Inhibition of *Streptococcus pyogenes* growth by native Australian plants: New approaches towards the management of impetigo, pharyngitis and rheumatic heart disease

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**ABSTRACT**

**Introduction:** *Streptococcus pyogenes* can cause a variety of diseases including streptococcal pharyngitis, impetigo and rheumatic heart disease, dependant on which tissue it infects. Many Australian plants have documented therapeutic properties as general antiseptics, but are yet to be tested for the ability to inhibit *S. pyogenes* growth. **Methods:** Solvent extracts were prepared using Australian plants with documented ethnobotanical usage to treat bacterial infections, or published antibacterial activity. The extracts were investigated by disc diffusion assay for the ability to inhibit the growth of a clinical strain of *S. pyogenes*. Their MIC values were determined to quantify and compare their efficacies. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *S. pyogenes* growth was inhibited by 24 of the 34 extracts tested. The *Eucalyptus* spp. extracts were particularly potent. MIC values of 341 and 88 µg/mL were determined for the aqueous and methanolic *E. baileyana* extracts respectively. Similarly, MIC values of 134 and 53 µg/mL were determined for the aqueous and methanolic *E. major* extracts respectively. The methanolic wattle seed extract, aqueous and methanolic lemon aspen extracts, aqueous native thyme extract, methanolic river mint extract and the methanolic native basil extract were similarly potent growth inhibitors, with MIC values ≤1000 µg/mL. Several other extracts (methanolic native tamarind, bush tomato, desert lime, native thyme, native sage and the *T. stipitata* leaf extracts, as well as the aqueous river mint, native basil, *T. stipitata* leaf extracts) displayed moderate growth inhibitory activity (MIC=1000-5000 µg/mL). All other extracts were either low potency *S. pyogenes* growth inhibitors or were devoid of inhibitory activity. The *E. baileyana* and *E. major* methanolic extracts, as well as the *E. baileyana* aqueous extract induced significant mortality in the *Artemia franciscana* bioassay, with LC₅₀ values substantially <1000 µg/mL. All other extracts were nontoxic, with LC₅₀ values >1000 µg/mL. **Conclusion:** The potent growth inhibitory bioactivity of the *Eucalyptus* spp., lemon aspen, wattle seed, native basil and river mint extracts against *S. pyogenes* demonstrates their potential for the treatment and prevention of *S. pyogenes* induced disease. However, the toxicity of the *Eucalyptus* spp. extracts may limit their use to topical treatments for pharyngitis and impetigo. As the lemon aspen, wattle seed, native basil and river mint extracts were nontoxic, they may also have wider uses in treating systemic illnesses such as rheumatic fever, rheumatic heart disease and cellulitis.

**Key words:** *Eucalyptus*, Antioxidant, Lemon aspen, Pharyngitis, Impetigo, Rheumatic heart disease, Antibacterial activity.

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**INTRODUCTION**

*Streptococcus pyogenes* is a gram-positive, facultative anaerobe and the etiological agent of a number of diseases in humans. In nature, the bacterium is present as part of the natural skin flora of humans and animals which under certain conditions can cause disease.¹ Associated diseases vary in symptoms and severity, ranging from superficial skin infections (impetigo and ecthyma) to rheumatic fever.²,²¹ Typically, streptococcal infections are localised on the epidermis or nasopharynx and/or oropharynx and are treated with antibiotics as required. However, due to the self-limiting nature of these infections and the increasing risk of antibiotic resistance unless complications arise monitoring and symptom treatment is often the preferred option. The probing of natural plant resources offers an alternate means of fighting streptococcal diseases through the prevention of bacterial growth.³

