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Original Article

Grevillea juncifolia Hook. and *Grevillea robusta* A. Cunn. Ex. R. Br. Methanolic Leaf and Flower Extracts Inhibit the Growth of Gram Positive and Gram Negative Bacteria

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ABSTRACT

Introduction: The development of multi-antibiotic resistant strains of bacteria has necessitated the search for new effective antibacterial therapies. Several *Grevillea* spp. were used traditionally to treat pathogenic illness and are rich in phytocompounds with antibacterial activity. Despite this, the antibacterial activity of Australian *Grevillea* spp. extracts have not been extensively examined. **Methods:** The ability of *G. juncifolia* and *G. robusta* leaf and flower extracts to inhibit the growth of gram-negative and gram-positive bacterial species and some fungi was investigated by disc diffusion assays. The growth inhibitory activity was further quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** The *G. juncifolia* and *G. robusta* extracts were good inhibitors of the growth of both gram-positive and gram-negative bacteria yet were completely ineffective against all fungal species tested. The leaf extracts generally had better antibacterial activity than the flower extracts. The *G. juncifolia* leaf extract was a particularly good inhibitor of *A. faecalis, P. fluorescens, Y. entercolitica* and *B. subtilis* growth, with MIC values of 62, 533, 736 and 682µg/mL respectively. The *G. robusta* leaf extract was a potent inhibitor of *B. cereus* and *B. subtilis* growth (145 and 83µg/mL respectively). That extract was also a good inhibitor of *A. faecalis, P. fluorescens, S. salford, S. aureus* and *S. epidermidis* growth, albeit with substantially higher MIC values. In contrast, none of the extracts inhibited fungal growth. All extracts were determined to be non-toxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for the treatment of bacterial infections. **Conclusion:** The lack of toxicity of the *G. juncifolia* and *G. robusta* extracts and their growth inhibitory bioactivity against gram-positive and gram-negative bacteria indicate their potential in the development of new antibiotic chemotherapies.

Key words: Australian plants, *Proteaceae*, Spider flower, Traditional medicine, Herbal medicine, Antibacterial, Antibiotic resistant bacteria, Toxicity.

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INTRODUCTION

The discovery of penicillin by Alexander Fleming in 1928 changed the way bacterial infections were treated and has resulted in substantially decreased mortalities towards many pathogenic infections. That discovery resulted in a major paradigm shift in the way that medical science sought to develop new antibiotic chemotherapies. Since that time, research has focussed on screening for microbially derived antibiotic agents to provide the majority of our first-generation drugs. Despite many significant advances in the treatment of pathogenic disease, bacteria have developed resistance to all of the antibiotics commonly used clinically.[1] Several medicinally important bacterial pathogens have become either extremely (XDR) or totally drug resistant (TDR) to common clinically used antibiotics[1] and there are now limited therapeutic options for the diseases caused by these pathogens. This problem is expected to 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.

Traditional medicines and herbal remedies have great potential for antimicrobial drug development and there has recently been a substantial increase in interest in this field.[3-5] The genus *Grevillea* (family *Proteaceae*) consists of approximately 360 species native to rainforest and open regions of Australia, New Guinea, New Caledonia and Sulawesi, with the greatest diversity occurring in Australia. *Grevilleas* are commonly referred to as spider flower trees due to the appearance of their flowers and are also often referred to as silky oaks. *Grevillea* flowers were used as a food source by Australian Aborigines. The flowers were sucked for their sweet nectar or used to make sweet drinks.[6] They also had roles as traditional bush medicines for Australian Aborigines.[6,7] The leaves were used to treat wounds and sores, skin diseases as well as diarrhoea and dysentery.[6,7] Many of these diseases are caused by bacterial pathogens. *Grevillea* spp. decoctions were also used as potent bacteriocides and are reputed to have broad-spectrum inhibitory activity.[6,7] Unfortunately most of our understanding of the antimicrobial potential of Australian *Grevillea* species is anecdotal, with few species being thoroughly studied. Indeed, we were only able to find two studies that have examined *Grevillea* spp. extracts for antibacterial activity.[8,9] Unfortunately, both of these studies screened for antibacterial activity using a single, relatively high extract concentration and did not determine MIC values, making it impossible to benchmark the efficacy of these extracts against other plant species and conventional antibiotics. Whilst much work is needed to determine the chemical composition of the *Grevillea* spp., *G. robusta* leaf methanolic extracts have been reported to contain the novel compounds graviquinone (Figure 1e), methyl-5-ethoxy-2-hydrocinnamate (Figure 1f) and cis-3-hydroxy-5-pentadecylcyclohedxanone (Figure 1g).[10] The same study also detected multiple other compounds including methyl 2, 5-dihydroxycinnamate (Figure 1h) gravicycle (Figure 1i) and quercetin (Figure 1j).

