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Original Article

Alpinia cearulea (R.Br.) Benth Leaf Extracts Inhibit the Growth of a Panel of Bacterial Pathogens

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ABSTRACT

Introduction: *Alpinia caerulea* is a perennial herb which grows in coastal regions of Australia. All parts of this species are consumed as a bushfood. Furthermore, *A. caerulea* is taxonomically related to several plant species with well documented antimicrobial properties. Despite this, *A. caerulea* leaf solvent extractions have not previously been examined for antibacterial properties. **Methods:** The antimicrobial activity of *A. caerulea* leaf solvent 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:** Methanolic and aqueous *A. caerulea* leaf extracts inhibited the growth of a range of gram positive and gram negative bacterial species. The methanolic extract was generally a more potent growth inhibitor than the aqueous extract. The methanolic and aqueous extracts were particularly potent inhibitors of *S. marcenscens* growth, with MIC values as low as 188 µg/mL for the methanolic extract. *P. mirabilis* (MICs of 785 and 976 µg/mL for the methanolic and aqueous extracts respectively) and *K. pneumoniae* growth (MICs of 1167 and 924 µg/mL for the methanolic and aqueous extracts respectively) were also particularly susceptible to the *A. caerulea* leaf extracts. The antibacterial activity of the methanolic and aqueous *A. caerulea* leaf extracts was further investigated by growth time course assays which showed significant growth inhibition in cultures of *S. marcenscens* and *P. mirabilis* 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. caerulea* leaf extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria indicate their potential in the development of antibiotic agents.

Key words: *Alpinia caerulea*, Zingiberaceae, Native ginger, Blue berry ginger, Rheumatoid arthritis, Ankylosing spondylitis, Antibacterial activity, Medicinal plants.

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INTRODUCTION

Alpinia caerulea (R.Br.) Benth. (family Zingiberaceae; synonym *Hellenia caerulea* R.Br.; commonly known as Australian native ginger and blue berry ginger) is a perennial herb which grows in the shaded understorey of rainforests and wet shlerophyll forests in eastern coastal regions of Australia, extending from Sydney to the tropical regions of far northern Queensland.[1] *A. caerulea* grows up to 2 m tall, with only the leaves growing above ground level (as with other Zingiberaceae spp., the stem grows below ground level). The sessile leaves (Figure 1a and Figure 1b) grow to approximately 40 cm long and 10 cm wide. Creamy white flowers (Figure 1c) develop into dark blue globular fruit capsules approximately 10-15 mm diameter (Figure 1d). The flesh inside the fruit is white and contains a single black seed. The first Australians used *A. caerulea* as a nutritious, flavoursome snack. The fruit pulp was consumed directly and the seeds were discarded. Alternatively, the fruit can be dried and ground and used as a flavouring agent. Whilst the fruit is most often cited as a bushfood, all parts of the plant are edible and have been used by Australian Aborigines as well as early colonial settlers in their cuisine. The leaves can be dried, ground and used both as a spice and as an herbal infusion. Wrapping food in the leaves imparts a gingery flavour and was sometimes used in traditional cookery. The centres of the new shoots have a distinct gingery flavour and can be used fresh in cooking as a ginger substitute. The below ground parts (roots and stem) have a stronger, more earthy ginger flavour and are used sparingly in cooking.

We were unable to find reports of the use of *A. caerulea* by Australian Aborigines as a traditional medicine. Instead, it appears to have been more valued as a culinary plant. However, the first Australians passed on traditional knowledge orally and there is a lack of written records from the early days of European colonisation which document the use of plants for therapeutic purposes. Therefore, much of the first Australian's traditional knowledge of medicinal plants has been lost. It is therefore possible that the first Australians may also have had therapeutic (as well as culinary) uses for *A. caerulea.* Notably, *A. caerulea* shares close taxonomic relationships with several other culinary plants from other regions of the world with well-established therapeutic properties. It is taxonomically related to *Alpinia galangal* (galangal), *Zingiber officinale* (common ginger), *Curcuma longa* (turmeric) and cardamom (*Elettaria* spp., *Amomum* spp.). All of these plants are members of family Zingiberaceae and all have well documented therapeutic properties.[2-5] It is possible that *A. caerulea* may contain similar phytochemicals and have similar therapeutic properties.

