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Original Article

Callistemon linearis Schrad. and J.C. Wendl. Extracts Inhibit the Growth of Gram-Positive Bacteria but have no Effect on Gram Negative Bacteria

Getmore Chikowe[1], Lindiwe Mpala[1], Ian Edwin Cock[1,2,*]
[1]School of Environment and Science, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland, AUSTRALIA.
[2]Environmental Futures Research Institute, Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland, AUSTRALIA.

ABSTRACT

Introduction: The development of multi-antibiotic resistant strains of bacteria has necessitated the search for new effective antibacterial therapies. Many *Callistemon* spp. were used traditionally to treat pathogenic illness and are rich in terpenoids with reported antibacterial activity. Despite this, the antibacterial activity of *C. linearis* leaf extracts has not been extensively examined. **Methods:** The ability of *C. linearis* 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 methanolic and aqueous *C. linearis* leaf extracts were good inhibitors of the growth of gram-positive bacteria yet were completely ineffective against gram-negative bacteria. The methanolic extract was a particularly good inhibitor of *B. cereus* and *S. pyogenes* growth, with MIC values of 610 and 354µg/mL respectively. The aqueous extract was also a good inhibitor of these bacteria (MICs of 927 and 660µg/mL respectively). Whilst the extracts also inhibited the growth of *S. aureus* and *S. epidermidis,* the MIC values (in the range 1200-1500µg/mL) were indicative of moderate inhibitory activity. The methanolic extracts were further investigated by growth time course assays that showed significant growth inhibition within 1h of exposure. All extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for the treatment of gram-positive bacterial infections. **Conclusion:** The lack of toxicity of the *C. linearis* leaf extracts and their growth inhibitory bioactivity against the gram-positive bacteria indicate their potential in the development of new antibiotic chemotherapies.

Key words: *Myrtaceae,* Bottlebrush trees, Traditional medicine, Antibacterial, Antibiotic resistant bacteria, Rheumatic fever, *Streptococcus pyogenes*, *Bacillus cereus*.

Correspondence:
Dr. Ian Edwin Cock
School of Environment and Science and Environmental Futures Research Institute Griffith University, 170 Kessels Rd, Nathan, Brisbane, Queensland, AUSTRALIA.
E-mail: I.Cock@griffith.edu.au
Phone no: +61 7 37357637
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INTRODUCTION

The discovery of penicillin by Alexander Flemming 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 *Callistemon* (family *Myrtaceae*) consists of 34 species endemic to Australia. Some species have also been introduced to other areas such as USA[6] and Africa[7] where they are often considered to be invasive species. They are closely related to *Melaleucas* which have similar leaf and flower morphology.[8,9] *Callistemons* are commonly referred to as 'bottlebrushes' due to the appearance of their flowers. They occur naturally in temperate regions of Australia, particularly on the east and south-west coasts. *Callistemon* flowers were used as a food source by Australian Aborigines. The flowers were sucked for their nectar or used to make sweet drinks.[10] They also had roles as traditional bush medicines for Australian Aborigines.[11,12] The leaves were used to cure respiratory tract infections. Unfortunately most of our understanding of the antimicrobial potential of Australian *Callistemon* species is anecdotal with few species being thoroughly studied. It has been postulated that terpenes in the leaves may be responsible for the efficacy of *Callistemons* in therapeutic treatments.[12] A recent report has demonstrated the antibacterial activity of a related *Callistemon* species (*Callistemon rigidus*).[13] Studies within our laboratory have also found antibacterial activity in methanolic extracts of *Callistemon citrinus* and *Callistemon salignus* leaves and flowers against multiple bacteria.[14,15]

Despite this, many *Callistemon* spp. are yet to be thoroughly evaluated for antibacterial activity. *Callistemon linearis* Schrad. and J.C. Wendl. (synonyms *Callistemon rigidus* R.Br.; *Melaleuca linearis* Schrad. and J.C. Wendl.; *Callistemon pinifolia* J.C. Wendl.; *Callistemon lanceolatus* (Sm.) Sweet; commonly known as narrow-leaved bottlebrush) is a member of family Myrtaceae. It produces bright red flowers (Figure 1a) and has lanceolate leaves (Figure 1b) as a defining characteristic of the species. This species is endemic to the eastern states of Queensland and New South Wales in Australia. Some previous studies have reported antibacterial activity for this species against a several bacterial species.[16] Whilst that study reported antibacterial activity against all of the species that it

