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Antibacterial Activity of Selected Australian Native Plant Extracts

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Abstract

Thirty nine methanolic extracts from twenty five Australian native plants were investigated for their antibacterial activity against two Gram-positive (*Bacillus cereus*, *Bacillus subtilis*) and two Gram negative (*Aeromonas hydrophilia*, *Pseudomonas fluorescens*) bacterial species using the disc diffusion assay. Twenty eight of the thirty nine extracts tested (72%) inhibited the growth of one or more bacteria. *B. cereus* was the most susceptible bacteria with twenty one extracts (54%) inhibiting its growth. In comparison, fifteen extracts (38%) inhibited the growth of *P. fluorescens*, thirteen extracts (33%) inhibited the growth of *B. subtilis*, and ten extracts (26%) inhibited the growth of *A. hydrophilia*. *Backhousia citriodora* and *Callistemon citrinus* were particularly effective antibacterial agents, being capable of inhibiting the growth of all four bacteria. *Acacia aulacocarpa*, *Buckinghamia celsissima*, *Callistemon salignus*, *Allocasuarina littoralis*, *Eucalyptus major*, *Leptospermum bracteata*, *Leptospermum juniperium* and *Syzygium australe* were also good antibacterial agents, each being capable of inhibiting the growth of the majority of bacteria tested.

Key words: Australian plants, antibacterial activity, medicinal plants, methanol extracts

1. Introduction

Bacterial resistance to currently used antibiotics is becoming a concern to public health (Monroe and Polk, 2000). The development of bacterial super resistant strains is resulting in currently used antibiotic agents failing to end many bacterial infections. For this reason the search is ongoing for new antimicrobial agents, either by the design and synthesis of new agents, or through the search of natural sources for as yet undiscovered antimicrobial agents (Bhavnani and Ballow, 2000). Herbal medications in particular have seen a revival of interest (Chariandy *et al.*, 1999) due to a perception that there is a lower incidence of adverse reactions to plant preparations compared to synthetic pharmaceuticals. Coupled with the reduced costs of plant preparations, this makes the search for natural therapeutics an attractive option.

Prior to European settlement in Australia, the Aboriginal people used a variety of plant medicines to help maintain their health (Barr *et al.*, 1993; Lassak and McCarthy, 1993). One of the most serious health threats was considered infection. Bacterial growth in the hot, often humid climates in which the Aborigines lived provide ideal growth conditions for bacteria. This resulted in the Aborigines searching for antibacterial agents to curb infection and speed up the healing process. In fact, more than 150 plants from nearly 60 widely varied botanical families were used by Australian Aborigines as antiseptic agents (Lassak and McCarthy, 1993). For example, Aborigines from Groote Eylandt in the Northern Territory of Australia used a warm infusion of the *Crinum asiaticum* bulb to disinfect wounds, followed by wrapping the wound with *Planchonia careya* bark (Lassak and McCarthy, 1993). As well as protecting the wound, the bark itself was also thought to have antibacterial properties.

Much of the information about the antimicrobial activities of Australian plants is anecdotal. Few of the Aboriginal medicinal plants have been scientifically investigated for their antimicrobial activities. One study (Palombo and Semple, 2001) examined a panel of plant extracts commonly used by Australian Aborigines and found approximately 20% of the samples tested were able to inhibit bacterial growth. This group has also demonstrated the antiviral activity of the same Australian plants (Semple *et al.*, 1998). There are many other Australian plants, some used by Australian Aborigines, that have not as yet been properly examined for antibacterial activity. This research was carried out to further extend these studies and screen a variety of other Australian plants for antimicrobial activity. The antimicrobial activities of thirty nine extracts from twenty five Australian plants against four bacteria is reported.

2. Materials and Methods

2.1 Plant Material

2.1.1 Collection of Plant Samples

Acacia aulacocarpa (leaves), *Acacia complanta* (leaves and flowers), *Astrotricha longifolia* (leaves and flowers), *Banksia colina* (leaves), *Allocasuarina littoralis* (leaves), *Eucalyptus baileyana* (leaves), *Eucalyptus major* (leaves and flowers), *Jacksonia scoparia* (leaves), *Leptospermum juniperium* (leaves and flowers), *Melaleuca quinquenervia* (leaves) and *Mirbelia oxylobiodes* (leaves and flowers) were collected from Toohey Forest, Brisbane, Australia and were identified with reference to a taxonomic key to Toohey Forest plants (Coutts and Catterall, 1980). *Backhousia citriodora* (leaves), *Grevillea robusta* (leaves and flowers) and *Macadamia integriflora* (leaves and flowers) were collected from verified trees on Logan campus of Griffith University. *Adansonia gregorii* (leaves and flowers), *Brachychiton acerifolius* (leaves and flowers), *Buckinghamia celsissima* (leaves), *Callistemon citrinus* (leaves and flowers), *Callistemon salignus* (leaves and flowers), *Davidsonia pruriens* var. *jerseyana* (fruit), *Grevillea juncifolia* (leaves and flowers), *Leptospermum bracteata* (leaves and flowers), *Syzygium australe* (leaves), *Syzygium leuhmannii* (leaves) and *Westringa fruticosa* (leaves and flowers) were collected from verified trees in the suburbs of Brisbane, Australia.