Despite their traditional use and the earlier studies confirming antibacterial activity against limited bacterial panels, many *Grevillea* spp. are yet to be thoroughly evaluated for antibacterial activity. *Grevillea juncifolia* Hook. (Figure 1a) (commonly known as honeysuckle grevillea, honey grevillea and honeysuckle spider flower) is a shrub that is native to

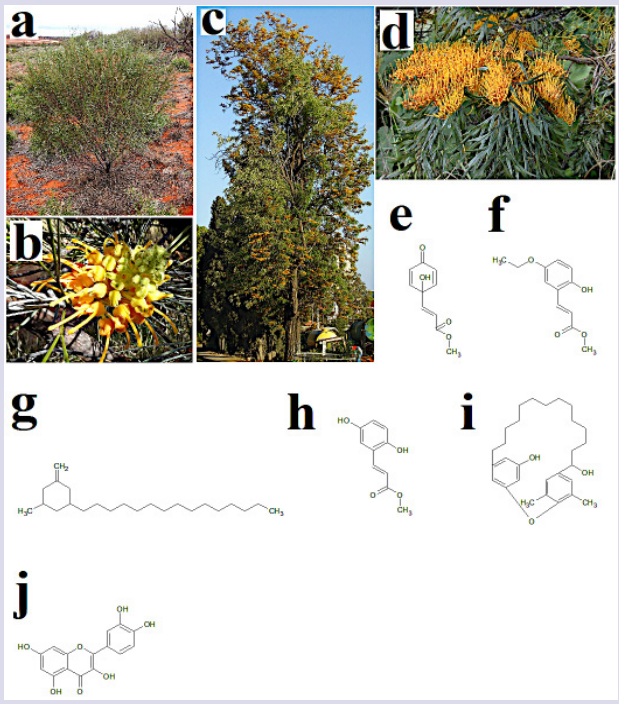

Figure 1: (a) *G. juncifolia* whole plant, (b) *G. juncifolia* flowers, (c) *G. robusta* whole plant, (b) *G. robusta* flowers, as well as the *G. robusta* phytochemical compounds (e) graviquinone, (f) methyl-5-ethoxy-2-hydrocinnamate, (g) cis-3-hydroxy-5-pentadecylcyclo-hedxanone, (h) methyl 2,5-dihydroxycinnamate (i) gravicycle and (j) quercetin.

inland regions of Australia. It produces yellow or orange flowers (Figure 1b), with flowering generally peaking between July and November each year. *Grevillea robusta* A. Cunn. Ex R. Br (Figure 1c) (commonly known as southern silky oak, silky oak, Australian silky oak) is a tall evergreen tree that grows to 40m tall. It produces yellowish-orange one-sided "toothbrush-like" flowers (Figure 1d) from September to November. The current study was undertaken to screen *G. juncifolia* and *G. robusta* leaf and flower extracts for the ability to inhibit the growth of panels of gram-positive and gram-negative bacterial pathogens and three fungal species.