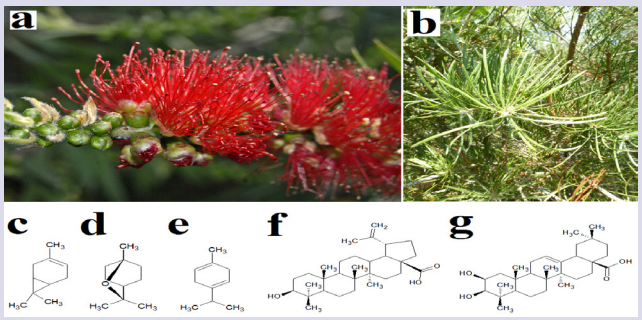

Figure 1: *C. linearis* (a) flower and (b) leaves, as well as the phytochemical compounds (c) 3-carene, (d) 1, 8-cineole, (e) γ -terpinene, (f) betulinic acid and (g) 2,3-dihydroxy olean-12-en-28-oic acid.

tested, it did not determine MIC values. A comparison of the potency of the extracts with other studies is therefore not possible. Further studies have examined the phytochemical composition of *C. linearis* leaves and have been reported to be particularly rich in the monoterpenoids 3-carene (Figure 1c), 1, 8-cineole (Figure 1d) and γ -terpinene (Figure 1e).[17] They are also rich in triterpenoids including betulinic acid (Figure 1f) and 2,3-dihydroxy olean-12-en-28-oic acid (Figure 1g).[18] Interestingly, many terpenoids have potent antibacterial activity[19,20] and this species may therefore be useful in blocking bacterial infections. The current study was undertaken to screen of *C. linearis* leaf extracts for the ability to inhibit the growth of a panel of gram-positive and gram-negative bacterial pathogens.

MATERIALS AND METHODS

Plant Collection and Extraction

Callistemon linearis Schrad. and J.C. Wendl. leaves 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) or sterile deionised water was added to individual 1g masses of the plant material and extracted for 24 hrs 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 *C. linearis* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by standard assays.[21-23]

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. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes feacalis*, *Bacillus cereus, Citrobacter freundii*, *Pseudomonas fluorescens*, *Salmonella newport, Serratia marcescens, Shigella sonneii, Staphylococcus aureus* and *Staphylococcus epidermidis* 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.

Evaluation of Antimicrobial Activity

Antimicrobial activity of the *C. linearis* leaf extracts was determined using a modified disc diffusion assay.[24-26] Briefly, 100µL of the 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 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) and chloramphenicol (10µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10µL of distilled water were used as a negative control.

Minimum Inhibitory Concentration (MIC) Determination

The minimum inhibitory concentration (MIC) of each extract against susceptible bacteria was determined as previously described.[26,27] Briefly, the *C. linearis* leaf 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.

Bacterial Growth Time Course Assay

Bacterial growth time course studies were performed as previously described.[28] 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 in triplicate.

Toxicity Screening

Artemia franciscana Nauplii Toxicity Screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.[29-31] 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.

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 *C. linearis* leaves with methanol and water yielded 438 and 325mg 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 studies showed that both extracts had similar phytochemical profiles. Both contained high levels of phenolic compounds and flavonoids, as well as moderate levels of triterpenoids and saponins. Lower levels of phytosterols, 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 *C. linearis* leaf extracts, aliquots (10µL) of each extract were screened in the disc diffusion assay. The *C. linearis* leaf extracts were ineffective at inhibiting the growth of all of 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 16mm (chloramphenicol against *E. coli*). A much different trend was noted against the gram-positive bacterial species. Indeed, the methanolic and aqueous extracts inhibited the growth of all gram-positive bacteria. The methanolic extract was a better *B. cereus* growth inhibitor than was the aqueous extract (Figure 3). Indeed, the methanolic extract produced ZOIs that were only slightly smaller than the ampicillin and chloramphenicol controls. 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 percentage of the overall extracts mass. Therefore, these extracts may be particularly promising as targets for antibiotic drug discovery.

