

Cinnamomum Oliveri F. M. Bailey Leaf Solvent Extractions Inhibit the Growth of a Panel of Pathogenic Bacteria

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ABSTRACT

Introduction: *Cinnamomum oliveri* F. M. Bailey is a rain forest tree native to Australia. Decoctions, infusions and essential oils produced from the leaves were used traditionally to treat a variety of bacterial diseases. Despite this, *C. oliveri* leaf extractions have not been rigorously examined for antibacterial properties against many pathogens. **Methods:** The antimicrobial activity of *C. oliveri* leaf extractions was investigated by disc diffusion and growth time course assays against a panel of pathogenic bacteria. The growth inhibitory activity was quantified by MIC determination. Toxicity was determined using the *Artemia franciscana* nauplii bioassay.

Results: *C. oliveri* leaf solvent extractions inhibited the growth of a wide range of bacterial species. Growth of both gram positive and gram negative bacteria was inhibited by the *C. oliveri* leaf extracts to approximately the same extent. The methanolic extracts were generally most potent growth inhibitors. The methanolic, aqueous and ethyl acetate *C. oliveri* leaf extracts were particularly potent inhibitors of *P. mirabilis* growth, with MIC values as low as 127 µg/mL (methanolic extract). *A. coli*, *K. pneumoniae* and *B. cereus* were also particularly susceptible to the methanolic, aqueous and ethyl acetate extracts, with MIC values generally substantially <1000 µg/mL. The antibacterial activity of the methanolic *C. oliveri* leaf extract was

further investigated by growth time course assays which showed significant growth inhibition in cultures of *E. coli*, *K. pneumoniae* and *P. mirabilis* within 1 h of exposure. All extracts were determined to be nontoxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for internal use as well as for topical uses. **Conclusions:** The lack of toxicity of the *C. oliveri* leaf extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria partially validate the traditional usage of these species to treat bacterial diseases and indicate their potential in the development of antiseptic agents.

Key words: Lauraceae, Oliver's Sassafras, Black Sassafras, Camphorwood, Cinnamon wood, Australian Plants, Antibacterial Activity, Medicinal Plants.

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INTRODUCTION

Plants produce a wide variety of compounds, which in addition to giving them characteristic pigment, odour and flavour characteristics, may also have antimicrobial properties.¹ For thousands of years, traditional plant derived medicines have been used in most parts of the world and their use in fighting microbial disease is becoming the focus of intense study.^{2,3} Whilst much of the research into traditional medicinal plant use has focused on Asian,⁴ African⁵ and South American⁶ plants, the therapeutic potential of the flora of Australia has been recognised for many thousands of years. The first Australians had well developed ethno-pharmacological systems and understood the therapeutic properties of a wide variety of aromatic Australian plants.⁷ Despite this, relatively few studies have rigorously examined the antibacterial activity of Australian native plants, although recently there has been increased study in this field.

Cinnamomum oliveri F.M.Bailey (family Lauraceae; commonly known as camphorwood, Oliver's sassafras, black sassafras and cinnamon wood) is a medium-large tree which is native to rainforest regions of north eastern Australia. The trees grow 25 m tall with buttressed trunks. The glossy green leaves (Figure 1a) are lanceolate with wavy margins and grow to 15 cm long by 4 cm wide. When broken, the leaves and twigs emit a sarsaparilla-like odour. White to cream flowers are produced in panicles approximately 6 mm long (Figure 1b). The flowers develop into small aromatic drupes to approximately 12 mm (Figure 1c) which ripen to a glossy black colour. Interestingly, Australian Aborigines used bark decoctions to treat diarrhoea and dysentery and several other bacterial diseases.⁷ Despite this, antibacterial studies of *Cinnamomum oliveri* leaves are lacking.

Several interesting phytochemical components have been identified in *C. oliveri* leaves. In particular, several terpenoids components have been isolated from *C. oliveri* leaf and bark and identified as camphor (Figure 1d), safrole (Figure 1e), eugenol (Figure 1f), methyl eugenol (Figure 1g)

and cinnamic aldehyde (Figure 1h). Growth inhibitory properties have previously been reported for many of these terpenoids against several bacteria.⁸ Despite these promising studies, examination of the antibacterial properties and phytochemistry of the leaves from *C. oliveri* is lacking. The current report was undertaken to screen *C. oliveri* leaf extracts for growth inhibitory properties against a panel of pathogenic bacteria.

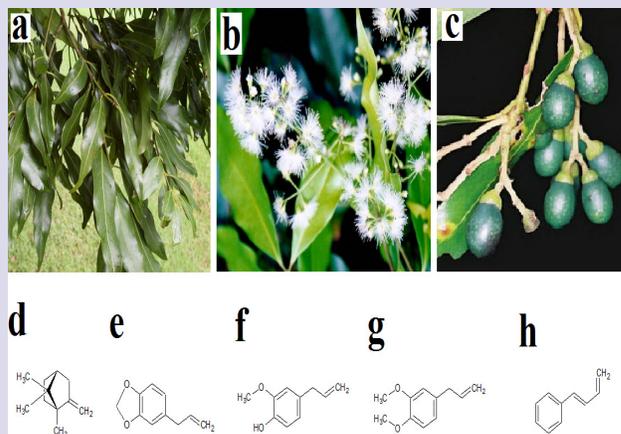


Figure 1: *C. oliveri* (a) leaves, (b) flower and (c) fruit, as well as the chemical structures of (d) camphor, (e) safrole, (f) eugenol, (g) methyl eugenol, (h) cinnamic aldehyde.