MATERIALS AND METHODS

Plant collection and extraction

Grevillea juncifolia Hook. and *Grevillea robusta* A. Cunn. ex R. Br. leaves and flowers were obtained from verified trees on the southside of Brisbane, Australia. The leaf samples were dried in a Sunbeam food dehydrator, ground to a coarse powder and stored at -30°C until use. A volume of 50mL of AR grade methanol (Ajax Fine Chemicals, Australia) was added to 1g of each plant material and extracted individually for 24 h at 4°C with gentle shaking. The extract was filtered through filter paper (Whatman No. 54) under vacuum, followed by lyophilisation. The resultant pellets were weighed to determine the extraction yield and subsequently 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 *Grevillea* spp. leaf and flower extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.[11-13]

Antibacterial screening

Test microorganisms

All media was purchased from Oxoid Ltd., Australia. The reference strains of *E. coli* (ATCC157293) and *Klebsiella pneumoniae* (ATCC31488) were purchased from American Tissue Culture Collection (ATCC), USA. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Aspergillus niger*, *Bacillus cereus, Bacillus subtilis, candidia albicans, Citrobacter freundi*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, *Sacchromyces cerevisiae*, *Salmonella salford, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis* and *Yersinia entercolitica* strains 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. The fungal strains were cultured in Sabourand broth (Oxoid, Australia).

Evaluation of antimicrobial activity

Antimicrobial activity of the *Grevillea* spp. leaf and flower extracts was determined using a modified disc diffusion assay.[14-16] Briefly, 100µL of the each bacterial suspension in log phase was spread onto individual nutrient agar plates (or Sabourand agar for the fungal strains) and the extracts were tested for antibacterial activity using 5mm sterilised filter paper discs. The discs were each infused with 10µL of the individual plant extract, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4°C for 2 h before incubation at 37°C for 24 h. The diameters of the zones of inhibition (ZOIs) were measured to the closest whole millimetre. Each assay was performed three times in triplicate (n=9). Mean values (± SEM) are reported in this study. Standard discs of ampicillin (10µg), chloramphenicol (10µg) and nystatin (100µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial and antifungal 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.[17-19] Briefly, the *Grevillea* spp. leaf and flower extracts were diluted in deionised water (1% DMSO) and tested across a range of concentrations. Discs were individually infused with 10µL of each extract, allowed to dry and placed onto the inoculated plates. The assay was completed as outlined above and graphs of the ZOI versus ln concentration were plotted for each extract. Linear regression was used to determine the MIC values of each extract.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.[20-22] Briefly, *A. franciscana* nauplii were incubated in the presence of the extracts, reference toxin (1mg/mL potassium dichromate) or artificial seawater (negative control) at 25±1°C under artificial light. All treatments were performed three times in triplicate (n=9). The number of dead were counted in each well at 24h and 48h. At the completion of the 48h exposure period, the remaining live nauplii were sacrificed and the total number of nauplii in each well were counted and used to calculate the % mortality per well. LC_{50} values were calculated for each treatment using probit analysis.

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

			GJL	GJF	GRL	GRF
	Mass of extracted material (mg)		264	334	378	312
	Concentration of resuspended extract (mg/mL)		26.4	33.4	37.8	31.2
Qualitative Phytochemical Tests	Phenols	Total phenols	+++	+++	+++	+++
		Water soluble phenols	+++	+++	+++	+++
		Insoluble phenols	++	++	++	++
	Saponins	Froth persistence	++	+	++	++
		Emulsion test	+	+	+	+
	Cardiac glycosides	Keller-Kiliani Test	-	-	-	-
	Triterpenoids	Salkowski Test	++	+	+	+
	Phytosterols	Acetic Anhydride Test	+	-	-	-
	Alkaloids	Meyer's Test	+	+	+	+
		Wagner's Test	+	+	+	+
		Draggendoff's Test	+	+	+	+
	Flavonoids	Kumar Test	+++	+++	+++	+++
	Tannins	Ferric Chloride Test	+	+	+	+
		Lead Acetate Test	+	+	+	+
	Anthraquinones	Free	-	-	-	-
		Combined	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay; GJL = *Grevillea juncifolia* leaf extract; GJF = *Grevillea juncifolia* flower extract; GRL = *Grevillea robusta* leaf extract; GRF = *Grevillea robusta* flower extract.