The methanolic and aqueous extracts were also effective inhibitors of *S. aureus* (Figure 4) and *S. epidermidis* (Figure 5) growth, albeit with substantially smaller ZOIs noted. As noted for *B. cereus*, the methanolic extract was a more potent inhibitor of the *Staphylococcus* spp. growth (as judged by ZOI). However, the size of these ZOIs is indicative of only moderate inhibitory activity. In contrast, the methanolic and aqueous

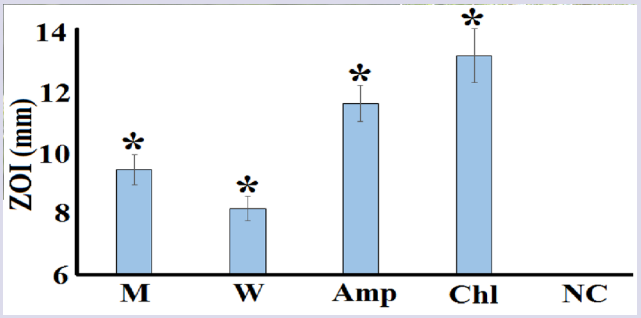

Figure 3: Growth inhibitory activity of *C. linearis* leaf extracts and reference antibiotics against *B. cereus* clinical isolate strain measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. *indicates values signidicantly different to the untreated control (*P*<0.01).

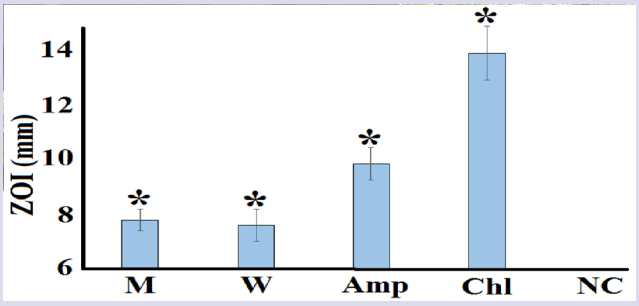

Figure 4: Growth Inhibitory activity of *C. linearis* leaf extracts and reference antibiotics against *S. aureus* clinical isolate strain measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. NC= negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. * indicates values signidicantly different to the untreated control (*P*<0.01).

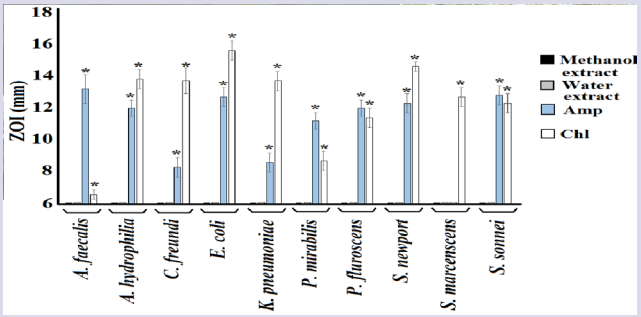

Figure 2: Growth Inhibitory Activity of *C. linearis* leaf extracts and reference antibiotics against gram-negative bacterial species measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. * indicates values signidicantly different to the untreated control (*P*<0.01).

