The Inhibitory Activity of *Banksia collina* R.Br. and *Banksia oblongifolia* Cav. Methanolic Leaf Extracts against a Panel of Bacterial Pathogens

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**ABSTRACT**

**Introduction:** The development of multi-antibiotic resistant strains of bacteria has necessitated the search for new, effective antibacterial therapies. *B. collina* and *B. oblongifolia* leaves were used by Australian Aborigines to treat bacterial infections. However, little research has been published on antibacterial activity of these species. **Methods:** The ability of *B. collina* and *B. oblongifolia* leaf extracts to inhibit the growth of gram-negative and gram-positive bacterial species was investigated by disc diffusion and growth time course assays. The growth inhibitory activity was further quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** The *B. collina* and *B. oblongifolia* leaf extracts were good inhibitors of the growth of both gram-positive and gram-negative bacteria. The *B. collina* and *B. oblongifolia* leaf extracts were particularly good inhibitors of *A. faecalis* growth (MICs of 225 and 486µg/mL respectively) and *B. cereus* growth (MICs of 515 and 875µg/mL respectively). The *B. collina* extract was also a good inhibitor of *B. subtilis* growth, whilst the *B. oblongifolia* extract was a moderate growth inhibitor (MIC values of 923 and 1250µg/mL respectively). A similar trend was noted for *Y. enterocolitica* growth inhibition (MICs of 518 and 1136µg/mL respectively). Whilst MIC values were also determined against other bacterial species, they generally indicated low-moderate activity. The *B. collina* and *B. oblongifolia* leaf extracts were further investigated by growth time course assays against *A. faecalis* and *B. cereus*. Interestingly, both extracts showed significant growth inhibition within 1h of exposure against both bacterial species. All extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for the treatment of bacterial infections. **Conclusion:** The lack of toxicity of the *B. collina* and *B. oblongifolia* leaf extracts and their growth inhibitory bioactivity against multiple bacterial species indicate their potential in the development of new antibiotic chemotherapies.

**Key words:** Protaceae, Hill Banksia, Golden candlesticks, *Banksia*, Fern-leaved Banksia, Traditional medicine, Antibacterial activity, Antibiotic resistant bacteria, MIC.

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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 antibiotic 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.4-6 Much of the research into traditional medicinal plant use has focused on Asian,6 African4,12 and South American13-14 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.

*Banksia collina* R.Br. (Figure 1a; synonyms *Banksia spinulosa* var. collina (R.Br.) A.S. George; commonly known as hill banksia or golden candlesticks) and *Banksia oblongifolia* Cav. (Figure 1b; synonyms *Banksia salicifolia* Cav. *Banksia latifolia* var. *minor* Maiden and Camfield, *Banksia robur* var. *minor* (Maiden and Camfield) Maiden and Betch, *Banksia integrifolia* var. *oblongifolia* (Cav.) Domini; common known as fern-leaved, dwarf or rusty banksia) are endemic Australian plants and members of family Protaceae. Both species are native to coastal regions of eastern Australia, extending from the central New South Wales Coast north to the central Queensland coast. Interestingly, several *Banksia* ssp. were used by the first Australians to treat bacterial infections.20,21 Furthermore, several studies have reported antibacterial activity for related *Banksia* ssp. Extracts produced from *Banksia interggrfolia* var. *aquilonia* have good inhibitory activity against *Bacillus cereus* and *Staphylococcus aureus* (MIC values of 312 and 78µg/mL respectively), as well as low-moderate activity against *Escherichia coli*.22 The same study reported that the same extract was ineffective against *Streptococcus pneumonia* and *Pseudomonas aeruginosa*. Studies into the antibacterial activity of many Australian *Banksia* ssp. are lacking. The phytochemistry of *Banksia* ssp. has been examined in the leaves of the related species *Banksia cocinea* R.Br. and *Banksia menziesii* R.Br.22 These species contain an abundance of anthocyanins including cyanidin-3-galactoside (Figure 1c), cyanidin-3-glucoside (Figure 1d), cyaniding-3,5-diglucoside Figure 1e), peonidin-3-galactoside (Figure 1f) and...
peonidin-3-glucoside (Figure 1g). Many similar flavonoids have good antibacterial activity.\textsuperscript{23} An examination of the antibacterial properties of Banksia spp. is therefore warranted. This study was undertaken to screen methanolic B. collina and B. oblongifolia leaf extracts for the ability to inhibit the growth of panels of gram-positive and gram-negative bacterial pathogens.