Statistical analysis

Data are expressed as the mean ± SEM of three independent experiments with internal triplicates (n=9). 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 *Grevillea* spp. leaves and flowers with methanol yielded 264-378mg of extracted material (Table 1). The 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 all of the extracts had similar phytochemical profiles. All contained high levels of phenolic compounds and flavonoids, as well as moderate levels of saponins. Lower levels of triterpenoids, alkaloids and tannins were also detected. Cardiac glycosides and anthraquinones were completely absent or below the detection thresholds for these assays.

Antimicrobial activity

To determine the growth inhibitory activity of the *Grevillea* spp. leaf and flower extracts, aliquots (10µL) of each extract were screened in the disc diffusion assay. Varied effects were noted for the *Grevillea* spp. leaf and flower extracts against the gram-negative bacterial species, athough the extracts were ineffective at inhibiting the growth of many the gram-negative bacterial species tested (Figure 2). In contrast, both positive control antibiotics (ampicillin and chloramphenicol) were effective growth inhibitors, with ZOI's of up to 14.3mm (ampicillin against *A. faecalis*). However, there was some notable activity against three gram-negative bacterial species. *A. faecalis* was particularly susceptible to the *Grevillea* spp. extracts, with ZOIs of 28.7, 20.7 and 15.7mm recorded for the *G. juncifolia* leaf and flower extracts and the *G. robusta* leaf extract respectively. Similarly, 18.3, 9.6 and 15.6mm ZOIs were noted for the *G. juncifolia* leaf and flower extracts and the *G. robusta* leaf extract respectively against *P. fluorescens*, whilst 17.3 and 8.6mm were measured

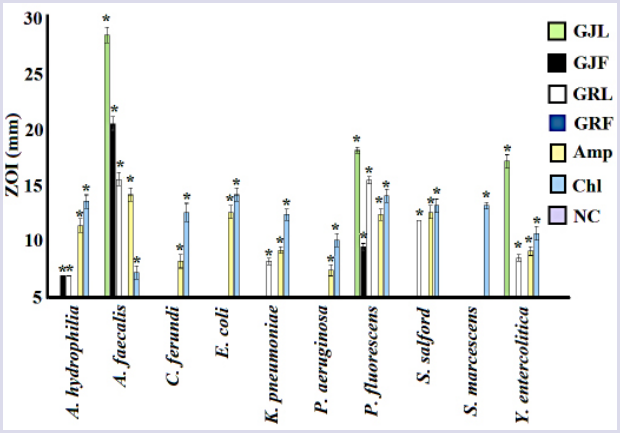

Figure 2: Growth inhibitory activity of *Grevillea* spp. leaf and flower extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. GJL = *G. juncifolia* leaf extract; GJF = G. *juncifolia* flower extract; GRL = *G. robusta* leaf extract; GRF = *G. robusta* flower extract; NC = negative control. All assays were completed three times, each with internal triplicates (n=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

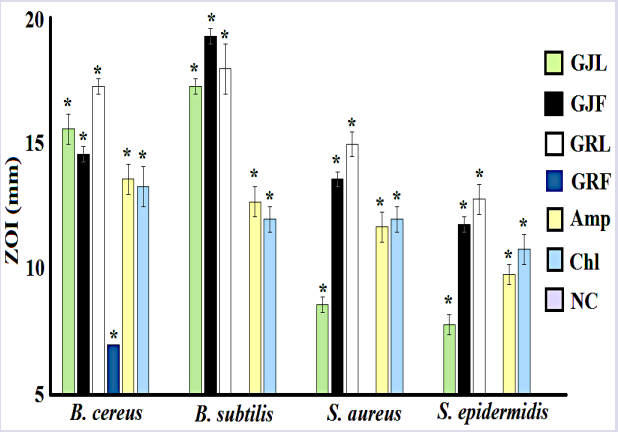

Figure 3: Growth inhibitory activity of *Grevillea* spp. leaf and flower extracts and reference antibiotics against gram-positive bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. GJL = *G. juncifolia* leaf extract; GJF = *G. juncifolia* flower extract; GRL = *G. robusta* leaf extract; GRF = *G. robusta* flower extract; NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM.