Several Australian plant species were selected for *S. pyogenes* growth inhibitory activity screening based on their usage in traditional medicine systems³ and/or their reported antibacterial activities⁴⁻⁹ (Table 1). Plants of the genus *Eucalyptus* are particularly well known for their antibiotic properties due to their high 1, 8-cineole contents.⁵,¹⁰ The first Australians crushed the leaves and inhaled the volatiles to treat coughs and colds.⁵,¹⁰ Fresh leaves or decoctions prepared from the leaves were also used as wound antiseptics and to treat skin and throat infections. Furthermore, *in vitro* studies have demonstrated the growth inhibitory properties of *Eucalyptus baileyana* and *Eucalyptus major* extracts towards a panel of bacterial species, indicating their therapeutic potential in treating pathogenic diseases.¹⁰,¹² Indeed, essential oils prepared from *Eucalyptus* spp. leaves remain a popular antiseptic agent, not only in Australia, but are also commonly sold in pharmacies internationally. While the ethnobotanical uses of many Australian plants in traditional Aboriginal medicine systems have been recorded, rigorous scientific studies are lacking for many species.⁵,¹⁰ Recent studies have reported potent broad spectrum bacterial growth inhibitory activity for *S. spinescens* extracts.¹² Despite this, *S. pyogenes* growth inhibitory activity. *Tasmannia lanceolata*¹⁴ and *Tasmannia stipitata* (family Winteraceae) extracts have potent broad spectrum antimicrobial activity for *in vitro*.¹ Furthermore, *T. lanceolata*¹⁵ and *T. stipitata* have also been reported to inhibit the proliferation of the gastrointestinal protozoal parasite *Giardia duodenalis*.⁶,¹¹ We were unable to find similar studies examining the therapeutic potential of *T. insipida*.

Recent studies have reported exceptionally high antioxidant content of the fruits of several Australian plant species.⁵,⁹,¹⁴ In particular, these...
studies reported the fruit of *Kunzea pomifera* F. Muell. (muntjies) and *Podocarpus elatus* R. Br. (Illawarra plum) to have similar antioxidant capacities to blueberries (which are themselves considered to have a high antioxidant capacity). *Acronychia acidula* F. Muell. (lemon aspen), *Citrus glauca* (Lindl.) Burkill (desert lime) and *Solanum aviculare* G. Forst. (bush tomato) have also been reported to have high antioxidant capacities.\(^1\)\(^4\)\(^-\)\(^9\)\(^-\)\(^2\(^0\) It has been postulated that the high antioxidant contents of some Australian native fruits may provide them with therapeutic effects.\(^1\)\(^4\)\(^-\)\(^9\)\(^-\)\(^2\(^0\) Similarly, a number of Australian culinary herbs including *Prostanthera rotundifolia* R. Br. (native thyme), *Prostanthera incise* R. Br. (native sage), and *Mentha australis* R. Br. (rivermint) have been reported to have high antioxidant capacities and potent growth inhibitory activity against bacteria associated with the induction of several autoimmune inflammatory diseases.\(^2\(^0\) Despite this relative wealth of information documenting antibacterial Australian plants, many are yet to be tested for the ability to inhibit *S. pyogenes* growth. The current study examines the growth inhibitory activity of extracts of selected Australian plants against *S. pyogenes*, and thus their potential in the prevention and treatment of streptococcal pharyngitis, impetigo, rheumatic fever and rheumatic heart disease.

**MATERIALS AND METHODS**

**Plant source and extraction**

*Acronychia acidula* F. Muell. (lemon aspen), *Podocarpus elatus* R. Br. (Illawarra plum), *Kunzea pomifera* F. Muell. (muntjies), *Diploglossis australis* Hook. f. (native tamarind), *Acacia victoriae* Benth. (wattle seed), *Citrus glauca* (Lindl.) Burkill (desert lime), *Solanum aviculare* G. Forst. (bush tomato), *Prostanthera incise* R. Br. (native sage), *Prostanthera rotundifolia* R. Br. (native thyme), *Ocimum tenuiflorum* L. (native basil) and *Mentha australis* R. Br. (river mint) were obtained from Taste of Australia Bush Food, Australia. Air dried *Tasmannia stipitata* R. Br. leaves and *Tasmannia stipitata* (Vick.) A. C. Smith leaves and berries were supplied and verified by the Queensland Bush foods Association, Australia. *Scaevola spinescens* R. Br. was supplied by Jeannie Crago of Outback Books Australia (a commercial supplier of *S. spinescens* tea) as a pre-dried and course milled whole plant material. *Eucalyptus baileyana* F. Muell. And *Eucalyptus major* (Maiden) Blakely plant materials were collected from Toohey Forest, Brisbane and were identified with reference to a taxonomic key to Toohey Forest plants.\(^2\(^1\) Voucher samples of all plant specimens are stored in the School of Natural Sciences, Griffith University (Australia). The plant materials were comprehensively dried in a Sunbeam food dehydrator and the dried plant materials were stored at -30°C. Prior to use, the plant materials were thawed and freshly ground to a coarse powder. Individual 1 g quantities of the ground plant material were weighed into separate tubes and 50 mL of water or methanol were added. All solvents were obtained from Ajax and were AR grade. The ground plant materials were individually extracted in each solvent for 24 h at 4°C and the extracts were transferred to the wells and incubated at 25 ± 1°C for 24 h. The minimum inhibitory concentration (MIC) of each extract against *S. pyogenes* was determined as previously described.\(^2\(^6\)\(^-\)\(^\)\(^2\(^8\) Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were infusid 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 *S. pyogenes* was determined as previously described.\(^2\(^6\)\(^-\)\(^\)\(^2\(^8\) Briefly, the plant extracts were diluted in deionised water and tested across a range of concentrations. Discs were infusid with 10 µL of the test dilutions, allowed to dry and placed onto the 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.