**MATERIALS AND METHODS**

Plant collection and extraction

*Banksia collina* R.Br. and *Banksia oblongifolia* Cav. leaves were obtained from verified plants in the Logan area south of Brisbane. The leaf samples were dried in a Sunbeam food dehydrator and stored at -30°C. Prior to use, the dried leaves were freshly ground to a coarse powder and 1g quantities were weighed into separate tubes. A volume of 50mL of AR grade methanol (Ajax Fine Chemicals, Australia) was added to 1g of the plant material and extracted for 24 hr 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 *B. collina* and *B. oblongifolia* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.\textsuperscript{24,25}

Antibacterial screening

**Test microorganisms**

All media was purchased from Oxoid Ltd., Australia. The reference strains of *E. coli* (ATCC157293), *Klebsiella pneumoniae* (ATCC31488), *Proteus mirabilis* (ATCC21721) and *Streptococcus pyogenes* (ATCC19615) were purchased from American Tissue Culture Collection (ATCC), USA. All other bacterial strains used in this study were clinical isolate microbial strains and 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 the *B. collina* and *B. oblongifolia* leaf extracts was determined using a modified disc diffusion assay.\textsuperscript{31-33} Briefly, 100µL of each bacterial suspension in log phase was spread onto individual nutrient agar plates and the extracts were tested for antibacterial activity using 6mm 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 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.

**Bacterial growth time course assay**

Bacterial growth time course studies were performed as previously described.\textsuperscript{24} Briefly, 3mL of the gram-positive bacterial species in nutrient broth were individually added to 27mL nutrient broth containing 3mL of 10mg/mL of the extract to give a final extract concentration of 1000µg/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 three times in triplicate (*n* = 9).

Toxicity screening

*Artemia franciscana* nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.\textsuperscript{32,33} 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 nauplii were counted in each well at 24, 48 and 72hr. At the completion of the 72hr 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\textsubscript{50} values were calculated for each treatment using probit analysis.

**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 *B. collina* and *B. oblongifolia* leaf extracts with methanol yielded 299 and 236mg of extracted material respectively (Table 1). The extracts were resuspended in 10mL of deionised water (containing 1% DMSO), resulting in an extract concentration shown in Table 1. Qualitative phytochemical screening studies showed that both extracts had similar phytochemical profiles. Both contained high levels of phenolic compounds and flavonoids. Lower levels of saponins, triterpenoids and tannins were also detected. Cardiac glycosides, phytosterols, alkaloids and anthraquinones were completely absent or below the detection thresholds for these assays.