Several interesting phytochemical components have been identified in *A. cearulea* extracts. In particular, 2 diterpenoids were isolated from *A. cearulea* fruit and identified as zerumin A (Figure 1e) and (E)-8(17), 12-labdadiene-15,16-dial (Figure 1f).[6] That study focussed on the anti-angiogenic activity of *A. cearulea* extracts and the isolated compounds and did not screen them for the ability to inhibit bacterial growth. Furthermore, we were unable to find any other studies examining the antibacterial properties of these compounds. A different study examined the phytochemistry and antibacterial properties of extracts prepared from the rhizome of the related species *Alpinia pahangensis* Ridley.[7] That study identified 2 different diterpenoids which were structurally similar to the *A. cearulea* fruit diterpenoids. Indeed, pahangensin B is a methylated form of zerumin A and pahangensin A is a dimeric form of pahangensin B. Interestingly that study tested pahangensin A and pahangensin B for antibacterial activity and reported potent growth inhibition. Indeed, pahangensin A was reported to have similar antibacterial potency as pure streptomycin and chloramphenicol against *S. aureus* and *B. subtilis* and substantially better potency than penicillin against

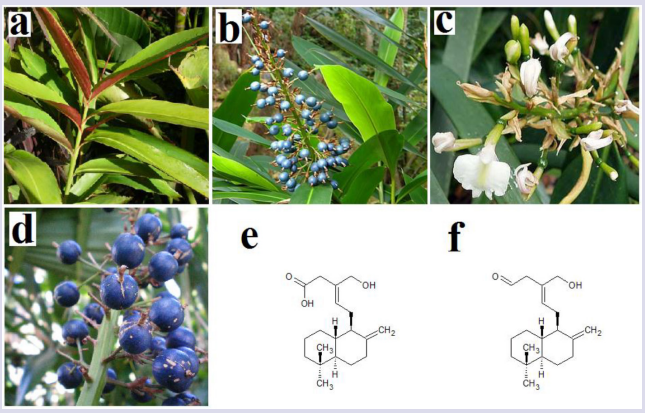

Figure 1: *A. cearulea* (a) leaves, (b) whole plant, (c) flowers and (d) fruit, as well as the chemical structures of the diterpenoid components (e) zerumin A, (f) (E)-8(17),12-labdadiene-15,16-dial.

B. cereus. Both *A. pahangensis* diterpenoids only inhibited gram positive bacterial growth and had no effect on gram negative bacteria. Despite these promising studies, examination of the antibacterial properties of *A. cearulea* extracts and isolated compounds is lacking. The current report was undertaken to screen *A. cearulea* leaf extracts for growth inhibitory properties against a panel of pathogenic bacteria.

MATERIALS AND METHODS

Plant collection and extraction

Alpinia cearulea (R.Br.) 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 dried leaves was freshly ground to a coarse powder and 1 g quantities were weighed into separate tubes. A volume of 50 mL methanol, sterile deionised water, ethyl acetate, chloroform or hexane was added to individual tubes and extracted for 24 h at 4°C with gentle shaking. All solvents were obtained from Ajax, Australia and were AR grade. The extracts was 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 10 mL sterile deionised water (containing 1% DMSO). The extracts were passed through 0.22 µm filter (Sarstedt) and stored at 4°C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *A. cearulea* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[8-10]