2.1.2 Preparation of Crude Extracts

Plant samples were dried in a Sunbeam food dehydrator and the dried material was ground to a coarse powder. 1 g of each of the samples of dried plant material was extracted extensively in 50 ml methanol (Ajax, AR grade) for 24 hours at 4 °C with gentle shaking. The extract was filtered through filter paper (Whatman No. 54) under vacuum followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellet was dissolved in 15 ml 20 % methanol. The extract was passed through 0.22 µm filter (Sarstedt) and stored at 4 °C.

2.2 Test Microorganisms

All media was supplied by Oxoid Ltd. Microbial strains were obtained from Tarita Morais, Griffith University. Stock cultures of *Aeromonas hydrophilia*, *Bacillus cereus*, *Bacillus subtilis* and *Pseudomonas fluorescens*, were grown in nutrient broth at 30 °C and were subcultured and maintained in nutrient broth at 4 °C.

2.3 Evaluation of Antimicrobial Activity

Antimicrobial activity of each plant extract and was determined using a modified Kirby-Bauer (Bauer *et al.*, 1966) disc diffusion method. Briefly, 100 µl of the test bacteria/fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml. 100 µl of the microbial suspension was spread onto nutrient agar plates.

The extracts were tested using 6 mm sterilised filter paper discs. Discs were impregnated with 10 µl of the test sample, allowed to dry and placed onto inoculated plates. The plates were allowed to stand at 4 °C for 2 hours before incubation with the test microbial agents at 30 °C for 24 hours. Following this incubation the diameters of the inhibition zones were measured in millimetres. All measurements were to the closest whole millimetre. Each antimicrobial assay was performed in at least triplicate. Mean values (\pm standard deviation) are reported in this study. Standard discs of ampicillin (2 µg) and chloramphenicol (10 µg) were obtained from Oxoid Ltd. and served as positive controls for antimicrobial activity. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

3. Results and Discussion

Thirty nine samples from twenty five Australian native plant species were extracted in methanol, dried and the weight of extracted material recorded (table 1). The weight of dried extractable material varied across samples, ranging from 83 mg (*L. juniperium* flowers) extracted per 1 g starting plant material up to 567 g (*C. citrinus* flowers) from the original 1 g of ground dried plant material. All extracts were resuspended in 15 ml of 20 % methanol, resulting in the crude test extract concentrations reported in table 1.

Table 1: Botanical names of plant species extracted, weight of dried extractable material and the concentrations of the extract used to determine antimicrobial activity.

Plant species	Plant part extracted	Dried extract (mg)	Test extract conc. (mg/ml)
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<i>Acacia aulacocarpa</i>	leaves	212	14.1
<i>Acacia complanta</i>	leaves	234	15.6
<i>Acacia complanta</i>	flowers	374	24.9
<i>Adansonia gregorii</i>	leaves	99	6.6
<i>Adansonia gregorii</i>	flowers	115	7.7
<i>Astrotricha longifolia</i>	leaves	223	14.9
<i>Astrotricha longifolia</i>	flowers	384	25.6
<i>Backhousia citriodora</i>	leaves	235	15.7
<i>Banksia collina</i>	leaves	299	19.9
<i>Brachychiton acerifolius</i>	leaves	409	27.3
<i>Brachychiton acerifolius</i>	flowers	105	7.0
<i>Buckinghamia celsissima</i>	leaves	395	26.3
<i>Callistemon citrinus</i>	leaves	561	37.4
<i>Callistemon citrinus</i>	flowers	567	37.8
<i>Callistemon salignus</i>	leaves	539	35.9
<i>Callistemon salignus</i>	flowers	525	35
<i>Allocasuarina littoralis</i>	leaves	376	25.1
<i>Davidsonia pruriens</i> var. <i>jerseyana</i>	fruit	362	24.1
<i>Eucalyptus baileyana</i>	leaves	218	14.5
<i>Eucalyptus major</i>	leaves	427	28.5
<i>Eucalyptus major</i>	flowers	533	35.5
<i>Grevillea juncifolia</i>	leaves	164	10.9
<i>Grevillea juncifolia</i>	flowers	334	22.3
<i>Grevillea robusta</i>	leaves	378	25.2
<i>Grevillea robusta</i>	flowers	312	20.8
<i>Jacksonia scoparia</i>	leaves	442	29.5
<i>Leptospermum bracteata</i>	leaves	192	12.8
<i>Leptospermum bracteata</i>	flowers	274	18.3
<i>Leptospermum juniperium</i>	leaves	246	16.4
<i>Leptospermum juniperium</i>	flowers	83	5.5
<i>Macadamia integriflora</i>	leaves	151	10.1
<i>Macadamia integriflora</i>	flowers	183	12.2
<i>Melaleuca quinquenervia</i>	leaves	355	23.7
<i>Mirbelia oxylobiodes</i>	leaves	306	20.4
<i>Mirbelia oxylobiodes</i>	flowers	314	20.9
<i>Syzygium australe</i>	leaves	402	26.8
<i>Syzygium leuhmannii</i>	leaves	122	8.1
<i>Westringa fruticosa</i>	leaves	418	27.9
<i>Westringa fruticosa</i>	flowers	425	28.3