**Toxicity screening**

**Reference toxin for toxicity screening**

Potassium dichromate (K₂Cr₂O₇) (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* nauplius bioassay.

**Artemia franciscana nauplius toxicity screening**

Toxicity was tested using an adapted *Artemia franciscana* nauplius lethality assay as previously described.\(^3\(^1\)\(^-\)\(^\)\(^3\(^3\) Briefly, 400 µL of seawater containing approximately 43 (mean 43.2, n=155, SD 14.5) *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 LC₅₀ 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.
Table 1: The medicinal usage, common names and known constituents of the native Australian plant species tested in this study

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>Part Used in This Study</th>
<th>Common Name/s</th>
<th>Traditional Medicinal Uses and Known Therapeutic Properties</th>
<th>Known Constituents</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acacia vivtoriae Benth.</td>
<td>seed</td>
<td>wattle</td>
<td>The seed was ground into a flour and used to make a bread. The seed has reported antibacterial and anticancer properties. Other Acacia spp. were used to treat allergies, rash and as an antiseptic.</td>
<td>Unknown, although other Acacia spp. contain high levels of tannins, terpenoids and saponins</td>
<td>5, 10</td>
</tr>
<tr>
<td>Acronychia acidula F. Muell.</td>
<td>fruit</td>
<td>lemon aspen</td>
<td>The fruit has a high antioxidant capacity and was mainly used as a nutritious food. The fruit has reported antibacterial and anticancer properties.</td>
<td>unknown although the related species Acronychia baueri has been reported to contain alkaloids</td>
<td>10</td>
</tr>
<tr>
<td>Citrus glauca (Lindl.) Burkill</td>
<td>fruit</td>
<td>desert lime</td>
<td>The fruit has a high antioxidant capacity and was mainly used as a nutritious food. The fruit has reported antibacterial and anticancer properties.</td>
<td>Unknown</td>
<td>16, 19, 20</td>
</tr>
<tr>
<td>Diploglottis australis Hook. f.</td>
<td>fruit</td>
<td>Australian native tamarind</td>
<td>The fruit has a high antioxidant capacity and was mainly used as a nutritious food. The fruit has reported antibacterial and anticancer properties.</td>
<td>Unknown</td>
<td>16, 19, 20</td>
</tr>
<tr>
<td>Eucalyptus baileyana F. Muell.</td>
<td>leaf</td>
<td>Bailey's stringybark</td>
<td>Used to treat rheumatism, swelling, inflammation, skin disorders stomach disorders, bactericide (wounds, sores)</td>
<td>high terpene content</td>
<td>5, 10, 12</td>
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<tr>
<td>Eucalyptus major (Maiden) Blakely</td>
<td>leaf</td>
<td>grey gum</td>
<td>Used to treat rheumatism, swelling, inflammation, skin disorders stomach disorders, bactericide (wounds, sores)</td>
<td>high terpene content</td>
<td>5, 10, 12</td>
</tr>
<tr>
<td>Kunzea pomifera F. Muell.</td>
<td>fruit</td>
<td>muntries</td>
<td>The fruit has a high antioxidant capacity and was mainly used as a nutritious food. The fruit has reported antibacterial and anticancer properties.</td>
<td>ellagitannins (including ellagic acid), gallotannins, terpenoids, purine analogues</td>
<td>5, 10, 21</td>
</tr>
<tr>
<td>Mentha australis R. Br.</td>
<td>leaf</td>
<td>river mint</td>
<td>The leaf has a high antioxidant capacity and was mainly used as a nutritious food. The leaf has reported antibacterial and anticancer properties.</td>
<td>high in essential oils; constituents have not been fully established.</td>
<td>10</td>
</tr>
<tr>
<td>Ocimum tenuiflorum L.</td>
<td>leaf</td>
<td>Australian native basil</td>
<td>The leaf has a high antioxidant capacity and was mainly used as a nutritious food. The leaf has reported antibacterial and anticancer properties.</td>
<td>unknown although the related species Ocimum sanctum is rich in polyphenolic compounds including eugenol</td>
<td>19</td>
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<tr>
<td>Podocarpus elatus R. Br.</td>
<td>fruit</td>
<td>Illawarra plum</td>
<td>The fruit has a high antioxidant capacity and was mainly used as a nutritious food. The fruit has reported antibacterial and anticancer properties.</td>
<td>unknown</td>
<td>16, 19, 20</td>
</tr>
<tr>
<td>Prostanthera incisa R. Br.</td>
<td>leaf</td>
<td>Australian native sage</td>
<td>The leaf has a high antioxidant capacity and was mainly used as a nutritious food. The leaf has reported antibacterial and anticancer properties.</td>
<td>high in essential oils; constituents have not been fully established.</td>
<td>10</td>
</tr>
<tr>
<td>Prostanthera rotundifolia R. Br.</td>
<td>leaf</td>
<td>Australian native thyme</td>
<td>The leaf has a high antioxidant capacity and was mainly used as a nutritious food. The leaf has reported antibacterial and anticancer properties.</td>
<td>high in essential oils, particularly 1,8-cineole</td>
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<tr>
<td>Scaevola spinescens R. Br.</td>
<td>leaf</td>
<td>currant bush, maroon bush, prickly fan flower</td>
<td>Used as an antiseptic (especially for skin disorders/sores), cancer, pain and urinary disorders. Antiviral properties have also recently been reported.</td>
<td>pentacyclic triterpenoids including lupeol, taraxerol, myricadiol, coumarins (including ammarin, nodakenetin), scaevoloside</td>
<td>1, 10, 13, 21, 22</td>
</tr>
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</table>
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**Table 2:** The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities of the Australian plant extracts.