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd., Australia. Reference strains of *Klebsiella pneumoniae* (ATCC31488), *Escherichia coli* (ATCC 25922) and *Proteus mirabilis* (ATCC21721) were purchased from American Tissue Culture Collection, USA. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus*, *Citrobacter freundii*, *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.[11-13] Briefly, 100 µL of each bacterial culture was grown in 10 mL of fresh nutrient broth until they reached a count of ~10^8 cells/mL. A volume of 100 µL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were infused with 10 µL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2 h before incubation at 30°C for 24 h. 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 (10 µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL 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.[14-15] Briefly, the *A. cearulea* leaf extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 µL 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. 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.[16] Briefly, 3 mL of *Proteus mirabilis* (ATCC21721) and *S. marcenscens* in nutrient broth were individually added to 27 mL nutrient broth containing 3 mL of 10 mg/mL methanolic or aqueous plant extract to give a final concentration of 1000 µg/mL in the assay. The tubes were incubated at 30°C with gentle shaking. The optical density was measured hourly at 550 nm for a 6 h 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 4 mg/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.[17-19] Briefly, 400 µL of seawater containing approximately 58 (mean 57.6, n = 75, SD 11.6) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL 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 400 µL 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 counted. The nauplii were considered dead if no movement of the appendages was detected within 10 sec. After 24 h, 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 1 g of dried and powdered *A. cearulea* leaf with solvents of varying polarity yielded dried extracts ranging from 54 mg (hexane extract) to 158 mg (methanol extract) (Table 1). The aqueous (126 mg) and chloroform extracts (117 mg) also yielded relatively high levels of extracted materials. The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1.

Qualitative phytochemical studies showed that the higher polarity methanol and water solvents extracted the greatest diversity and highest levels of phytochemicals. Both contained high levels of phenolics and flavonoids and low-moderate levels of saponins, triterpenoids and tannins. The ethyl acetate extract contained similar phytochemical classes, albeit generally at lower levels. 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 and other techniques are required to further examine the nature of these nonpolar components.

Antimicrobial activity

To determine the growth inhibitory activity of the *A. cearulea* leaf extracts against the panel of pathogenic bacteria, aliquots (10 µL) of each extract were screened in the disc diffusion assay. The methanolic and aqueous *A. cearulea* leaf extracts inhibited 3 of the 10 (30 %) gram negative bacterial species screened in this study (Figure 2). The methanolic *A. cearulea* leaf extract was generally a more potent growth inhibitor than the aqueous extract against most bacterial species (as assessed by the sizes of the zones of inhibition). The inhibition of *S. marcenscens* growth inhibition was particularly noteworthy, with the methanolic and aqueous extracts producing 10.0 ± 1.0 and 8.6 ± 0.6 mm respectively. This inhibition was particularly noteworthy compared to the inhibition by the ampicillin control (10 µg: inhibition zones of 6.6 ± 0.3 mm). *P. mirabilis* (8.3 ± 0.3 mm and 7.9 ± 0.6 mm for the methanolic and aqueous extracts respectively) and *K. pneumoniae* growth (7.3 ± 0.3 mm and 7.5 ± 0.5 mm for the methanolic and aqueous extracts respectively) were also susceptible to inhibition by the *A. cearulea* leaf extracts. The ethyl acetate extract also inhibited the growth of the same bacteria, albeit generally with substantially smaller inhibition zones than were recorded for methanolic and aqueous extracts. The chloroform and hexane extracts were generally devoid of growth inhibitory activity.

Gram positive bacteria were also susceptible to the *A. cearulea* leaf extracts (Figure 3). Indeed, a higher proportion of the gram positive bacteria screened were susceptible compared to the gram negative bacteria. Of the 4 gram positive bacterial strains tested, 3 (75 %) were inhibited by both the methanolic and aqueous *A. cearulea* leaf extracts. With the exception of *S. pyogenes*, all of the susceptible gram positive bacteria were also inhibited by the ethyl acetate *A. cearulea* leaf extract. The chloroform and hexane extracts were completely devoid of bacterial growth inhibitory activity against all gram positive bacterial species.

