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Original Article

Alphitonia excelsa (Fenzl) Benth. Leaf Extracts Inhibit the Growth of a Panel of Pathogenic Bacteria

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ABSTRACT

Introduction: *Alphitonia excelsa* (Fenzl) Benth. is a large tree that is native to the east coast and northern regions of Australia. Decoctions and infusions produced from the leaves were used by the first Australians to treat a variety of bacterial diseases. Despite this, *A. excelsa* leaf extractions have not been rigorously examined for antibacterial properties against many pathogens. **Methods:** The antimicrobial activity of *A. excelsa* leaf extractions was investigated by disc diffusion and growth time course assays against a panel of pathogenic bacteria. The growth inhibitory activity was quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Polar to mid-polarity *A. excelsa* leaf solvent extractions inhibited the growth of a wide range of bacterial species. Growth of both gram positive and gram negative bacteria was inhibited by the *A. excelsa* leaf extracts to approximately the same extent. The methanolic extracts were the most potent growth inhibitor against all bacteria tested. The methanolic, aqueous and ethyl acetate *A. excelsa* leaf extracts were particularly potent inhibitors of *P. mirabilis* growth, with MIC values as low as 118µg/mL for the methanolic extract. *A. hydrophilia, E. coli, K. pneumonia, S. marcenscens* and *B. cereus* were also particularly susceptible to the methanolic extract, with MIC values generally substantially <1000 µg/mL. The antibacterial activity of the methanolic *A. excelsa* leaf extract was further investigated by growth time course assays which showed significant growth inhibition in cultures of *P. mirabilis* and *B. cereus* within 1 h of exposure. All extracts were determined to be nontoxic in the Artemia franciscana nauplii bioassay, indicating their safety for internal use as well as for topical uses. **Conclusion:** The lack of toxicity of the *A. excelsa* leaf extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria partially validate the traditional usage of this species to treat bacterial diseases and indicate their potential in the development of antiseptic agents.

Key words: Rhamnaceae, Soap tree, Red ash, Australian plants, Antibacterial activity, Medicinal plants.

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INTRODUCTION

Despite many significant advances in the treatment of disease, illnesses caused by bacterial pathogens remain difficult to treat effectively. Many bacterial strains have gained resistance genes and have become either extremely (XDR) or totally drug resistant (TDR) to many antibiotics.[1] There are now limited therapeutic options for the diseases caused by these pathogens and it is likely that this problem will worsen in the future as bacteria exchange resistance genes and more strains become multi-drug resistant (MDR). The development of alternative antibacterial treatment modalities has become crucial and is considered by the World Health Organisation (WHO) to be one of the most serious challenges facing medical science.[2] For a number of reasons reviewed elsewhere,[1] it is unlikely that the previous methods of antibiotic discovery/development will be as successful in the future and new treatment modalities are urgently required.

Plants produce a wide variety of secondary compounds that may provide them with antimicrobial properties.[3] Traditional plant derived medicines have been used in most parts of the world for a variety of therapeutic purposes, including fighting microbial disease. Indeed, the ability of plant extracts to block the growth of pathogenic bacteria has become the focus of much recent study.[4,5] Much of the research into traditional medicinal plant use has focused on Asian,[6,7] African[8-12] and South American[13,14] plants. However, the therapeutic potential of the flora of Australia has also received recent attention. The first Australians had well-developed medicinal systems and understood the therapeutic properties of a wide variety of Australian plants and how to use them effectively.[15] Whilst studies have reported antibacterial activity for some Australian plant species,[16-19] the antibacterial activity of many Australian native plants remains unexamined.

