Antibacterial Activity of Selected Australian Native Plant Extracts
I Cock

Citation
I Cock. Antibacterial Activity of Selected Australian Native Plant Extracts. The Internet Journal of Microbiology. 2007 Volume 4 Number 2.

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.

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 Aboriginals 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 Aboriginals, 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.
MATERIALS AND METHODS

PLANT MATERIAL

COLLECTION OF PLANT SAMPLES

Acacia aulacocarpa (leaves), Acacia compta (leaves and flowers), Astronotricha 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 celsisima (leaves), Callistemon citrinus (leaves and flowers), Callistemon salignus (leaves and flowers), Davidsonia pruriens var. jerseymia (fruit), Grevillea junifolia (leaves and flowers), Leptospermum bracteata (leaves and flowers), Syzygium australe (leaves), Syzygium leuhamnii (leaves) and Westringa fruticosa (leaves and flowers) were collected from verified trees in the suburbs of Brisbane, Australia.

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 of 20 % methanol. The extract was passed through 0.22 µm filter (Sarstedt) and stored at 4 °C.

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.

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 (± 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.

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.
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).

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 have been observed, with Gram-positive bacteria being more susceptible to plant extracts. This may be due to differences in the cell wall structure or metabolism of these bacteria, which may affect their sensitivity to plant compounds.
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
centrations 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. flavescens. 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
genuses 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.

ACKNOWLEDGEMENTS
Financial support for this work was provided by the School
of Biomolecular and Physical Sciences, Griffith University,
Australia.

References
r-0. Barr, A, Chapman, J, Smith, N, Wightman, G, Knight, T, Mills, L, Andrews, M, Alexander, V, 1993, Traditional Medicines in the Northern Territory or Australia by Aboriginal communities of the Northern Territory, Conservation Commission of the Northern Territory, Darwin
r-2. Bhavnani, SM and Ballow, CH, 2000, New agents for Gram-positive bacteria, Current Opinion in Microbiology, 3, 528-534
r-3. Chiariandy, CM, Seaforth, CE, Phelps, RH, Pollard, GV and Khabay, BP, 1999, Screening of medicinal plants from Trinidad and Tobago for antimicrobial and insecticidal properties, Journal of Ethnopharmacology, 64, 265-270
r-4. Couts, RH and Catterall, CP, 1980, Identifying the plants of Toohey Forest, Ecos Educational Publishers, Nambour, Australia
r-7. Lassak, EV and McCarthy, T, 1993, Australian Medicinal Plants, Reed publishers, Australia
Antibacterial Activity of Selected Australian Native Plant Extracts

67-70


Author Information

I.E. Cock
Biomolecular and Physical Sciences, (Genomics Research Centre), Logan Campus, (Gold Coast Campus), Griffith University