<table>
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<tr>
<th>Extract</th>
<th>Mass of Dried Extract (mg)</th>
<th>Concentration of Resuspended Extract (µg/mL)</th>
<th>Total Phenolics</th>
<th>Water Soluble Phenolics</th>
<th>Water Insoluble Phenolics</th>
<th>Cardiac Glycosides</th>
<th>Sapons</th>
<th>Terpenes</th>
<th>Triterpenes</th>
<th>Phytosteroids</th>
<th>Alkaloids (Mayer Test)</th>
<th>Alkaloids (Wagner Test)</th>
<th>Flavonoids</th>
<th>Tannins</th>
<th>Free Anthraquinones</th>
<th>Combined Anthraquinones</th>
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</table>
+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. NTW = aqueous native tamarind extract; NTM = methanolic native tamarind extract; BTW = aqueous bush tomato extract; BTM = methanolic bush tomato extract; DLM = methanolic desert lime extract; DLM = methanolic desert lime extract; MW = aqueous muntries extract; MM = methanolic muntries extract; WSW = aqueous wattle seed extract; WSM = methanolic wattle seed extract; LAW = aqueous lemon aspen extract; LAM = methanolic lemon aspen extract; THW = aqueous native thyme extract; THM = methanolic native thyme extract; IPW = aqueous Illawarra plum extract; IPM = methanolic Illawarra plum extract; NSW = aqueous native sage extract; NSM = methanolic native sage extract; RMW = aqueous river mint extract; RMM = methanolic river mint extract; NBW = aqueous native basil extract; NBM = methanolic native basil extract; EBLW = aqueous E. baileyana leaf extract; EBLM = methanolic E. baileyana leaf extract; EMLW = aqueous E. major leaf extract; EMLM = methanolic E. major leaf extract; TSLW = aqueous S. spinescens leaf extract; TSLM = methanolic S. spinescens leaf extract; TSILW = aqueous T. stipitata leaf extract; TSILM = methanolic T. stipitata leaf extract; TSBW = aqueous T. stipitata berry extract; TSBM = methanolic T. stipitata berry extract.