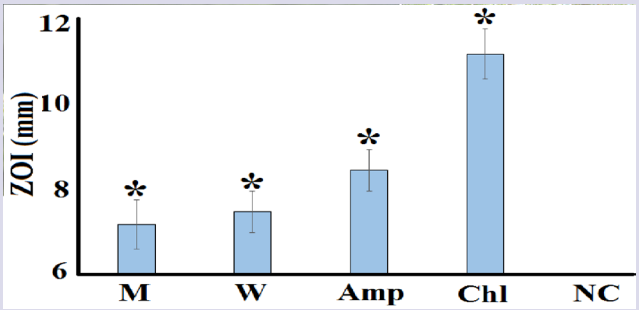

Figure 5: Growth inhibitory activity of *C. linearis* leaf extracts and reference antibiotics against *S. epidermidis* clinical isolate strain measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. * indicates values signidicantly different to the untreated control (*P*<0.01).

extracts were particularly good inhibitors of *S. pyogenes* growth (Figure 6). Indeed, ZOIs of 16 and 12.3mm were noted for the methanolic and aqueous extracts respectively against *S. pyogenes*. This bacterium was quite resistant to ampicillin, with a ZOI of approximately 7mm. The ZOIs of both extracts were also substantially bigger than that of the chloramphenicol control (~9mm) indicating that these extracts have good inhibitory activity against resistant *S. pyogenes* strains. As *S. pyogenes* can cause a wide variety of diseases including pharyngitis, impetigo and rheumatic fever depending on the tissue that it infects, these extracts may be particularly useful as targets for antibiotic discovery.

The antimicrobial efficacy was further quantified by determining the MIC value. The methanolic and aqueous extracts were particularly good inhibitors of *S. pyogenes* growth, with MIC values of 354 and 660µg/mL respectively. Both extracts were also good inhibitors of *B. cereus* growth, with MIC values of 610 and 927µg/mL respectively. Higher MIC values (1200-1500µg/mL) were noted against both *Staphylococcus* spp. Whilst these MIC values demonstrate that the *C. linearis* leaf extracts have potential in the control of *Staphylococcus* spp. infections, they indicate only moderate potency.

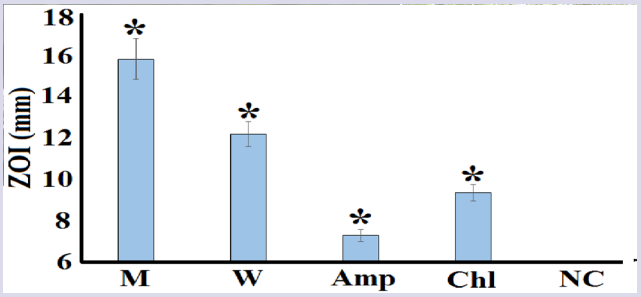

Figure 6: Growth inhibitory activity of *C. linearis* leaf extracts and reference antibiotics against *S. pyogenes* (ATCC19615) measured as ZOIs (mm) ± SEM. Ampicillin (Amp) and chloramphenicol (Chl) standard discs (10µg) were used as positive controls. NC = negative control. All assays were completed three times, each with internal triplicates (*n*=9) and the results are expressed as mean zones of inhibition (mm) ± SEM. * indicates values signidicantly different to the untreated control (*P*<0.01).

Bacterial Growth Time Course Assay

The antibacterial activity of the *C. linearis* leaf methanolic and aqueous leaf extracts was further investigated against the four gram-positive bacterial species by bacterial growth time course assays in the presence and absence of the extracts. The starting concentration of the extract used in these assays was 1000µg/mL. The *C. linearis* leaf methanolic and aqueous leaf extracts both significantly inhibited *B. cereus* within 1h of exposure, indicating a rapid antimicrobial action. The absorbance of the *B. cereus* culture (And thus the bacterial growth) remained substantially lower than the untreated control for the entire 6h incubation period. Indeed, the turbidity had not significantly increased throughout the 6h growth period, indicating that the extracts may be bacteriocidal at the concentrations tested (Figure 7a). The methanolic and aqueous extracts were also was rapid in its inhibition of *S. aureus* (Figure 7b) and *S. epidermidis* (Figure 7c) growth, with a significant decrease in bacterial growth also noted within the first hour of incubation. However, in contrast to the inhibition of *B. cereus* growth, the inhibition of the *Staphylococcus* spp. growth had returned to similar levels to that of the untreated control by the end of the 6h incubation period (as judged by turbidity). This may indicate that the aqueous *C. linearis* leaf methanolic and aqueous leaf extracts had bacteriostatic effects at the tested concentration, rather than bactericidal effects. The inhibition of *S. pyogenes* growth (Figure 7d) followed similar trends to those noted for *B. cereus*, indicating that the extracts may be bactericidal at the tested concentration.