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 and aqueous *A. cearulea* leaf extracts were potent growth inhibitors of several bacterial species (as judged by MIC; Table 2). *S. marcenscens* was the most susceptible bacteria to the *A. cearulea* leaf extracts, with an MIC value of 188 µg/mL (<2 µg infused into the disc) recorded for the methanolic extract. The aqueous and ethyl acetate extracts were also potent *S. marcenscens* growth inhibitors, with MIC values of 405 and 846 µg/mL respectively. *P. mirabilis* and *K. pneumoniae* were also highly susceptible to the methanolic and aqueous *A. cearulea* leaf extracts, generally with MIC values <1000 µg/mL. As *P. mirabilis* infection is a common cause of

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the *A. cearulea* 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	158	15.8	+++	+++	+++	-	++	++	+	-	+	-	-	+++	+++	+	+	-	-
Water	126	12.6	+++	+++	+++	-	+	+	+	-	+	-	-	+++	+++	+	+	-	-
Ethyl Acetate	67	6.7	+	+	++	-	+	++	++	-	+	-	-	++	++	-	-	-	-
Chloroform	117	11.7	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Hexane	54	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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

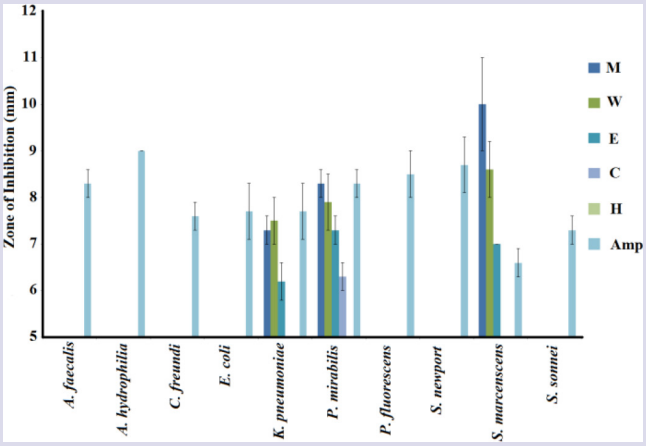

Figure 2: Growth inhibitory activity of *A. cearulea* leaf extracts against the gram negative bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 µg) control. All determinations were performed in at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

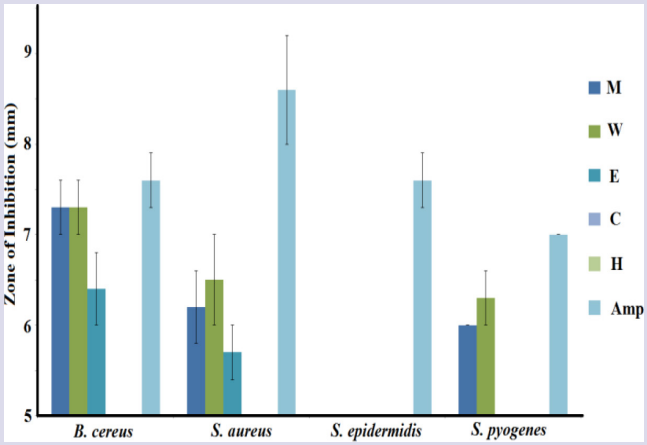

Figure 3: Growth inhibitory activity of *A. cearulea* leaf extracts against the gram positive bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 µg) control. All determinations were performed in at least triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.

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

Bacteria	M	W	E	C	H
Gram negative bacteria					
A. faecalis	-	-	-	-	-
A. hydrophilia	-	-	-	-	-
C. freundi	-	-	-	-	-
E. coli	-	-	-	-	-
K. pneumoniae	1167	924	1255	-	-
P. mirabilis	785	976	1085	2038	-
P. fluroscens	-	-	-	-	-
S. newport	-	-	-	-	-
S. marcenscens	188	405	846	-	-
S. sonnei	-	-	-	-	-
Gram positive bacteria					
B. cereus	1078	826	1140	-	-
S. aureus	1855	1450	3408	-	-
S. epidermidis	-	-	-	-	-
S. pyogenes	2275	1638	-	-	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no inhibition. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract.

urinary tract infections and has also been identified as a trigger of rheumatoid arthritis,[20-21] the aqueous and methanolic *A. cearulea* leaf extracts have potential for the prevention of these diseases in genetically susceptible individuals. Similarly, *K. pneumoniae* is a microbial trigger of ankylosing spondylitis.[22-23] indicating the potential of the aqueous and methanolic *A. cearulea* leaf extracts in the prevention and treatment of this disease. The aqueous and methanolic *A. cearulea* leaf extracts also displayed moderate growth inhibitory activity towards the gram positive bacteria *B. cereus*, *S. aureus* and *S. pyogenes*, with MIC values in the 1000-2300 µg/mL range. Moderate to low growth inhibition (or no inhibition) was noted for all other extract/bacterium combinations.