**RESULTS**

**Liquid extraction yields and qualitative phytochemical screening**

Extraction of 1 g of the native Australian plant materials with the solvents yielded dried plant extracts ranging from 25 mg (aqueous native sage extract) to 524 mg (methanolic muntries extract) (Table 2). Methanol was a better extractant than water, with substantially higher extraction for most plant materials. However, this trend was not observed in the wattle seed and S. spinescens extracts, which had substantially higher yields in the aqueous extracts compared to the methanolic extracts.

An extensive range of phytochemicals was detected in both the methanolic and aqueous extracts for all plant species tested by qualitative phytochemical screening (Table 2). Both solvents typically extracted high levels of phenolics (both water soluble and water insoluble phenolics) for all plant materials. Additionally, all extracts generally contained high levels of flavonoids and moderate to high levels of saponins. Low to moderate levels of triterpenoids were present in most extracts, with the exception of the native thyme, Eucalyptus spp, and S. spinescens extracts. Similarly, cardiac glycosides, alkaloids, and tannins were detected in some, but not all of the extracts. Furthermore, when these classes of compound were detected, they were generally only in low to moderate abundance. All extracts were generally devoid of detectable levels of phytosteroids and anthraquinones.

**Antimicrobial activity**

To assess the inhibitory activity of the crude plant extracts against *S. pyogenes*, 10 µL aliquots of each extract were screened using a disc diffusion assay. The bacterial growth was inhibited by 24 of the 34 extracts tested (~71%) (Figure 1). The methanolic *E. baileyana* and *E. major* extracts were the most potent inhibitors of *S. pyogenes* growth (as judged by zones of inhibition), with inhibition zones of 12.3 ± 0.6 and 13.3 ± 0.6 mm respectively. This compares favourably with the ampicillin control, which had an inhibition zone of 12.0 ± 1.0 mm. Whilst less potent than the corresponding methanolic extracts, the aqueous *E. baileyana* and *E. major* extracts were also good *S. pyogenes* growth inhibitors, with 10 and 8.3 ± 0.3 mm inhibitory zones respectively.

The antimicrobial efficacy was further quantified by determining the MIC values (Table 3). Several extracts were potent inhibitors of *S. pyogenes* growth, with MIC values <1000 µg/mL (<10 µg infused into the disc). The *Eucalyptus* spp. extracts were particularly potent. Indeed, MIC values of 341 (3.4 µg infused into the disc) and 88 µg/mL (8.8 µg infused into the disc) were determined for the aqueous *E. baileyana* extracts respectively. Similarly, MIC values of 134 (1.3 µg infused into the disc) and 53 µg/mL (5.3 µg infused into the disc) were determined for the aqueous and methanolic *E. major* extracts respectively. This compares well with the ampicillin control, which was tested at 10 µg infused into the disc. The methanolic wattle seed extract, aqueous and methanolic lemon aspen extracts, aqueous native thyme extract, methanolic river mint extract and the methanolic native basil extract were similarly potent growth inhibitors, with MIC values ≤1000 µg/mL. MIC values indicative of moderate inhibitory activity (1000–5000 µg/mL) were determined for many of the other extracts (methanolic native tamarind, bush tomato, desert lime, native thyme, native basil, *T. stipitata* leaf extracts). All other extracts were either low potency *S. pyogenes* growth inhibitors (MIC >5000 µg/mL) or were devoid of inhibitory activity.

**Quantification of toxicity**

All extracts were initially screened at 2000 µg/mL in the assay (Figure 2). For comparison, the reference toxin potassium dichromate (1000 µg/mL) was also assessed in the bioassay. The potassium dichromate reference toxin was rapid in its onset, inducing nauplii death within the first 3 h of exposure, with 100% mortality evident in the subsequent 4-5 h (unpublished results). The majority of the Australian plant extracts also induced significant *Artemia* nauplii toxicity, with ≥50% mortality rates at 24 h. Indeed, only the aqueous desert lime, muntries, native thyme, native sage, river mint and native basil extracts, as well as both *S. spinescens* extracts, induced <50% mortality following 24 h exposure. Thus, only these extracts were deemed nontoxic, whilst all others were deemed toxic.