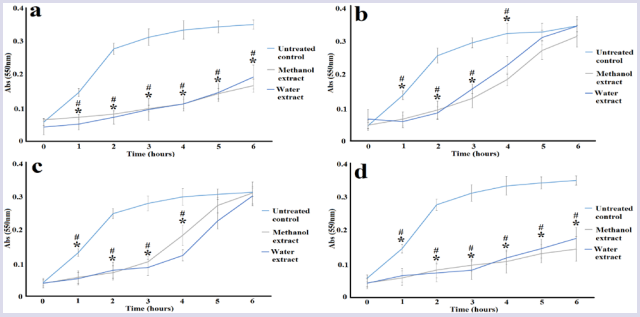

Figure 7: Bacterial growth curves for the *C. linearis* leaf methanolic extract against (a) *B. cereus,* (b) *S. aureus,* (c) *S. epidermidis* and (d) *S. pyogenes.* All bioassays were performed three times in triplicate (*n*=9) and are expressed as mean ± SEM. * = methanolic extract results that are 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).

Quantification of Toxicity

The toxicity of the *C. linearis* leaf extracts was initially tested at 2mg/mL in the *A. franciscana* nauplii bioassay (Figure 8). The mortality in the presence of all extracts was not significantly different to that of the untreated control at 24h and thus were deemed to be non-toxic. Extracts with 24h LC_{50} values >1000µg/mL have previously been defined as non-toxic.[30,31] 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 low for the *C. linearis* leaf extracts at 48h. Indeed, the % mortality induction was substantially <50% for all extracts at all times tested and therefore it was not possible to determine LC_{50} values for any of the *C. linearis* leaf extracts (Table 2).

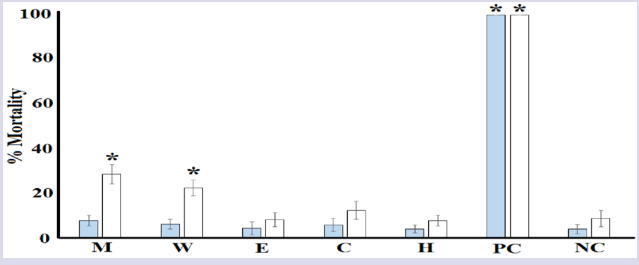

Figure 8: The Lethality of the *C. linearis* leaf extracts (2000µg/mL), Potassium dichromate control (1000µg/mL) and seawater (negative control). Shaded bars represent the mortality induced by the *C. linearis* leaf extracts following 24h exposure; open bars represent the mortality induced by the *C. linearis* leaf extracts following 48h exposure. 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). * indicates values significantly different to the untreated control (*P*<0.01).

DISCUSSION

Despite the initial potency of many antibiotic chemotherapies, recent in-

Table 1: The Mass of Dried Extracted Material, the Concentration after Resuspension in Deionised Water and Qualitative Phytochemical Screenings of the *C. linearis* Leaf Extracts.

			Methanolic extract	Aqueous extract
		Mass of extracted material (mg)	438	325
		Concentration of resuspended extract (mg/mL)	43.8	32.5
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.

creases 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.[32] *Callistemon* spp. are good candidates for the development of new antibacterial drugs as they were used by Australian Aborigines to treat bacterial infections.[11,12] Furthermore, several studies have reported that *Callistemon citrinus* and *Callistemon salignus* leaf and flower extracts are potent inhibitors of multiple bacterial species.[14,15] This study has extended these earlier studies by testing *C. linearis* against an extended panel of bacterial pathogens.