Alphitonia excelsa (Fenzl) Benth. (Figure 1a; Family Rhamnaceae; commonly known as soap tree or red ash) in an endemic Australian plant that grows in open savannah and forest regions from southern New South Wales through to the northern regions of Queensland and Northern Territory. *A. excelsa* was used by the first Australians to treat wound infections, diarrhoea and dysentery, eye infections and skin diseases.[20,21] The antibacterial activity of *A. excelsa* is poorly studied, although one study reported bacteriostatic effects for a methanolic leaf extract against *Micrococcus luteus* and *Salmonella typhimurium*.[22] The same study reported that the extract was completely ineffective against *Bacillus subtilis*, *S. aureus, E. coli* and *Candida albicans*. However, that study examined the growth inhibitory activity of a single high dose (10mg/mL). As an MIC at that dosage in many assay systems would be considered to be inactive, the value of this study is doubtful. Furthermore, MIC values were not determined in that study, making a comparison with the efficacy in other studies impossible. Several studies have also reported antibacterial activity for related *Excelsa* spp. and for compounds isolated from those species. Two triterpenoid saponins isolated from *Alphitonia xerocarpus* Baill. inhibited the growth of *S. aureus* and *Enterococcus faecalis*, with MIC values of 4 and 16µg/mL respectively.[23] In contrast, the triterpenoids were completely inactive against *E. coli* and *P. aeruginosa*.

Whilst investigations into the chemical composition of *A. excelsa* leaves are generally lacking, several interesting phytochemical components have been identified in other *Alphitonia* spp. In particular, several triterpenoid components have been identified in *Alphitonia petriei* (Braid

and C.T. White), including emmolic acid (Figure 1d), alphitolic acid (Figure 1e), *trans*-coumaroyl alphitolic acid (Figure 1f), *cis*-coumaroyl alphitolic acid (Figure 1g), betulinic acid (Figure 1h) and emmolic acid acetate (Figure 1i).[24] Growth inhibitory properties have previously been reported for many triterpenoids against several bacteria.[25] Despite these promising studies, examination of the antibacterial properties and phytochemistry of the leaves from *A. excelsa* is lacking. The current report was undertaken to screen *A. excelsa* leaf extracts for growth inhibitory properties against a panel of pathogenic bacteria.

MATERIALS AND METHODS

Plant collection and extraction

Alphitonia excelsa (Fenzl) Benth. leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. Leaf samples were dried in a Sunbeam food dehydrator and stored at -30°C. Prior to use, the leaves were freshly ground to a coarse powder and 1g quantities were weighed into separate tubes. A volume of 50mL methanol, sterile deionised water, ethyl acetate, chloroform or hexane was added to individual tubes and extracted for 24 hr at 4°C with gentle shaking. All solvents were obtained from Ajax, Australia and were AR grade. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10mL sterile deionised water (containing 1% DMSO). The extracts were passed through 0.22µm filter (Sarstedt) and stored at 4°C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *A. excelsa* leaf extracts for the presence of phenolic compounds, flavonoids, saponins, triterpenoids, phytosteroids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[26-28]

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd., Australia. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Salmonella newport, Serratia marcescens*, *Shigella sonneii*, *Staphylococcus aureus, Staphylococcus epidermidis* and *Streptococcus pyonenes* were obtained from Ms. Michelle Mendell and Ms. Jane Gifkins, Griffith University. All stock cultures were subcultured and maintained in nutrient broth at 4°C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion assay.[29-31] Briefly, 100µL of each bacterial culture was grown in 10mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. A volume of 100µL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 5mm sterilised filter paper discs. Discs were infused with 10µL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2hr before incubation at 37°C for 24hr. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10µg) and chloramphenicol (2µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10µL of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of each extract against susceptible bacteria was determined as previously described.[32,33] Briefly, the *A. excelsa* extracts were diluted in deionised water (containing 1% DMSO) and tested across a range of concentrations. Discs were infused with 10µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was completed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Ln linear regression was used to determine the MIC values of each extract.