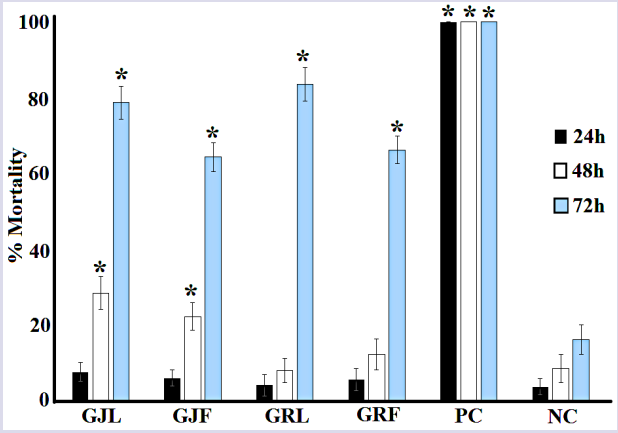

Figure 4: The lethality of the *Grevillea* spp. leaf and flower extracts (2000µg/mL), potassium dichromate control (1000µg/mL) and seawater (negative control) following 24, 48 and 72 h exposure. GJL = *Grevillea juncifolia* leaf extract; GJF = *Grevillea juncifolia* flower extract; GRL = *Grevillea robusta* leaf extract; GRF = *Grevillea robusta* flower extract. All bioassays were performed three times in triplicate (*n*=9) and are expressed as mean ± SEM. * indicates results that are significantly different to the untreated (seawater) control at the equivalent exposure time ($P<0.01$).

for the *G. juncifolia* and *G. robusta* leaf extracts when tested against *Y. entercolitica*.

A different trend was noted against the gram-positive bacterial species. With the exception of the *G. robusta* flower extract, all extracts were strong inhibitors of the growth of all gram-positive bacteria (Figure 3). Indeed, ZOIs in the range 15-20mm were measured for these extracts against both *Bacillus* spp. These ZOIs compared particularly favourably to the ampicillin and chloramphenicol controls, which produced ZOIs in the range 12-14mm. This is noteworthy as both of these controls were tested at relatively high doses (10µg/disc). Furthermore, the control antibiotics are pure compounds, whereas the extracts are crude mixtures and the active compound(s) would be expected to be a minor % of the overall extracts mass. Therefore, these extracts may be particularly promising as targets for antibiotic drug discovery.

Grevillea spp. leaf and flower extracts were also effective inhibitors of *S. aureus* and *S. epidermidis* growth (Figure 3). However, the size of these ZOIs is indicative of only moderate inhibitory activity. Interestingly, all extracts were completely devoid of antifungal activity, with ZOIs not significantly different to those determined for the negative control (results not shown). In contrast, the nystatin control was a good inhibitor of the growth of all fungal species, indicating that the assay was functioning correctly.

The antimicrobial efficacy was further quantified by determining the MIC value against each susceptible bacterial species (Table 2). The leaf extracts of each species were generally better inhibitors of bacterial growth than the corresponding flower extracts for both *Grevillea* species. The *G. juncifolia* leaf extract was a particularly potent inhibitor of *A. faecalis, P. fluorescens* and *Y. entercolitica* (MICs of 62, 533 and 736µg/mL respectively). However, there were some notable exceptions to these trends. The *G. juncifolia* flower extract was a more potent growth inhibitor of all gram-positive bacterial species than the corresponding leaf extract. In comparison, the *G. juncifolia* leaf extract was a substantially more potent inhibitor of gram-negative bacterial growth than was the flower extract, whilst the *G. robusta* leaf extract was much more potent against the gram-positive bacteria than the *G. robusta* flower extract.