Bacterial growth time course assay

The antibacterial activity of the *A. cearulea* leaf extracts was further investigated in the *P. mirabilis* and *S. marcenscens* by bacterial growth time course assays in the presence and absence of the *A. cearulea* extracts. Only the effect of the methanolic and aqueous extracts on the bacterial growth time course were evaluated as these extracts were the most potent bacterial growth inhibitors (as judged by MIC). Furthermore, the time course studies only examined the effect of the extracts on the *P. mirabilis* and *S. marcescens* as these were the most susceptible bacterial species. The starting concentration of the extract used in these assays was 1000 µg/mL. The methanolic and aqueous *A. cearulea* leaf extracts significantly inhibited *P. mirabilis* (Figure 4a) and *S. marcescens* growth (Figure 4b) within 1 h, indicating a rapid antimicrobial action. Whilst *P. mirabilis* growth was inhibited for 4-5 h of the time course, the bacteria were 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. cearulea* leaf extract at the concentrations tested. A similar inhibitory time course was evident for *S. marcescens* growth in the presence of aqueous *A. cearulea* extract. *S. marcescens* growth was inhibited for 4-5 h of the time course, although the bacterium overcame the inhibition by 6 h. In contrast, inhibition of *S. marcescens* growth by the methanolic *A. cearulea* leaf extract was substantially more profound, with growth still significantly inhibited by the end of the 6 h time course study. This may indicate that the methanolic *A. cearulea* leaf extract has bactericidal activity against *S. marcescens* at the dose tested. Indeed, the turbidity at 6 h was not greatly increased from the starting turbidity.

Quantification of toxicity

The toxicity of the *A. cearulea* leaf extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 µg/mL (Figure 5). All extracts induced low levels of mortality at 24 h, similar to the % mortality seen for the seawater control. By 48 h, the aqueous and

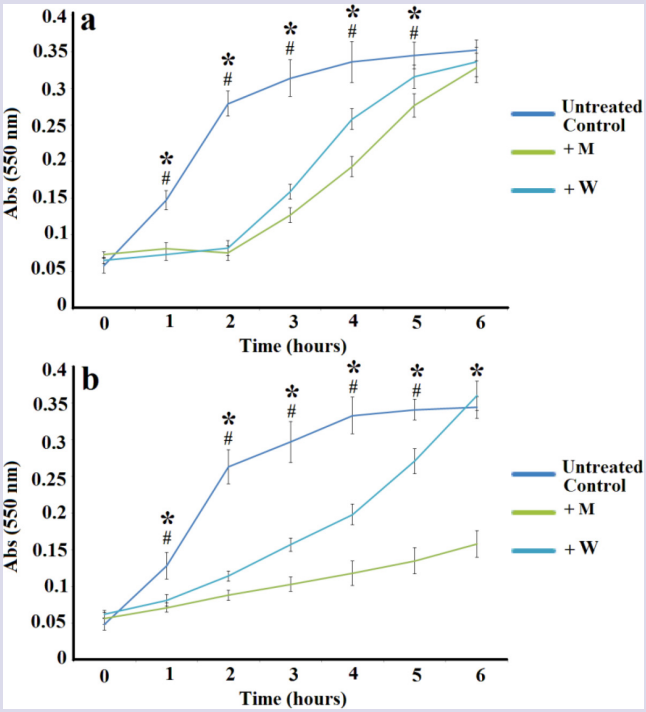

Figure 4: Bacterial growth curves for the methanolic and aqueous *A. cearulea* leaf extracts against (a) *P. mirabilis* and (b) *S. marcenscens*. All bioassays were performed in at least triplicate and are expressed as mean ± SEM. * = results that are significantly different between the growth in the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