Bacterial growth time course assay

Bacterial growth time course studies were performed as previously described.[30,34] Briefly, 3mL of the log phase *P. mirabilis* and *B. cereus* bacterial cultures in nutrient broth were added individually to 27mL nutrient broth containing 3mL of 10mg/mL methanolic plant extract to give a final concentration of 1000 µg/mL in the assay. The tubes were incubated at 37°C with gentle shaking. The optical density was measured hourly at 550nm for a 6h incubation period. Control tubes were incubated under the same conditions but without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 4mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.[35-37] Briefly, 400µL of seawater containing approximately 54 (mean 53.7, $n = 75$, SD 9.6) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at 25±1°C under artificial light (1000 Lux). A 400µL seawater negative control was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead were counted. The nauplii were considered dead if no movement of the appendages was detected within 10 sec. After 24hr, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was determined using probit analysis.

Statistical analysis

Data are expressed as the mean ± SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a P value <0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1g of dried and powdered *A. excelsa* leaves with solvents of varying polarity yielded dried extracts ranging from 45mg (hexane extract) to 347mg (methanolic extract) (Table 1). The aqueous (285mg) and chloroform extracts (140mg) also yielded relatively high levels of extracted materials. The dried extracts were resuspended in 10mL of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1. Qualitative phytochemical studies

showed that the high to mid polarity methanol and water solvents extracted the greatest diversity and highest levels of phytochemicals. These extracts contained high levels of polyphenolics and saponins, moderate levels of flavonoids, as well as low levels of alkaloids and tannins. The methanolic extract also contained high levels of phytosterols. Interestingly, despite extracting relatively large amounts of material, the chloroform and hexane extracts were devoid of all classes of phytochemicals screened. Due to their nonpolar nature, these extracts would be expected to contain high levels of lipids, hydrocarbons etc. As our qualitative phytochemical studies did not screen for these compounds, they were not detected. Other techniques are required to further examine the nature of these non-polar components.

Antimicrobial activity

To determine the growth inhibitory activity of the *A. excelsa* leaf extracts against the panel of pathogenic bacteria, aliquots (10µL) of each extract were screened in the disc diffusion assay. The *A. excelsa* leaf extracts inhibited the growth of several gram negative bacterial species (Figure 2). Of the 10 gram negative bacterial strains tested, 6 (60%) were inhibited by then methanolic *A. excelsa* leaf extract. *P. mirabilis* and *S. marcenscens* were most susceptible to the methanolic *A. excelsa* extract, with ZOIs substantially >8mm. The aqueous and ethyl acetate extracts also inhibited bacterial growth, albeit with substantially smaller ZOIs, indicating lower potency. The growth of most of the gram positive bacteria was also inhibited by the *A. excelsa* leaf extracts (Figure 3). The growth of 3 of the 4 g positive bacteria species screened (75%) was inhibited by the *A. excelsa* leaf extracts, with only *S. pyogenes* being unaffected. However, in general, only the methanolic *A. excelsa* leaf extract inhibited the growth of the gram positive bacteria.

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against the microbial species which were determined to be susceptible. The methanolic, aqueous and ethyl acetate *A. excelsa* leaf extracts were good growth inhibitors of several bacterial species (as judged by MIC; Table 2). *P. mirabilis* was the most susceptible bacteria to the *A. excelsa* leaf extracts, with MIC values as low as 118µg/mL for the methanolic extract (approximately 1µg infused into the disc) recorded for against this bacterium. The MIC values determined for the aqueous and ethyl acetate extract against *P. mirabilis* also indicate moderate growth inhibition (1238 and 984µg/mL respectively). As *P. mirabilis* infection is a common cause of urinary tract infections and has also been identified as a trigger of rheumatoid arthritis,[11,38] the methanolic *A. excelsa* extract has potential for the prevention and treatment of these diseases in genetically susceptible individuals. Furthermore, the methanolic *A. excelsa* leaf extracts were also a good inhibitor of *K. pneumomiae* growth (MIC<1000µg/mL). As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,[39,12] these extracts may also be useful in the prevention and treatment of this disease.