The greater susceptibility of Gram-positive bacteria to the *C. linearis* leaf extracts noted in this study is in agreement with previously reported results for South American,[33,34] African[35,36] and Australian[37] plant extracts. Results within our laboratory have also confirmed the greater susceptibility of Gram-positive bacteria towards other Australian plant extracts.[38-40] The Gram-negative bacterial cell wall outer membrane is thought to act as a barrier to many substances including antibiotics.[41] The uptake of the *Callistemon* extract antibiotic compounds by gram-negative bacteria is presumably affected by the cell wall outer membrane. In contrast, other studies have demonstrated that Gram-negative bacteria are often more susceptible to plant extracts from different Australian plant species.[42-44]

Whilst an investigation of the phytochemistry of the *C. linearis* leaf 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.[19] For example, the flavonoids kaempferol and myricetin have potent growth inhibitory activity against a panel of bacteria.[45] Similarly, quercetin, rutin and their corresponding glycosides inhibit the growth of *Pseudomonas maltophilia* and *Enterobacter cloacae*.[46] 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.[19] Similarly, the antibacterial activities for several sesquiterpenoids including α-cubebene, copaene and caryophyllene have been reported.[19] Furthermore, many tannin compounds have bacterial growth inhibitory activity. Gallotannins inhibit the growth of a broad spectrum of bacterial species[47] through a variety of mechanisms including binding cell surface molecules including lipotoichoic acid and proline-rich cell surface proteins,[48,49] and by inhibiting glucosyltransferase enzymes.[50] Elligitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.[47,49] Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.[47,49] Thus, it is likely that multiple compounds within the *C. linearis* leaf extracts are contributing to the antibacterial activity reported here.

The findings reported here also indicate that the extracts examined were non-toxicity (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.[30,31] However, further studies are required to determine whether this is also true for the *C. linearis* leaf extracts examined in these studies. The results of this study indicate that the *C. linearis* 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.

Table 2: Minimum Inhibitory Concentrations (µg/mL) of the *C. linearis* Leaf Extracts against each Bacterial Strain and LC_{50} Values (µg/mL) against *Artemia* Nauplii.

Organism	Exposure time (h)	MIC or LC_{50} (ug/mL)	
		Methanolic extract	Aqueous extract
B. cereus (clinical isolate)	24	610	927
S. aureus (clinical isolate)	24	1188	1355
S. epiedermidis (clinical isolate)	24	1463	1292
S. pyogenes (ATCC19615)	24	354	660
Artemia nauplii	24	CND	CND
	48	CND	CND

Numbers indicate the mean MIC or LC_{50} values of three independent experiments in triplicate (n=9). CND indicates that an LC_{50} could not be determined as the mortality did not exceed 50% at any concentration tested.

CONCLUSION

The growth inhibitory activity of the *C. linearis* leaf extracts against the gram-positive bacteria, particularly against *S. pyogenes* and their lack of toxicity indicate their potential for the treatment of all manifestations of streptococcal 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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CONFLICT OF INTEREST

The authors report no conflicts of interest.

ABBREVIATIONS

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

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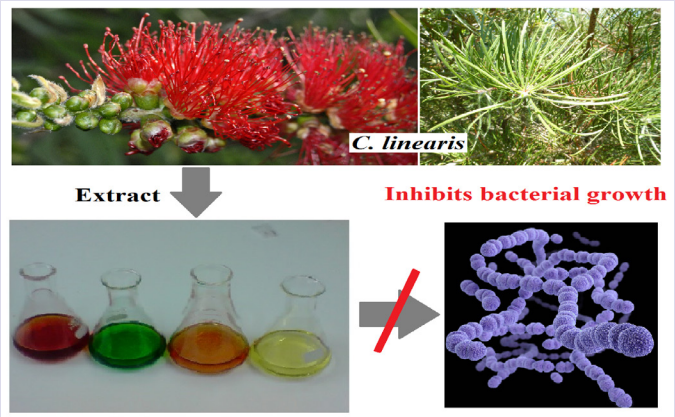
PICTORIAL ABSTRACT



SUMMARY

- Methanolic and aqueous *C. linearis* 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 undertaken against the gram-positive bacterial species.
- Toxicity of the *C. linearis* extracts was determined using the *Artemia* nauplii toxicity bioassay.

ABOUT AUTHORS

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

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

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