A	A	A	A	A	A	A
	Water	A	A	A	A	A	A	A
	Ethyl acetate	A	A	A	A	A	A	A
S. Quadrifida (leaves)	Methanol	NA	NA	NA	NA	NA	NA	NA
	Water	A	A	BS	A	BS	BS	A

Not active (NA) indicates no inhibition of growth (pink colour). Bacteriostatic (BS) indicates a reduction in cell growth of between a 1 and 3 log decrease in cell numbers. Active (A) indicates a greater than 3-log decrease in cell number or no cell growth after plating of extract wells on agar plates.

the leaves of *E. elleryana* was bacteriostatic against *B. sutilis, S. aureus* and *E. coli* but active against the remainder of the bacteria tested. The methanol extract of the fruits of *P. pubescens* was only active against *M. luteus*, *C. albicans* and MRSA and bacteriostatic against the other microorganisms. As stated, the ethyl acetate extract of the fruits of *P. pubescens* was active against all bacteria studied but the water extract from the fruits had similar activity to the methanol extract except it was also active against *B. subtilis*. The methanol extract of the leaves of *E. vitiflora* had the same range of activity as the methanol extract of the fruit of *P. pubescens*, displaying antimicrobial activity against *M. luteus, C. albicans* and MRSA, with a bacteriostatic effect against the other bacteria tested. The ethyl acetate extract of the leaves of *E. vitiflora* showed similar activity and was also found to be active against *B. subtilis*. The methanol extract of the leaves of *F. coronata* had good bacteriostatic properties. It was active against only *M. luteus* but bacteriostatic against the remaining bacteria tested.

Generally the plant extracts were more active against the gram-positive bacteria which are consistent with other reported papers (Paz et al., 1995; Vlietinck et al., 1995). This is probably due to differences in the structure of the cell walls of gram-negative bacteria compared to gram-positive bacteria. For example, the methanol extract of the bark of *F. coronata* was not active against the gram-negative bacteria and *B. subtilis* but was bacteriostatic against the rest of the microorganisms tested.

A number of extracts displayed only selective activity against one or more microorganisms whereas some extracts only displayed a bacteriostatic effect against the microorganisms tested. The majority of the extracts had much greater antimicrobial activity against the gram-positive bacteria and a large number were bioactive against the multi-drug resistant MRSA bacteria. This study has demonstrated the potential of these Aboriginal plants as antibacterial products and, as such, they may

contain novel compounds that could be used to treat infections such as MRSA. Further investigation is currently being carried out in an attempt to isolate the bioactive compounds present.

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