The other bacteria for which the growth inhibitory activity was quantified by MIC determination are generally associated with food poisoning and diarrhoea. Strong growth inhibitory activity was also noted for the methanolic *A. excelsa* leaf extract against *A. hydrophilia* (MIC 225µg/mL), *E. coli* (MIC 415µg/mL), *B. cereus* (MIC 563µg/mL) and *S. aureus* (MIC 927µg/mL). The methanolic extract was also a moderate inhibitor of *S. newport* (MIC 1204µg/mL). Therefore, the methanolic extract has potential for the treatment of diarrhoea and gastrointestinal disease. Two other non-gastrointestinal bacteria were also included in this study. *S. marcenscens,* is an opportunistic pathogen that is most commonly associated with hospital acquired infections, particularly catheter associated bacteremia, urinary tract infections and wound infections. The methanolic, aqueous and ethyl acetate *A. excelsa* leaf extracts were all good inhibitors of *S. marcenscens* growth, with MICs of 155, 863 and 822µg/mL respectively. Thus, these extracts may also be useful for the treatment of hospital acquired bacterial infections.

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the *A. excelsa* leaf extracts.

Extract	Mass of Dried Extract (mg)	Resuspended Extract (mg/mL)	Phenols			Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids				Flavanoids		Tannins		Anthraquinones	
			Total Phenolics	Water Soluble	Water Insoluble	Keller-Kiliani Test	Froth Persistence	Emulsion test	Salkowski Test	Acetic Anhydride Test	Meyers Test	Wagners Test	Draggendoffs Test	Shinoda Test	Kumar test	Ferric Chloride Test	Lead Acetate Test	Free	Combined
Methanol	347	34.7	+++	+++	+++	-	++	+	+	+++	++	+	+	++	++	++	+	-	-
Water	285	28.5	+++	+++	++	-	+++	+	-	-	-	-	-	++	++	+	+	-	-
Ethyl Acetate	48	4.8	-	-	+	-	-	-	-	-	+	+	+	+	+	-	-	-	-
Chloroform	140	14.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexane	45	4.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay.

Bacterial growth time course assay

The most susceptible gram negative (*P. mirabilis*) and gram positive (*B. cereus*) bacterial species were selected for further study by growth time course assays in the presence and absence of the extract. Only the effect of the methanolic extract on the bacterial growth time courses was evaluated as this extract was generally more potent than the other *A. excelsa* extracts. The starting concentration of the extract used in these assays was 1000µg/mL. The methanolic *A. excelsa* extract significantly inhibited *P. mirabilis* (Figure 4a) and *B. cereus* (Figure 4b) growth within 1h, indicating a rapid antimicrobial action. Whilst both *P. mirabilis* and *B. cereus* growth was inhibited for at least the first 4 hr of the time course, the bacteria were generally able to overcome this inhibition by 6h, with the recorded turbidity not significantly different to that of the untreated control. This indicates that the growth inhibition of these bacteria was bacteriostatic for the methanolic *A. excelsa* extract at the concentrations tested against these bacteria.

Quantification of toxicity

The toxicity of the *A. excelsa* extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 1000µg/mL

Table 2: Minimum bacterial growth inhibitory concentration (µg/mL) of the *A. excelsa* leaf extracts against susceptible bacterial species.

Bacterial species	*A. excelsa* Extract				
	Methanol	Water	Ethyl Acetate	Chloroform	Hexane
Gram negative					
A. faecalis	-	-	-	-	-
A. hydrophilia	225	326	280	-	-
C. freundi	-	-	-	-	-
E. coli	415	826	684	-	-
K.pneumoniae	866	-	-	-	-
P. mirabilis	118	1238	984	-	-
P. fluroscens	-	-	-	-	-
S. newport	1204	-	-	-	-
S. marcenscens	155	863	822	-	-
S. sonnei	-	-	-	-	-
Gram positive					
B. cereus	563	3280	1350	-	-
S. aureus	927	3870	-	-	-
S. epidermidis	1428	-	-	-	-
S. pyogenes	-	-	-	-	-

Numbers indicate the mean MIC values of triplicate determinations. - indicates no inhibition.