Antimicrobial activity of the extracts was determined by disc diffusion assays. Twenty nine of the thirty nine extracts tested (72%) showed antibacterial activity against one or more bacteria. Indeed, of the twenty five plant species tested only 3 species had no inhibitory activity towards any of the bacteria tested in any of their extracts (*D. pruriens* var. *jerseyana*, *S. leuhmannii*, *W. fruticosa*). With respect to *D.pruriens* (fruit) and *S. leuhmannii* (leaves), only a single plant part was available for extraction and testing. Perhaps these plants may have also shown antibacterial activity if leaves and/or flowers were also tested. Two species (*B. citriodora*, and *C. citrinus*) were particularly versatile, being capable of inhibiting the growth of all four bacteria tested. These results were not surprising. The antibacterial activity of *B. citriodora* is well known (Dupont *et al.*, 2006; Wilkinson *et al.*, 2003) and recent studies have reported on the antibacterial activity of extracts from a different species of *Callistemon* (*C. rigidus*) (Sanjai and Charu, 2006).

Table 2: Antibacterial activity of plant extracts. Numbers indicate the mean diameters of inhibition of triplicate experiments \pm standard deviation. – indicates no growth inhibition. NM indicates zone of inhibition not measured.

Plant species	Plant part extracted	Antibacterial activity against			
		A. hydrophilia	P. fluorescens	B. cereus	B.subtilis
				9.0 \pm	
<i>Acacia aulacocarpa</i>	leaves	7.7 \pm 0.6	10.6 \pm 0.3	1.0	-
<i>Acacia complanta</i>	leaves	-	-	-	-
<i>Acacia complanta</i>	flowers	-	-	-	8.0 \pm 0
<i>Adansonia gregorii</i>	leaves	-	-	-	-
				9.0 \pm	
<i>Adansonia gregorii</i>	flowers	-	10.3 \pm 0.3	1.0	-
<i>Astrotricha longifolia</i>	leaves	6.0 \pm 0	-	-	-
<i>Astrotricha longifolia</i>	flowers	-	-	-	-
				7.6 \pm	
<i>Backhousia citriodora</i>	leaves	8.3 \pm 0.6	7.6 \pm 0.3	0.3	7.6 \pm 0.3
<i>Banksia collina</i>	leaves	-	-	-	8.7 \pm 1.2
<i>Brachychiton acerifolius</i>	leaves	-	-	-	-
<i>Brachychiton acerifolius</i>	flowers	-	-	7.0 \pm 0	-
				10.6 \pm	
<i>Buckinghamia celsissima</i>	leaves	8.3 \pm 1.2	-	0.3	13.6 \pm 0.3
				14.6 \pm	
<i>Callistemon citrinus</i>	leaves	6.0 \pm 0	9.6 \pm 0.3	0.3	19.3 \pm 0.3
				17.3 \pm	
<i>Callistemon citrinus</i>	flowers	6.0 \pm 0	15.6 \pm 0.3	0.3	18.0 \pm 1.0
				13.7 \pm	
<i>Callistemon salignus</i>	leaves	-	-	1.5	13.6 \pm 0.3
<i>Callistemon salignus</i>	flowers	-	18.3 \pm 0.3	15.6 \pm	17.3 \pm 0.3