Antimicrobial activity

To determine the growth inhibitory activity of the *B. collina* and *B. oblongifolia* leaf extracts, aliquots (10µL) of each extract were screened in the disc diffusion assay. *B. collina* and *B. oblongifolia* leaf extracts were effective at inhibiting the growth of 4 of the 5 (80%) gram-negative bacterial species tested (Figure 2). For all of the inhibited bacteria, the *B. collina* extract was a substantially more potent inhibitor of bacterial growth than the *B. oblongifolia* extract. Only *E. coli* was completely resistant to the *B. collina* and *B. oblongifolia* leaf extracts. In contrast, *A. faecalis* was highly susceptible to the *B. collina* and *B. oblongifolia* leaf extracts, with ZOIs of 17.3 and 11.6mm. This compared well to the ZOIs of the...
control antibiotics, indicating that this extract may be particularly useful in the development of future antibiotic therapies. The ampicillin control was a potent inhibitor of *A. faecalis* growth, with a ZOI of 15.3mm. This bacterium was relatively resistant to chloramphenicol, with only 6.6mm ZOIs recorded. Notably, the control antibiotics were tested at a relatively high dosage (10μg/disc) of pure antibiotic. In contrast, the extracts were crude and the antibacterial component(s) would be expected to contain a relatively low % of the bioactive compound(s). Similar results, albeit with smaller ZOIs, were noted for the *collina* and *oblongifolia* leaf extracts against *K. pneumonia*, *P. mirabilis* and *Y. enterocolitica*. The gram-positive bacterial species were also susceptible to the *collina* and *oblongifolia* leaf extracts. The growth of 3 of the 5 (60%) gram-positive bacterial species tested were susceptible to at least one of the extracts (Figure 3). As noted for the gram-negative bacteria, the *collina* extract was generally a substantially better inhibitor of gram-positive bacterial growth than the *oblongifolia* extracts. *B. cereus* was the most susceptible to the inhibitory effects of the extracts, with ZOIs of nearly 10.6 and 9.2mm measured respectively (Figure 3). These ZOIs are comparable to those of the pure ampicillin and chloramphenicol (14.6 and 11.3mm respectively). This is noteworthy as the antibiotic 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 contain a minor % of the overall extracts mass. Therefore, these extracts may be particularly promising as targets for antibiotic drug discovery. The *collina* and *oblongifolia* leaf extracts were also effective inhibitors of *B. subtilis* growth (Figure 3), albeit with a smaller ZOI noted (8.8 and 7.6mm respectively). The *collina* (but not the *oblongifolia*) leaf extract also inhibited *S. pyogenes* growth, albeit with a ZOI that is indicative of low to moderate inhibitory activity. As *S. pyogenes* can cause a wide variety of diseases including pharyngitis, impetigo and rheumatic fever depending on the tissue that it infects, the *collina* extract may be useful as targets for antibiotic discovery. In contrast, both *Staphylococcus* spp. were resistant to the *collina* and *oblongifolia* leaf extracts.

The antimicrobial efficacy was further quantified by determining MIC values. The *collina* and *oblongifolia* leaf extracts were particularly good inhibitors of *A. faecalis* (MICs of 225 and 486μg/mL respectively) and *B. cereus* growth (MICs of 515 and 875μg/mL respectively). The *collina* extracts were also a good inhibitor of *B. subtilis* growth, whilst the *oblongifolia* extract was a moderate growth inhibitor (MIC values of 923 and 1250μg/mL respectively). A similar trend was noted for *Y. enterocolitica* growth inhibition (MICs of 518 and 1136μg/mL respectively). Whilst MIC values were also determined against other bacterial species, they generally indicated moderate-low activity.

**Bacterial growth time course assay**

The antibacterial activity of the *collina* and *oblongifolia* leaf extracts was further investigated against *A. faecalis* and *B. cereus* by bacterial growth time course assays in the presence and absence of the extracts (Figure 4). The starting concentration of the extract used in these assays was 1000μg/mL. The *collina* and *oblongifolia* leaf extracts both significantly inhibited *A. faecalis* within 1hr of exposure, indicating a rapid antimicrobial action (Figure 4a). The absorbance of the *A. faecalis* culture remained substantially lower than the untreated control for the first 4 hr of exposure. After that time, the absorbance increased to approximately the same level as the control, indicating that the *collina* and *oblongifolia* leaf extracts are bacteriostatic rather than bacteriocidal at the concentrations tested. Similar trends were noted when the *collina* and *oblongifolia* leaf extracts were tested against *B. cereus* (Figure 4b). Again, the absorbance of the *B. cereus* culture (and thus the bacterial growth) remained substantially lower than the untreated control for the first 4 hr of exposure and then increased to approximately the same level as the control, indicating that *collina* and *oblongifolia* leaf extracts may be bacteriostatic at the concentrations tested (Figure 4b).

**Quantification of toxicity**

The toxicity of the *collina* and *oblongifolia* leaf extracts extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 5). The mortality in the presence of both extracts was not significantly different to that of the untreated control at 24hr and thus they were deemed to be non-toxic. Extracts with 24h LC50 values >1000μg/mL have previously been defined as non-toxic. In contrast, the potassium dichromate positive control induced substantial mortality within 4hr (results not shown), with 100% mortality induction seen by 24hr. The

**Figure 1:** (a) *B. collina*, (b) *B. oblongifolia*, as well as the anthocyanins (c) cyanidin-3-galactoside, (d) cyanidin-3-glucoside, (e) cyaniding-3,5-diglucoside, (f) peonidin-3-galactoside and (g) peonidin-3-glucoside.