To further evaluate the effect of toxin concentration on the induction of mortality, the extracts were serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay. Table
Table 3: Minimum inhibitory concentration (µg/mL) of the plant extracts against *S. pyogenes* and LC$_{50}$ values (µg/mL) in the *Artemia nauplii* bioassay

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Numbers indicate the mean MIC and LC$_{50}$ values of triplicate determinations. - indicates no inhibition. NTW=aqueous native tamarind extract; NTM=methanolic native tamarind extract; BTW=aqueous bush tomato extract; BTM=methanolic bush tomato extract; DLW=aqueous desert lime extract; DLM=methanolic desert lime extract; MW=aqueous muntries extract; MM=methanolic muntries extract; WSW=aqueous wattle seed extract; WSM=methanolic wattle seed extract; LAW=methanolic lemon aspen extract; LAM=methanolic lemon aspen extract; THW=aqueous native thyme extract; THM=methanolic native thyme extract; IPW=aqueous Illawarra plum extract; IPM=methanolic Illawarra plum extract; NSW=aqueous native sage extract; NSM=methanolic native sage extract; RMW=aqueous river mint extract; RMM=methanolic river mint extract; NBW=aqueous native basil extract; NBM=methanolic native basil extract; EBLW=aqueous *E. baileyana* leaf extract; EBLM=methanolic *E. baileyana* leaf extract; EMLW=aqueous *E. major* leaf extract; EMLM=methanolic *E. major* leaf extract; SSLW=aqueous *S. spinescens* leaf extract; SSLM=methanolic *S. spinescens* leaf extract; TILW=aqueous *T. insipida* leaf extract; TILM=methanolic *T. insipida* leaf extract; TSLW=aqueous *T. stipitata* leaf extract; TSLM=methanolic *T. stipitata* leaf extract; TSBW=aqueous *T. stipitata* berry extract; TSBM=methanolic *T. stipitata* berry extract. PD=potassium dichromate control; SW=seawater control. ND=the indicated test was not performed.
Figure 1: Growth inhibitory activity of Australian plant extracts against the S. pyogenes clinical isolate measured as zones of inhibition (mm).

NTW = aqueous native tamarind extract; NTM = methanolic native tamarind extract; BTW = aqueous bush tomato extract; BTM = methanolic bush tomato extract; DLW = aqueous desert lime extract; DLM = methanolic desert lime extract; MW = aqueous muntries extract; MM = methanolic muntries extract; WSW = aqueous wattle seed extract; WSM = methanolic wattle seed extract; LAW = aqueous lemon aspen extract; LAM = methanolic lemon aspen extract; TWH = aqueous native thyme extract; THM = methanolic native thyme extract; IPW = aqueous Illawarra plum extract; IPM = methanolic Illawarra plum extract; NSW = aqueous native sage extract; NSM = methanolic native sage extract; RMW = aqueous river mint extract; RMM = methanolic river mint extract; NBW = aqueous native basil extract; NBM = methanolic native basil extract; EBLW = aqueous E. baileyana leaf extract; EBLM = methanolic E. baileyana leaf extract; EMLW = aqueous E. major leaf extract; EMLM = methanolic E. major leaf extract; SSLW = aqueous S. spinescens leaf extract; SSLM = methanolic S. spinescens leaf extract; TILW = aqueous T. stipitata leaf extract; TILM = methanolic T. stipitata leaf extract; TLW = aqueous T. insipida leaf extract; TLM = methanolic T. insipida leaf extract; AMP = ampicillin (10 µg) control. Results are expressed as mean zones of inhibition (mm) ± SEM.

3 shows the LC₅₀ values of the extracts towards A. franciscana. No LC₅₀ values are reported for the aqueous desert lime, aqueous mints, native thyme, native sage, river mint and native basil extracts, as well as both S. spinescens extractas <50% mortality was seen across all concentrations tested. With the exception of the aqueous E. major leaf extract, all Eucalyptus spp. extracts generally had LC₅₀ values < 1000 µg/mL. All other extracts yielded LC₅₀ values substantially >1000 µg/mL following 24 h exposure. As extracts with LC₅₀ values of > 1000 µg/mL towards Artemia nauplii are deemed to be nontoxic, all extracts except the aqueous and methanolic E. baileyana extracts and the methanolic E. major leaf extract were deemed to be nontoxic.