Table 2: Minimum inhibitory concentrations (µg/mL) of the *Grevillea* spp. leaf and flower extracts against each susceptible bacterial strain and LC_{50} values (µg/mL) against *Artemia* nauplii.

Organism	Exposure time (h)	MIC or LC_{50} (ug/mL)			
		GJL	GJF	GRL	GRF
A. hydrophilia	24	-	2285	1788	-
A. faecalis	24	62	276	886	-
K. pneumoniae	24	-	-	1146	-
P. fluorescens	24	533	1260	829	-
S. salford	24	-	-	869	-
Y. entercolitica	24	736	-	1185	-
B. cereus	24	1055	463	145	2360
B. subtilis	24	682	226	83	-
S. aureus	24	1065	686	380	-
S. epiedermidis	24	1387	1046	623	-
Artemia nauplii	24	CND	CND	CND	CND
	48	CND	CND	CND	CND
	72	785	923	662	880

Numbers indicate the mean MIC or LC_{50} values of three independent experiments in triplicate (n=9). GJL = *G. juncifolia* leaf extract; GJF = *G. juncifolia* flower extract; GRL = *G. robusta* leaf extract; GRF = *G. robusta* flower extract; - indicates that the extract was inactive at all concentrations tested; CND indicates that an LC_{50} could not be determined as the mortality did not exceed 50% at any concentration tested.

Quantification of toxicity

The toxicity of the *Grevillea* spp. extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 4). The mortality in

the presence of all extracts was not significantly different to that of the untreated control at 24h and thus all extracts were deemed to be non-toxic. Extracts with 24h LC_{50} values >1000µg/mL have previously been defined as non-toxic.[20,21] In contrast, the potassium dichromate positive control induced substantial mortality within 4h (results not shown), with 100% mortality induction seen by 24h. The mortality induction remained relatively low for the *Grevillea* spp. extracts at 48h. Indeed, the % mortality induction was substantially <50% for all extracts following 48h exposure and therefore it was not possible to determine LC_{50} values for any of the *Grevillea* spp. extracts at 24 or 48h (Table 2). In contrast, the mortality was substantially increased for all extracts at 72h. However, as toxicity in this assay is defined following 24h exposure, all extracts were deemed to be nontoxic.

DISCUSSION

Despite the initial potency of many antibiotic chemotherapies, recent increases in bacterial resistance to many antibiotics 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.[23] *Grevillea* spp. are good candidates for the development of new antibacterial drugs as they were used by Australian Aborigines to treat bacterial infections.[6,7] Furthermore, several studies have reported that *Grevillea* spp. leaf and flower extracts are potent inhibitors of multiple bacterial species.[8,9] This study has extended these earlier studies by testing *Grevillea* spp. leaf and flower extracts against an extended panel of bacterial pathogens and three fungal species.

The greater susceptibility of gram-positive bacteria to the *Grevillea* spp. leaf and flower extracts extracts noted in this study is in agreement with previously reported results for South American.[24,25] African[26,27] and Australian[28] plant extracts. Results within our laboratory have also confirmed the greater susceptibility of gram-positive bacteria towards other Australian plant extracts.[29-31] The gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including antibiotics.[32] In contrast, other studies have demonstrated that gram-negative bacteria are often more susceptible to plant extracts from different Australian plant species.[33-35]