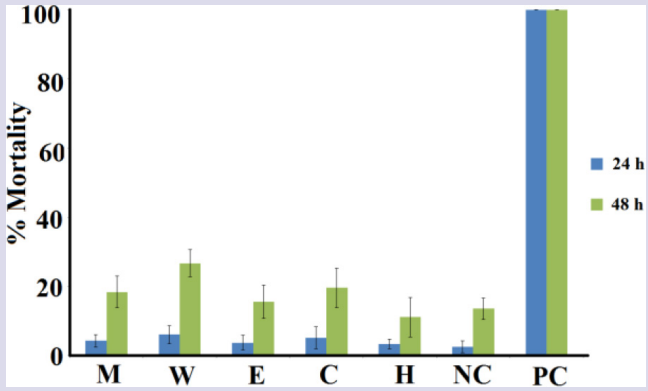

Figure 5: The lethality of the *A. cearulea* leaf extracts (2000 µg/mL), potassium dichromate (1000 µg/mL) and a seawater control. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; NC = negative (seawater) control; PC = positive control (1000 µg/mL potassium dichromate). All bioassays were performed in at least triplicate and are expressed as mean ± SEM.

methanolic extracts had begun to induce mortality significantly higher than that in the untreated control. However, the level of mortality was still substantially < 50 % toxicity at 48 h for all extracts. Thus, all of the *A. cearulea* leaf extracts were deemed to be nontoxic. Extracts with an LC_{50} >1000 µg/mL towards *Artemia* nauplii have previously been defined as being nontoxic.[19] In contrast, the potassium dichromate positive control induced mortality within 4 h (results not shown), with 100 % mortality induction seen by 24 h.

DISCUSSION

Pharmacognostic and natural product remedies are becoming increasingly sought after in the treatment and prevention of disease due to the failure of current drug regimens to effectively treat many diseases. This study reports on the growth inhibitory properties of *A. cearulea* leaf extracts against a panel of pathogenic bacteria and on their toxicity. Both gram positive and gram negative bacteria were susceptible to the *S. formosa* leaf extracts, although the gram positive bacteria had greater susceptibility (as judged by the number of bacteria inhibited). Indeed, the methanolic and aqueous extracts each inhibited 75 % of the gram positive bacterial species screened, compared to 30 % of the gram negative bacteria. This is consistent with many previous studies with other plant species which report a greater susceptibility of gram positive bacteria towards solvent extracts for South American,[24] African[25] and Australian.[26-28] plant extracts.

Our study examined the ability of *A. cearulea* leaf extracts to inhibit the growth of a panel of medicinally important bacterial pathogens. The methanolic and aqueous extracts were identified as being particularly potent inhibitors of *S. marcenscens* growth, with MIC values of 188 and 405 µg/mL respectively. As *S. marcenscens* is a common cause of hospital-acquired infections, as well as urinary tract infections and wound infections,[22] *A. cearulea* leaf extracts may provide useful leads in the treatment of these diseases. The methanolic and aqueous extracts were also potent inhibitors of *P. mirabilis* growth, with MIC values of 785 and 976 µg/mL respectively. As *P. mirabilis* can trigger rheumatoid arthritis in genetically susceptible individuals,[20-21] these extracts have potential for the development of rheumatoid arthritis preventative therapies. The methanolic and aqueous extracts also had moderate to good *K. pneumoniae* growth inhibitory properties, with MIC values of 1167 and 924 µg/mL respectively. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,[22-23] these extracts may also be useful in the prevention of that disease. The methanolic and aqueous *A. cearulea* leaf extracts also were moderate inhibitors of several other bacterial pathogens. Both the aqueous and methanolic *A. cearulea* leaf extracts were good inhibitors of *B. cereus*, *S. aureus* and *S. pyogenes* growth, with MIC values generally 1000-2300 µg/mL. As these bacteria have been implicated in a number of gastrointestinal and skin diseases, the aqueous and methanolic *A. cearulea* leaf extracts also may have applications in the treatment of these diseases.