**DISCUSSION**

Previous studies have reported potent bacterial growth inhibitory activity for all of the native Australian plant species screened in our study against a variety of pathogenic bacterial species. Extracts prepared from Australian fruits and culinary herbs, S. spinescens leaf extracts and Tasmannia spp. leaf and berry extracts have previously been reported to have inhibitory activity against extensive panels of pathogenic bacteria. With the exception of the S. spinescens, T. insipida and the T. stipitata berry extracts, all species screened in our study inhibited the growth of S. pyogenes. In contrast, the bacterial growth inhibitory properties of the Eucalyptus spp. have been reported against a narrower range of pathogenic bacteria. The Eucalyptus spp. extracts displayed the most potent S. pyogenes growth inhibitory activity of the extracts tested in our study. Indeed, an MIC of 53 µg/mL was determined for the methanolic E. major leaf extract. However, despite being the most promising S. pyogenes growth inhibitory extracts, the methanolic and aqueous E. baileyana and methanolic E. major leaf extracts displayed substantial toxicity, with LC₅₀ values as low as 455 µg/mL (methanolic E. baileyana leaf extract). Extracts with LC₅₀ values < 1000 µg/mL towards Artemia nauplii are defined as being toxic, which may impact on their therapeutic potential. As the LC₅₀ values are within the therapeutic ranges that would be required for S. pyogenes growth inhibition (determined by MIC), studies using human cell lines are required to further evaluate the safety of these extracts. However, even if the Eucalyptus spp. extracts are subsequently deemed unsafe for ingestion, they may still be useful S. pyogenes growth inhibitory agents. Impetigo is a cutaneous skin disease often resulting from S. pyogenes infection. Topical application of the extracts may prove effective in treating this form of the disease. Streptococcal pharyngitis is caused by Streptococcus spp. infection on the pharynx surface. Therefore, gargling with solutions containing Eucalyptus spp. extracts may prove effective in treating this disease. However, streptococcal induced rheumatic fe-
ver and rheumatic heart disease result from systemic infections, mainly affecting joint and cardiac tissue. Treatment of these diseases requires ingestion of antibiotics and anti-inflammatory drugs. The toxic nature of the Eucalyptus spp. extracts may preclude their use in the treatment of these diseases and instead limit them to topical applications.

Whilst an investigation of the phytochemistry of the Eucalyptus spp. extracts was beyond the scope of our study, plants of the genus Eucalyptus are well known for their high terpenoid contents. In particular, high 1, 8-cineole contents was reported for several Eucalyptus spp. Potent bacterial growth inhibitory activity has been reported for 1, 8-cineole against a panel of pathogenic bacteria. Another study reported MIC values for 1, 8-cineole against Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli of between 16 and 256 µg/mL. That study did not screen 1, 8-cineole against S. pyogenes. Eucalyptus spp. are also rich in a variety of other mono-and sesquiterpenoids. Some of these terpenoids have been previously reported to have potent broad spectrum antibacterial activity and therefore may contribute to the S. pyogenes inhibitory activity.

Another commonality between the inhibitory Eucalyptus spp. extracts was that all contained relatively high levels of flavonoids and tannins. Many studies have reported potent growth inhibitory activities for a wide variety of flavonoids against extensive bacterial panels. A number of tannin compounds have bacterial growth inhibitory activity. Gallotannins have been reported to inhibit the growth of a broad spectrum of bacterial species through a variety of mechanisms including binding cell surface molecules including lipoteichoic acid and proline-rich cell surface proteins, and by inhibiting glucosyltransferase enzymes. Ellagitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL and by inhibiting glucosyltransferase enzymes. Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls. Thus, it is likely that multiple compounds within the Eucalyptus spp. extracts are contributing to the growth inhibition of S. pyogenes.

Lemon aspen extracts, as well as the methanolic wattle seed, native basil and river mint extracts, were also potent S. pyogenes growth inhibitors, with MIC values substantially <1000 µg/mL. In contrast with the Eucalyptus spp. extracts, these extracts were nontoxic (LC₅₀ values substantially >1000 µg/mL). Thus, the therapeutic usage of these extracts need not be limited to external uses. Whilst the Eucalyptus spp. extracts may have greater efficacy in topical application, lemon aspen and wattle seed extracts would be more acceptable for systemic streptococcal induced rheumatic fever and rheumatic heart disease. A previous study has reported LC-MS evaluations of lemon aspen extracts prepared in the same
CAMERON LEE et al.: Australian plant extracts inhibit Streptococcus pyogenes growth

way as those screened in our study.48 A number of interesting phyto-
compounds with antibacterial activity were detected. The identification of gingerol, rutin, luteolin, dihydrokaempferol, ellagic acid (and methyl-
ated ellagic acid derivatives) and chlorogenic acid was particularly note-
worthy. Whether these compounds contribute to the S. pyogenes growth
inhibitory activity reported here is yet to be determined. The S. pyogenes growth inhibitory activity reported here is particularly noteworthy for the development of future antibiotic therapeutics.