**Figure 2:** Growth inhibitory activity of the *collina* and *oblongifolia* leaf extracts extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chlor) standard discs (10μg) were used as positive controls. All assays were completed three times, each with internal triplicates (n=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. * indicates results that were significantly different to the untreated control (*p*<0.01).
Cock.: Antibacterial activity of Australian Banksia spp.

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Figure 3: Growth inhibitory activity of the B. collina and B. oblongifolia leaf extracts extracts and reference antibiotics against gram-positive bacterial species measured as ZOI (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. 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. * indicates results that were significantly different to the untreated control (P<0.01).

Figure 4: Bacterial growth curves the B. collina and B. oblongifolia leaf extracts against (a) A. faecalis and (b) B. cereus. All bioassays were performed three times in triplicate (n=9) and are expressed as mean ± SEM. BC = B. collina extract; BO = B. oblongifolia extract; * = methanolic extract results that are significantly different between the treated and the untreated control growth; # = aqueous extract results that are significantly different between the treated and the untreated control growth (P<0.01).

Figure 5: The lethality of the B. collina and B. oblongifolia leaf extracts (2000µg/mL), potassium dichromate control (1000µg/mL) and seawater (negative control) following 24, 48 and 72 hr exposure. BC = Banksia collina extract; BO = Banksia oblongifolia extract; PC = potassium dichromate control; NC = negative (seawater) control. 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).

mortality induction remained low for the B. collina and B. oblongifolia leaf extracts at 48hr. Indeed, the % mortality induction was substantially <50% for all extracts at all times tested and therefore it was not possible to determine LC50 values for any of the B. collina and B. oblongifolia leaf extracts (Table 2).

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.34 The first Australians used several Banksia spp. to treat multiple diseases and infections caused by bacterial pathogens.15,20 Despite this, limited scientific evaluations have rigorously evaluated the antibacterial properties of other Banksia spp. To the best of our knowledge, this is the first study to report bacterial growth inhibitory activity of B. collina and B. oblongifolia.

The ability of the B. collina and B. oblongifolia 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.35,36 In our study, the gram-negative and gram-positive bacteria were approximately equally susceptible to the B. collina and B. oblongifolia extracts. In contrast, many previous studies have reported substantially greater susceptibility for gram-positive bacteria to South American,11,12 African,11,12 and Australian37 plant extracts. Results within our laboratory have also confirmed the greater susceptibility of gram-positive bacteria towards many other Australian plant extracts.38,39 The gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including several antibiotics.40 In contrast, other studies have demonstrated that gram-negative bacteria are often as susceptible (or more susceptible) to plant extracts from different Australian plant species.41,42

Whilst an investigation of the phytochemistry of the B. collina and B. oblongifolia leaf extracts was beyond the scope of this study, moderate to
Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water and qualitative phytochemical screenings of the \textit{B. collina} and \textit{B. oblongifolia} leaf extracts.

<table>
<thead>
<tr>
<th>Mass of extracted material (mg)</th>
<th>\textit{B. collina}</th>
<th>\textit{B. oblongifolia}</th>
</tr>
</thead>
<tbody>
<tr>
<td>299</td>
<td>236</td>
<td></td>
</tr>
</tbody>
</table>

Concentration of resuspended extract (mg/mL)

| Total phenols | +++ | +++ |
| Water-soluble phenols | +++ | +++ |
| Insoluble phenols | ++ | ++ |
| Froth persistence | + | + |
| Keller-Kiliian Test | - | - |
| Salkowski Test | + | + |

Cardiac glycosides

| Acetic Anhydride Test | - | - |
| Meyer’s Test | - | - |
| Wagner’s Test | - | - |
| Dragendorff’s Test | - | - |