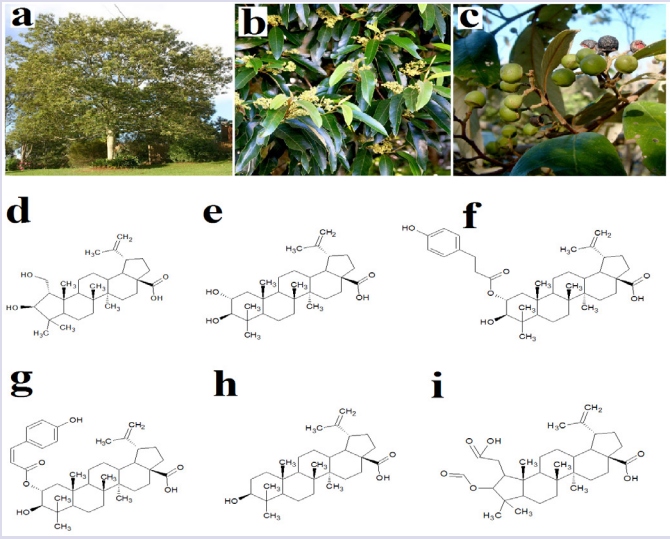

Figure 1: *A. excelsa* (a) whole tree, (b) leaves and flowers and (c) fruit, as well as the chemical structures of (d) emmolic acid, (e) alphitolic acid, (f) *trans*-coumaroyl alphitolic acid, (g) *cis*-coumaroyl alphitolic acid, (h) betulinic acid, (i) emmolic acid acetate.

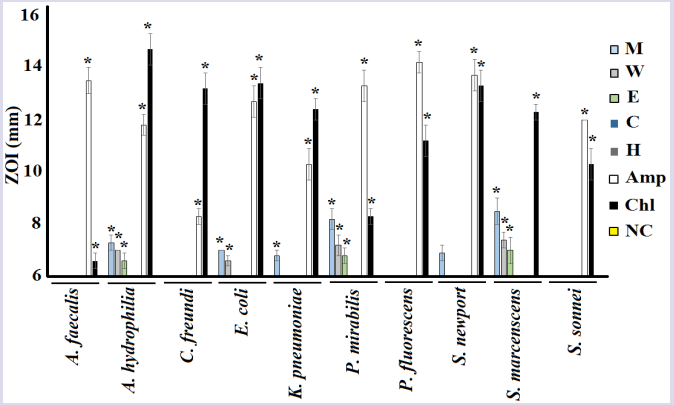

Figure 2: Growth inhibitory activity of *A. excelsa* leaf extracts against the gram negative bacterial species measured as zones of inhibition (mm) ± SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10µg) control; Chl = chloramphenicol (2µg) control; * = results that are significantly different to the untreated control (P<0.5). All determinations were in at least triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.

(Figure 5). All extracts induced <50% mortality at 24 and 48hr and were thus deemed to be non-toxic. In contrast, the potassium dichromate positive control induced mortality within 4h (results not shown), with 100% mortality induction seen by 24hr. All extracts were determined to be nontoxic, with LC_{50} values substantially greater than 1000µg/mL following 24 hr exposure. Extracts with an LC_{50} of greater than 1000µg/mL towards *Artemia* nauplii have previously been defined as being nontoxic.[38]

DISCUSSION

Despite the initial potency of many antibiotic chemotherapies, recent increases in bacterial resistance has made the development of new antibiotic therapies a high priority.[1] A parallel decrease in the introduction of new antibiotic therapies in recent years has further compounded this problem. As a result, interest in re-evaluating medicinal plants for new antibiotic chemotherapies has escalated substantially. The first Australians used *A. excelsa* to treat multiple diseases and infections caused by bacterial pathogens.[20,21] Despite this, limited scientific evaluations have rigorously evaluated the antibacterial properties of *A. excelsa*.