Whilst a detailed investigation of the phytochemistry of the *A. cearulea* leaf extracts was beyond the scope of our study, qualitative screening was used to determine the classes of compounds present. Several commonalities were noted: the most potent aqueous and methanolic extracts all contained relatively high levels of phenolics and flavonoids. Many studies have reported potent antibacterial activities for a wide variety of polyphenolic compounds, including many flavonoids.[29] Bioactivity driven isolation of active components is required to further identify the bioactive components and to evaluate the mechanism of bacterial growth inhibition.

It is likely that other phytochemical classes may also contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies indicated that tannins were present in the *A. cearulea* leaf extracts in low to moderate levels. Many studies have reported potent growth inhibitory activities for a number of tannin compounds. Gallotannins have been reported to inhibit the growth of a broad spectrum of bacterial species[30] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins[31-32] and by inhibiting glucosyltransferase

enzymes.[33] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.[30,32] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[31,33] Thus, it is likely that *A. cearulea* leaf tannins may contribute to the inhibition of bacterial growth reported in our study. Triterpenoids were also detected in low to moderate levels in the inhibitory extracts. Many terpenoids have very good antibacterial activity[30] and it is therefore likely that these compounds may also contribute to the growth inhibitory properties reported here.

Our findings also demonstrate that all of the *A. cearulea* leaf extracts were nontoxic towards *Artemia franciscana* nauplii, with 24 h LC_{50} values substantially > 1000 µg/mL. Extracts with LC_{50} values > 1000 µg/mL towards *Artemia* nauplii are defined as being nontoxic.[19] 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. Whilst these studies have demonstrated the potential of the *A. cearulea* 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 some gastrointestinal and skin 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. cearulea* leaf extracts as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates than they are safe for internal as well as topical treatments. Further studies aimed at the purification and identification of bioactive components are needed to examine the mechanisms of action of these agents.

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CONFLICT OF INTEREST

The authors 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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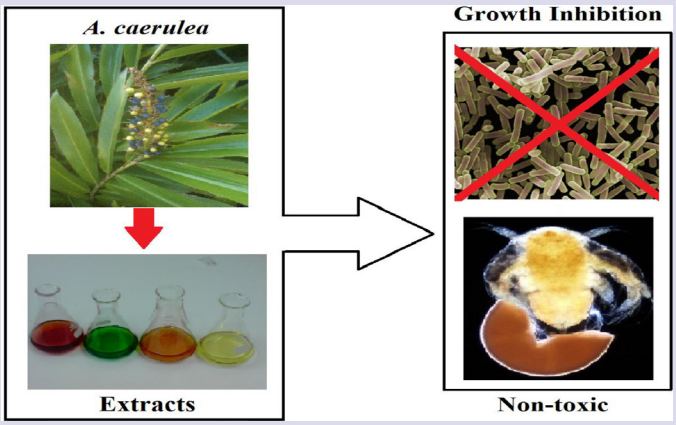
PICTORIAL ABSTRACT



SUMMARY

- Methanolic and aqueous *A. caerulea* leaf extracts inhibited the growth of a range of gram positive and gram negative bacterial species.
- The methanolic and aqueous extracts were potent inhibitors of *S. marcenscens* growth, with MICs of 188 and 405 µg/mL respectively.
- *P. mirabilis* and *K. pneumoniae* were also highly susceptible to the methanolic and aqueous extracts (MICs in the range 750-1250 µg/mL).
- The growth of *B. cereus*, *S. aureus* and *S. epidermidis* were also inhibited by the methanolic and aqueous extracts, albeit with higher MIC values.
- All *A. caerulea* leaf extracts were nontoxic.

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Ms Getmore Chikowe completed her BSc at Griffith University in life sciences. Following graduation, she undertook a research project in Dr Ian Cock's laboratory in the School of Natural Sciences at Griffith University. The project examined the growth inhibitory properties of a variety of Australian native plants against an extensive panel of bacterial pathogens.

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.

Ms Lindiwe Mpala completed her BSc at Griffith University in Life Sciences. Following graduation, she undertook a research project in Dr Ian Cock's laboratory in the School of Natural Sciences at Griffith University. The project examined the growth inhibitory properties of a variety of Australian native plants against an extensive panel of bacterial pathogens.