Flavonoids

| Kumar Test | +++ | +++ |
| Ferric Chloride Test | + | + |

Tannins

| Lead Acetate Test | + | + |

Anthraquinones

| Combined | Free | - |

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

Table 2: Minimum inhibitory concentrations (µg/mL) of the \textit{B. collina} and \textit{B. oblongifolia} leaf extracts against each bacterial strain and LC50 values (µg/mL) against \textit{Artemia nauplii}.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Exposure time (h)</th>
<th>MIC or LC50 (µg/mL)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>\textit{B. collina}</td>
<td>\textit{B. oblongifolia}</td>
</tr>
<tr>
<td>A. faecalis</td>
<td>24</td>
<td>225</td>
</tr>
<tr>
<td>E. coli</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>24</td>
<td>1134</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>24</td>
<td>1426</td>
</tr>
<tr>
<td>Y. entercolitica</td>
<td>24</td>
<td>518</td>
</tr>
<tr>
<td>B. cereus</td>
<td>24</td>
<td>515</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>24</td>
<td>923</td>
</tr>
<tr>
<td>S. aureus</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>24</td>
<td>-</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>24</td>
<td>1352</td>
</tr>
<tr>
<td>\textit{Artemia nauplii}</td>
<td>48</td>
<td>CND</td>
</tr>
<tr>
<td>72</td>
<td>1343</td>
<td>1580</td>
</tr>
</tbody>
</table>

Numbers indicate the mean MIC or LC50 values of three independent experiments (n=9). - indicates that an neither extract did not inhibit bacterial growth at any concentration tested; CND indicates that an LC50 could not be determined as the mortality did not exceed 50% at any concentration tested.

high levels of polyphenolics and flavonoids were noted in the extracts by qualitative phytochemical screening. Lower levels of saponins, triterpenoids and tannins were also detected. Previous studies have also reported that \textit{Banksia} spp. are a relatively rich source of anthocyanin flavonoids.22 Flavonoids have well established bacterial growth inhibitory activities.23 The flavonoids kaempferol and myricetin have been reported to be potent growth inhibitors of a panel of bacterial pathogens.43 Similarly, queretin, rutin and their corresponding glycosides inhibit the growth of \textit{Pseudomonas maltophilia} and \textit{Enterobacter cloacae}.44 It is therefore likely that the \textit{B. collina} and \textit{B. oblongifolia} leaf extract flavonoids may contribute to the antibacterial activity reported in this study. However, it is likely that other phytochemical classes in these extracts may also contribute to the antibacterial activity.

The antimicrobial activity of terpenoids has also been extensively documented. Monoterpenoids including α-pinene, β-pinene, sabine, myrcene, terpinene, limonene, piperitone and β-phellandrene inhibit the growth of a panel of bacteria, including several antibiotic resistant strains of \textit{Enterobacteriaceae}.23 The antibacterial activities for several sesquiterpenoids including α-cubebene, copaene and caryophyllene have been reported.23 Similarly, many tannin compounds have bacterial growth inhibitory activity. Gallotannins inhibit the growth of a broad spectrum of bacterial species through a variety of mechanisms including binding cell surface molecules including lipotioichoic acid and proline-rich cell surface proteins, and by inhibiting glucosyltransferase enzymes.46 Ellagitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.41 Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.45,46 Thus, it is likely that multiple compounds within the \textit{B. collina} and \textit{B. oblongifolia} leaf extracts are contributing to the antibacterial activity reported here.

The findings reported here also indicate that the extracts examined were non-toxic (LC50 >1000 µg/mL) in the \textit{Artemia nauplii} bioassay. Whilst

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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. However, further studies are required to determine whether this is also true for the B. collina and B. oblongifolia leaf extracts examined in these studies. The results of this study indicate that the B. collina and B. oblongifolia leaf 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 B. collina and B. oblongifolia leaf extracts against gram-positive and gram-negative bacteria and their lack of toxicity indicate their potential for the development of novel chemotherapies to treat a variety of diseases caused by bacterial pathogens. Further studies aimed at the purification of the bioactive components are needed to examine the mechanisms of action of these agents.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

DMSO: Dimethyl sulfoxide; LC₅₀: The concentration required to achieve 50 % mortality; MIC: Minimum inhibitory concentration; ZOI: Zone of inhibition.

REFERENCES


PICTORIAL ABSTRACT

- Methanolic B. collina and B. oblongifolia leaf 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 was tested.
- The antibacterial activity was quantified by determining the MIC values of each extract.
- Growth time course studies were also undertaken against A. faecalis and B. cereus.
- Toxicity of the B. collina and B. oblongifolia leaf extracts was determined using the Artemia nauplii toxicity bioassay.

ABOUT AUTHORS

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 Ferdinandi-diana (Kakadu plum), Australian Acacias, Syzygiuums, Petalostigmas and Xanthorrhoea Johnsonii (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.