Our study examined the ability of *A. excelsa* leaf extracts to inhibit the growth of a panel of medicinally important bacterial pathogens. The methanolic extract was a particularly potent inhibitor of *P. mirabilis* with an MIC value of 118µg/mL. The aqueous, ethyl acetate extract and chloroform extracts were also moderate *P. mirabilis* growth inhibitors, albeit with higher MIC values (1238 and 984µg/mL respectively). As *P. mirabilis* can trigger rheumatoid arthritis in genetically susceptible individuals,[11,39] these extracts have potential for the development of rheumatoid arthritis inhibitory therapies. The methanolic extract also displayed good *K. pneumoniae* growth inhibitory properties (MIC 866µg/mL). As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,[39,12] this extract may also be useful in the prevention and treatment of ankylosing spondylitis. The methanolic *A. excelsa* leaf extract was also a good inhibitor of bacterial pathogens associated with food poisoning and diarrhoea. Strong growth inhibitory activity was also noted for the methanolic *A. excelsa* leaf extract against *A. hydrophilia* (MIC 225µg/mL), *E. coli* (MIC 415µg/mL), *B. cereus* (MIC 563µg/mL) and *S. aureus* (MIC 927µg/mL). The methanolic extract was also a moderate inhibitor of *S. newport* (MIC 1204µg/mL). Therefore, the methanolic extract has potential for the treatment of diarrhoea and gastrointestinal disease. Similarly, the methanolic *A. excelsa* leaf extract was also a good inhibitor of the growth of the opportunistic pathogen *S. marcenscens*. The bacterium is commonly associated with hospital acquired infections, particularly catheter associated bacteremia, urinary tract infections and wound infections. The methanolic, aqueous and ethyl acetate *A. excelsa* leaf extracts were all good inhibitors of *S. marcenscens* growth, with MICs of 155, 863 and 822µg/mL respectively. Thus, these extracts may also be useful for the treatment of hospital acquired bacterial infections.

The ability of the *A. excelsa* leaf extracts to inhibit the growth of both gram-positive and gram-negative bacteria is in agreement with previous reports of the antibacterial activity of other Australian plant species.[40,41] In this study, the gram-negative and gram-positive bacteria were approximately equally susceptible to the *A. excelsa* leaf extracts. In contrast, many previous studies have reported substantially greater susceptibility for gram-positive bacteria to South American,[42,43] African[11,39] and Australian[44] plant extracts. Results within our laboratory have also confirmed the greater susceptibility of gram-positive bacteria towards many other Australian plant extracts.[44,45] The gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including several antibiotics.[46] In contrast, other studies have demonstrated that gram-negative bacteria are often as susceptible

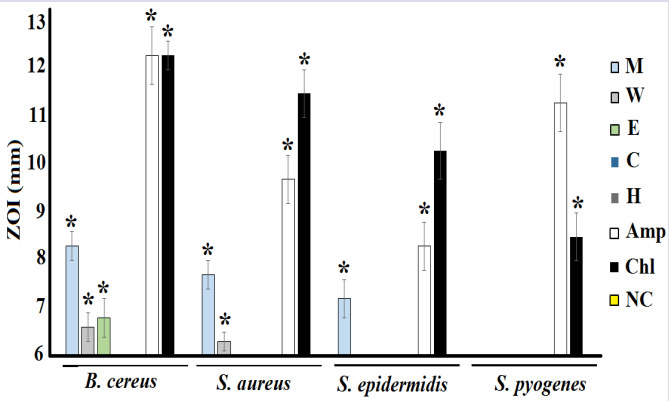

Figure 3: Growth inhibitory activity of *A. excelsa* leaf extracts against the gram positive bacterial species measured as zones of inhibition (mm) ± SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10µg) control; Chl = chloramphenicol (2µg) control; * = results that are significantly different to the untreated control (P<0.5). All determinations were in at least triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.