Aside from the obvious antibiotic applications to directly treat localised throat (pharyngitis) and skin infections (impetigo),43,48 a number of sub-
stantially more serious illnesses are caused by acute and chronic S. pyo-
genoses infections and may also benefit from treatment with these extracts.

When S. pyogenes invades and colonises deeper tissue it can lead to er-
sipelas and cellulitis, conditions characterised by localised red, swollen and painful areas, and often by fever and lethargy.43,48 If not promptly
reated, bacteria can spread to other areas via the bloodstream which may result in serious tissue damage and autoimmune diseases such as
glomerulonephritis (inflammation of the glomeruli in the kidneys),
lymphedema (inflammation of lymph nodes), septic arthritis and rheu-
matic fever (inflammation of cardiac tissue).43,48 Furthermore, acute S.
pyogenes infections of subcutaneous tissues can induce the potentially
deadly disease necrotizing fasciitis.43 These conditions are not only highly
debilitating, but may also be life threatening and new, more effective
regimens could potentially prolong and increase the quality of
life as well as reducing the burden on the health system.

CONCLUSION

The results of this study demonstrate the potential of Eucalyptus spp.,
lemon aspen, wattle seed, native basil and river mint extracts to block the
growth of S. pyogenes. The toxicity of the Eucalyptus spp. extracts may
limit their clinical usage to topical applications. However, the nontoxicity of the lemon aspen fruit, wattle seed, native sage and river mint extracts
indicates their potential in the treatment of all manifestations of strep-
tococcal disease, including systemic treatment. Further studies aimed at
the purification of the bioactive components are needed to examine the
mechanisms of action of these agents.

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CONFLICTS OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS USED

DMSO: Dimethyl sulfoxide; LC:
50% mortality; MIC: Minimum inhibitory concentration.
SUMMARY

- E. major extracts were particularly potent S. pyogenes growth inhibitors, with MIC values of 134 and 53 µg/mL respectively.
- Aqueous and methanolic E. baileyana extracts were also potent inhibitors of S. pyogenes growth with MIC values of 341 and 88 µg/mL respectively.
- The lemon aspen extracts as well as the methanolic wattle seed, native basil and river mint extracts were also potent growth inhibitors with MIC values substantially <1000 µg/mL.
- All extracts except the Eucalyptus spp. Extracts were nontoxic in the Artemia nauplii assay.

ABOUT AUTHORS

Mr Cameron Lee: Completed his Bachelor of Science (BSc) in 2015 and is currently concluding his honours year. His research involves the investigation of thermostable anaerobes that utilize toxic metals in anaerobic respiration (including uranium and arsenic). He has extensive experience in anaerobic cultivation/isolation and in numerous analytical techniques associated with heavy metal analysis.

Dr Wright: Received his PhD in 2014, for his work investigating the manganese reduction and oxidation characteristics of environmental bacteria. He is currently a postdoctoral researcher at Griffith University, Australia, where he is working on several projects both in the areas of geomicrobiology and pharmacognosy. His present research interests are the use of bacteriogenic manganese oxides in the bioremediation of metal-contaminated sites as well as the use of Australian native plants in the treatment and prevention of various pathogenic bacteria.

Megan Arnold: Is currently undertaking her PhD in Tropical Parasitology at Griffith University’s Eskitis Institute for Drug Discovery with a focus on the identification and development of novel chemoprophylactic agents for malaria. Her other research interests include investigating Australian high antioxidant plants for their antibacterial capabilities.

Dr Anthony Greene: Is a senior lecturer and researcher at Griffith University, Brisbane Australia. He obtained his PhD in Microbiology from the University of New South Wales and focuses on extreme environments, Bioremediation and Geomicrobiology. His specific interests include the microbial ecology of thermophilic, saline and alkaliphilic environments and the mechanisms and industrial potential of extremophilic bacteria contained therein.

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 ferrandiana (Kakadu plum), Australian Acacias, Szyzygiums, Petalostigmas and Xanthorrhoea johnsonii (grass trees). This range of projects has resulted in nearly 200 publications in a variety of peer reviewed journals.

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