					0.6	
<i>Allocasuarina littoralis</i>	leaves	-	18.0 ± 0	9.6 ± 0.3		9.3 ± 0.3
<i>Davidsonia pruriens</i> var. <i>jerseyana</i>	fruit	-	-	-		-
<i>Eucalyptus baileyana</i>	leaves	-	7.0 ± 0	9.3 ± 0.3		-
<i>Eucalyptus major</i>	leaves	-	15.3 ± 0.3	12.0 ± 1.0		10.0 ± 0
<i>Eucalyptus major</i>	flowers	-	23.3 ± 1.2	12.6 ± 0.3		13.3 ± 0.3
<i>Grevillea juncifolia</i>	leaves	-	-	7.0 ± 0		-
<i>Grevillea juncifolia</i>	flowers	-	-	7.0 ± 0		-
<i>Grevillea robusta</i>	leaves	-	-	-		-
<i>Grevillea robusta</i>	flowers	-	-	10.6 ± 0.3		-
<i>Jacksonia scoparia</i>	leaves	-	-	7.0 ± 0		-
<i>Leptospermum bracteata</i>	leaves	-	15.3 ± 0.3	-		-
<i>Leptospermum bracteata</i>	flowers	-	21.3 ± 0.3	11.0 ± 1.0		9.6 ± 0.3
<i>Leptospermum juniperium</i>	leaves	7.3 ± 0.6	-	7.6 ± 0.3		9.0 ± 1.0
<i>Leptospermum juniperium</i>	flowers	-	-	-		-
<i>Macadamia integriflora</i>	leaves	-	-	-		-
<i>Macadamia integriflora</i>	flowers	6.7 ± 0.6	-	-		-
<i>Melaleuca quinquenervia</i>	leaves	-	9.3 ± 0.3	-		-
<i>Mirbelia oxylobiodes</i>	leaves	7.7 ± 0.6	-	9.0 ± 1.0		-
<i>Mirbelia oxylobiodes</i>	flowers	-	8.6 ± 0.3	-		-
<i>Syzygium australe</i>	leaves	8.0 ± 0	10.0 ± 0	8.3 ± 0.3		-
<i>Syzygium leuhmannii</i>	leaves	-	-	-		-
<i>Westringa fruticosa</i>	leaves	-	-	-		-
<i>Westringa fruticosa</i>	flowers	-	-	-		-
	Ampicillin	-	-	-		22.7 ± 0.6
	Chloramphenicol	17.3 ± 0.3	21.0 ± 0	25.6 ± 0.3		NM
	water control	-	-	-		-

Gram-positive bacteria (*B. cereus* and *B. subtilis*) were the most susceptible to growth inhibition by the plant extracts. The greater susceptibility of Gram-positive bacteria has been previously reported for South American (Paz *et al.*, 1995), African (Kudi *et al.*, 1999; Vlietinck *et al.*, 1995) and Australian (Palombo and Semple, 2001) plant extracts. Susceptibility differences between Gram-positive and Gram-negative bacteria may be due to cell wall structural differences between these classes of

bacteria. The Gram-negative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (Tortora *et al.*, 2001). *B. cereus* was especially susceptible, being inhibited by extracts from twenty one of the twenty nine plants tested (72%).

In contrast to the Palombo and Semple (2001) report, many of the Australian native plant extracts examined in the present report also had significant activity towards Gram-negative bacteria. These differences relate to the different species studied, but may also relate to the extract concentrations tested. Some of the extracts tested in this study were tested at concentrations as high as 30-40 mg extracted material per ml. No mention is made in the Palombo and Semple report (2001) of the concentrations of extracted material tested so the possibility exists that lower doses were used in those studies. However, the concentrations tested in this study were comparable to the 36 mg/ml extracts used by Kudi *et al.* (1999) and the 10mg/ml extracts tested by Paz *et al.* (1995) and are substantially lower than the doses tested (100 mg/0.2ml) by Vlietinck *et al.* (1995).

The *Callistemon* extracts tested exhibited the greatest antimicrobial activities (as determined by the diameters of the zones of inhibition) towards the most susceptible bacteria, *B. cereus*. These extracts also displayed good antibacterial activity towards Gram-negative bacteria, particularly *P. fluorescens*. *E. major* and *L. bracteata* were also notable for their strong antibacterial activity against Gram-negative bacteria.

Noteworthy was the apparent trend that flower extracts generally were more potent inhibitors of bacterial growth than were leaf extracts from the same plant. In only one case (*L. juniperium*) were leaf extracts found to have better antimicrobial activities than flower extracts from the same plant. In all other cases, when both leaves and flowers were extracted, flower extracts were better antimicrobial agents. Unfortunately flower extracts were not able to be tested for all species to determine whether this trend holds up due to the unavailability of the flowers of some plants. Further studies may determine whether this trend also applies to other plants.

The results of this study provide further evidence of the antimicrobial activities of some Australian native plants. This study indicates that *A. aulacocarpa*, *B. citriodora*,

B. celsissima, *A. littoralis*, as well as members of the *Callistemon*, *Eucalyptus*, *Leptospermum* and *Syzygium* genera are particularly worthy of further study due to the range of bacteria they are capable of inhibiting. Further evaluation of the antibacterial properties of these extracts against a more extensive panel of microbial agents is warranted. Likewise, purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents. Whilst the extracts examined in this report appear promising as antimicrobial agents, caution is needed before these compounds can be applied to medicinal purposes and as food additives to inhibit spoilage. In particular, toxicity studies are needed to determine the suitability of these extracts for these purposes.

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