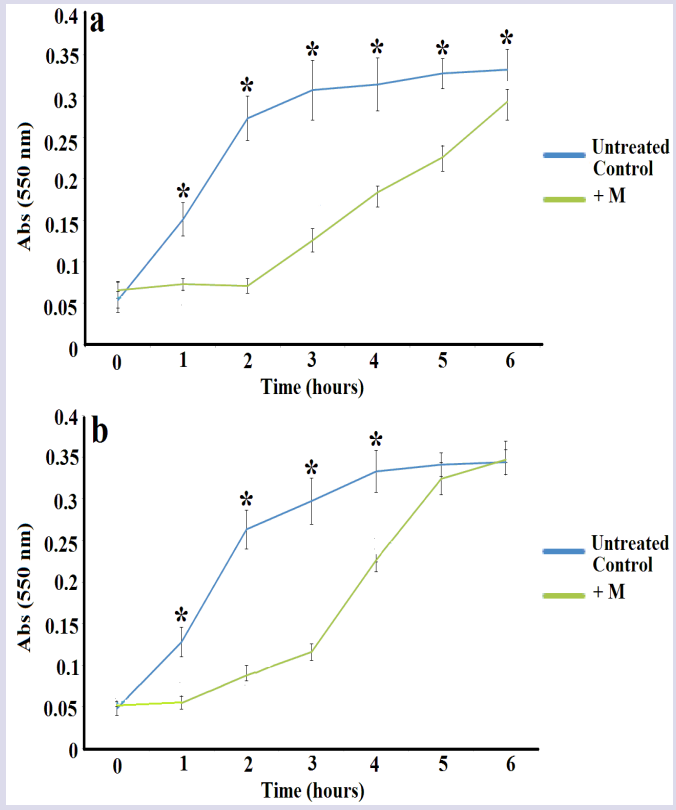

Figure 4: Bacterial growth curves for the methanolic and aqueous *A. excelsa* leaf extract against (a) *P. mirabilis*, (b) *B. cereus*. All bioassays were performed in at least triplicate and are expressed as mean ± SEM. * = growth results in the presence of the methanolic extract that are significantly different to the untreated control growth (p<0.01).

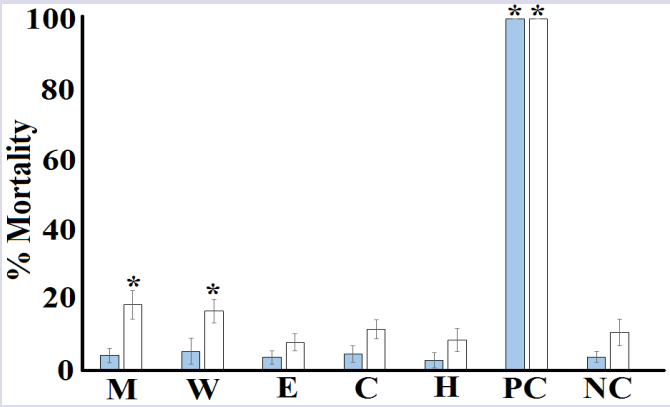

Figure 5: The lethality of the *A. excelsa* leaf extracts (1000µg/mL), potassium dichromate (1000µg/mL) and a seawater control. Blue bars represent the % mortality following 24hr exposure to the extract/toxin. White bars represent the % mortality following 48hr exposure to the extract/toxin. NC = negative (seawater) control; PC = positive control (1000µg/mL potassium dichromate). All bioassays were performed in at least triplicate and are expressed as mean ± SEM. * indicates results that are significantly different to the negative controls (P<0.5).

(or more susceptible) to plant extracts from different Australian plant species.[30,28]

Whilst a detailed investigation of the phytochemistry of the *A. excelsa* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Some commonalities were noted: the most potent aqueous and methanolic extracts all contained relatively high levels of total phenolics and flavonoids. It is likely that these and other phytochemical classes may contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies also indicated that triterpenoids, phytosterols and saponins were present in the *A. excelsa* leaf extracts. Furthermore, previous studies have also identified a number of triterpenoids in *Alphitonia* spp. Extracts.[24] Many studies have reported potent antibacterial activities for a wide variety of these compounds.[25] Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of bacterial growth inhibition.