Whilst an investigation of the phytochemistry of the *Grevillea* spp *Grevillea* spp. extracts was beyond the scope of our study, high levels of polyphenolics and flavonoids, as well as moderate levels of triterpenoids and saponins were noted in the extracts in the qualitative phytochemical screening study. Lower levels of tannins were also detected. Flavonoids have well established bacterial growth inhibitory activities.[36] For example, the flavonoids kaempferol and myricetin have potent growth inhibitory activity against a panel of bacteria.[37] Similarly, quercetin, rutin and their corresponding glycosides inhibit the growth of *Pseudomonas maltophilia* and *Enterobacter cloacae*.[38] The antimicrobial activity of terpenoids has also been extensively documented. Monoterpenoids including α-pinene, β-pinene, sabinene, mycrene, terpinene, limonene, piperitone and β-phellandrene inhibit the growth of a panel of bacteria including several antibiotic resistant strains of Enterobacteriaceae.[36] Similarly, the antibacterial activities for several sesquiterpenoids including α-cubebene, copaene and caryophyllene have been reported.[36] Furthermore, many tannin compounds have bacterial growth inhibitory activity. Gallotannins inhibit the growth of a broad spectrum of bacterial species[39] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[40,41] and by inhibiting glucosyltransferase enzymes.[42] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.[39,41] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[39,41] Thus, it is likely that multiple compounds within the *Grevillea* spp. leaf and flower extracts are contributing to the antibacterial activity reported here.

Our findings also indicate that the extracts examined were non-toxic (LC_{50} >1000 µg/mL) in the *Artemia* nauplii bioassay. Whilst toxicity was assessed in this study with the test organism *A. franciscana*, toxicity towards *A. franciscana* has previously been shown to correlate well with toxicity towards human cells for many toxins.[20,21] However, further studies are required to determine whether this is also true for the *Grevillea* spp. leaf and flower extracts examined in these studies. The results of this study indicate that the *Grevillea* spp. leaf and flower extracts may be good candidates for antimicrobial drug discovery and further examination is warranted. Whilst the extracts examined in this report have potential as bacterial growth inhibitors, caution is needed before these compounds can be applied to medicinal purposes. Purification and identification of the bioactive components is needed to examine the mechanisms of action of these agents.

CONCLUSION

The growth inhibitory activity of the *Grevillea* spp. leaf and flower extracts against the gram-negative and gram-positive bacteria and their lack of toxicity towards *Artemia* nauplii indicate their potential for the development of new antibiotic drugs.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The author reports no conflicts of interest.

ABBREVIATIONS

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

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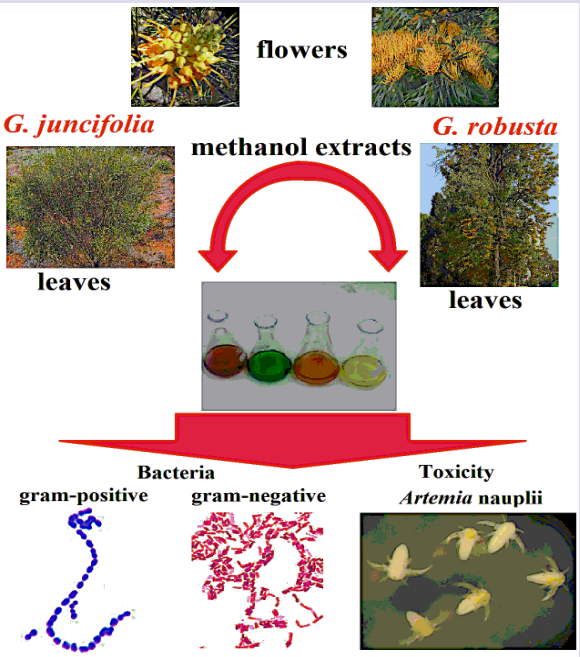
PICTORIAL ABSTRACT



SUMMARY

- Methanolic *G. juncifolia* and *G. robusta* leaf and flower extracts were screened for the ability to block the growth of a panel of bacteria.
- The growth inhibition of both gram-positive and gram-negative bacteria and three fungi was tested.
- The antibacterial activity was quantified by determining the MIC values of each extract.
- Toxicity of the *G. juncifolia* and *G. robusta* extracts was determined using the *Artemia* nauplii toxicity bioassay.

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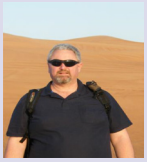

Dr. Ian Cock leads a research team in the Environmental Futures Research Institute and the School of Environment and Science at Griffith University, Australia. His research involves bioactivity and phytochemical studies into a variety of plant species of both Australian and international origin, including *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.