It is likely that other phytocompounds may also contribute to the antibacterial properties of *A. excelsa*. Monoterpenoids including α-pinene, β-pinene, sabinene, mycrene, terpinene, limonene, piperitone and β-phellandrene also inhibit the growth multiple bacteria, including several antibiotic resistant strains of Enterobacteriaceae.[25] The antibacterial activities for several sesquiterpenoids including α-cubebene, copaene and caryophyllene have also been reported.[25] Similarly, many tannin compounds have bacterial growth inhibitory activity. Gallotannins inhibit the growth of a broad spectrum of bacterial species[47] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[43,48] and by inhibiting glucosyltransferase enzymes.[45] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5µg/mL.[49,50] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[49,51] Thus, it is likely that multiple compounds within the *A. excelsa* leaf extracts are contributing to the antibacterial activity reported here.

The findings of this study also demonstrate that the *A. excelsa* leaf extracts were nontoxic towards *Artemia franciscana* nauplii, with LC_{50} values substantially >1000µg/mL. Extracts with LC_{50} values >1000µg/mL towards *Artemia* nauplii are defined as being nontoxic.[37] Whilst our preliminary toxicity studies indicate that these extracts may be safe for therapeutic use, studies using human cell lines are required to further evaluate the safety of these extracts. Furthermore, whilst these studies have demonstrated the potential of the *A. excelsa* leaf extracts in the development of future antibiotic chemotherapeutics for the prevention and treatment of urinary tract infections, autoimmune diseases (particularly rheumatoid arthritis and ankylosing spondylitis) and gastrointestinal diseases, more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSION

The results of this study demonstrate the potential of the *A. excelsa* leaf extracts as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates than they are safe for internal as well as topical treatment. Further studies aimed at the purification and identification of bioactive components are required to examine the mechanisms of action of these agents.

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CONFLICT OF INTEREST

The author report no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; **LC_{50}:** The concentration required to achieve 50 % mortality; **MIC:** minimum inhibitory concentration.

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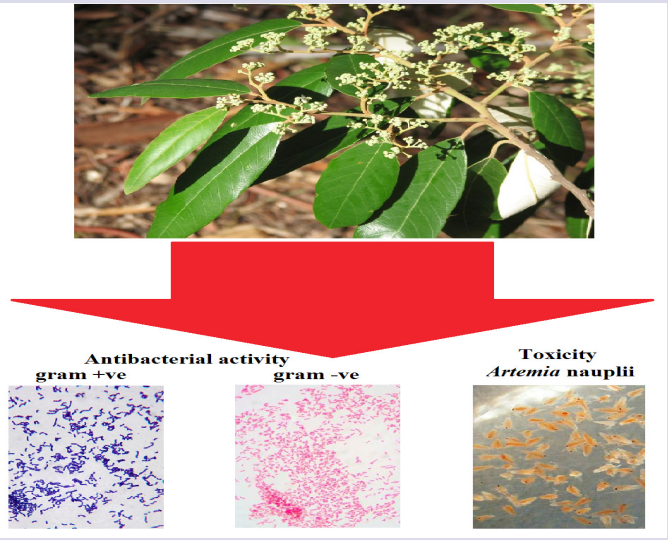
SUMMARY

- *A. excelsa* leaf extracts were screened for the ability to block the growth of a panel of bacterial pathogens.
- The growth inhibition of both gram-positive and gram-negative bacteria was tested.
- The antibacterial activity was quantified by determining the MIC values of each extract.
- Growth time course studies were also undertaken against *P. mirabilis* and *B. cereus*.
- Toxicity of the *A. excelsa* leaf extracts was determined using the Artemia nauplii toxicity bioassay.

PICTORIAL ABSTRACT



ABOUT AUTHORS

Dr. Ian Cock leads a research team in the Environmental Futures Research Institute and the School of Natural Sciences at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including *Aloe vera*, South Asian and South American tropical fruits, as well as Australia plants including *Scaevola spinescens, Pittosporum phylliraeoides, Terminalia ferdinandiana* (Kakadu plum), Australian *Acacias, Syzygiums, Petalostigmas* and *Xanthorrhoea johnsonii* (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.