MATERIALS AND METHODS

Plant collection and extraction

Cinnamomum oliveri F.M. Bailey leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. Leaf samples were dried in a Sunbeam food dehydrator and stored at -30 °C. Prior to use, the leaves were freshly ground to a coarse powder and 1 g quantities were weighed into separate tubes. A volume of 50 mL methanol, sterile deionised water, ethyl acetate, chloroform or hexane was added to individual tubes and extracted for 24 h at 4 °C with gentle shaking. All solvents were obtained from Ajax, Australia and were AR grade. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant pellets were dissolved in 10 mL sterile deionised water (containing 1 % DMSO). The extracts were passed through 0.22 µm filter (Sarstedt) and stored at 4 °C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *C. oliveri* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.⁹⁻¹¹

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd., Australia. Clinical isolate microbial strains of *Aeromonas hydrophilia*, *Alcaligenes faecalis*, *Bacillus cereus*, *Citrobacter freundii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas fluorescens*, *Salmonella newport*, *Serratia marcescens*, *Shigella sonnei*, *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Streptococcus pyogenes* were obtained from Ms Michelle Mendell and Ms Jane Gifkins, Griffith University. All stock cultures were subcultured and maintained in nutrient broth at 4 °C.

Evaluation of antimicrobial activity

Antimicrobial activity of all plant extracts was determined using a modified disc diffusion assay.¹²⁻¹⁴ Briefly, 100 µL of each bacterial culture was grown in 10 mL of fresh nutrient broth until they reached a count of $\sim 10^8$ cells/mL. A volume of 100 µL of the bacterial suspension was spread onto nutrient agar plates and extracts were tested for antibacterial activity using 5 mm sterilised filter paper discs. Discs were infused with 10 µL of the plant extracts, allowed to dry and placed onto the inoculated plates. The plates were allowed to stand at 4 °C for 2 h before incubation at 30 °C for 24 h. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was performed in at least triplicate. Mean values (\pm SEM) are reported in this study. Standard discs of ampicillin (10 µg) were obtained from Oxoid, Australia and were used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL of distilled water were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration (MIC) of each extract against susceptible bacteria was determined as previously described.^{15,16} Briefly, the *C. oliveri* extracts were diluted in deionised water and tested across a range of concentrations. Discs were infused with 10 µL of the test dilutions, allowed to dry and placed onto inoculated plates. The assay was completed as outlined above and graphs of the zone of inhibition versus concentration were plotted for each extract. Linear regression was used to determine the MIC values of each extract.

Bacterial growth time course assay

Bacterial growth time course studies were performed as previously described.^{17,18} Briefly, 3 mL of the *E. coli*, *K. pneumoniae* and *P. mirabilis* bacterial cultures in nutrient broth were added individually to 27 mL nutrient broth containing 3 mL of 10 mg/mL methanolic plant extract to give a final concentration of 1000 µg/mL in the assay. The tubes were incubated at 30 °C with gentle shaking. The optical density was measured hourly at 550 nm for a 6 h incubation period. Control tubes were incubated under the same conditions but without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($K_2Cr_2O_7$) (AR grade, Chem-Supply, Australia) was prepared as a 4 mg/mL solution in distilled water and was serially diluted in artificial seawater for use in the *Artemia franciscana* nauplii bioassay.

Artemia franciscana nauplii toxicity screening

Toxicity was tested using an adapted *Artemia franciscana* nauplii lethality assay.¹⁹⁻²¹ Briefly, 400 µL of seawater containing approximately 46 (mean 46.3, n = 75, SD 11.6) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 µL of diluted plant extracts or the reference toxin were transferred to the wells and incubated at $25 \pm 1^\circ\text{C}$ 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 seconds. After 24 h, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was determined using probit analysis.

Statistical analysis

Data are expressed as the mean \pm SEM of at least three independent experiments. One way ANOVA was used to calculate statistical significance between control and treated groups with a *P* value < 0.01 considered to be statistically significant.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of 1 g of dried and powdered *C. oliveri* leaves with solvents of varying polarity yielded dried extracts ranging from 53 mg (ethyl acetate extract) to 164 mg (chloroform extract) (Table 1). The aqueous (145 mg) and methanolic extracts (104 mg) also yielded relatively high levels of extracted materials. The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1. Qualitative phytochemical studies showed that the high to mid polarity methanol, water and ethyl acetate solvents extracted the greatest diversity and highest levels of phytochemicals. These extracts contained high levels of water soluble phenolics and flavonoids, and low levels of triterpenoids. The aqueous and ethyl acetate extracts also contained low levels of alkaloids. Interestingly, despite extracting relatively large amounts of material, the chloroform and hexane extracts were devoid of all classes of phytochemicals screened. Due to their nonpolar nature, these extracts would be expected to contain high levels of lipids, hydrocarbons etc. As our qualitative phytochemical studies did not screen for these compounds, they were

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

Extract	Mass of Dried Extract (mg)	Resuspended Extract (mg/mL)	Phytochemical Screenings																
			Total Phenolics	Phenols Water Soluble Water Insoluble	Cardiac Glycosides Keller-Kiliani Test	Saponins Froth Persistence Emulsion test	Triterpenes Salkowski Test	Phytosteroids Acetic Anhydride Test	Alkaloids Meyers Test Wagners Test Draggendorffs Test	Flavanoids Shinoda Test Kumar test	Tannins Ferric Chloride Test Lead Acetate Test	Anthraquinones Free Combined							
Methanol	104	10.4	+++	+++	+	-	-	-	+	++	-	-	-	++	+++	-	-	-	-
Water	145	14.5	++	++	+	-	++	+	+	-	+	+	+	++	++	-	-	-	-
Ethyl Acetate	53	5.3	++	++	+	-	-	-	+	+	+	+	+	+	+	-	-	-	-
Chloroform	164	16.4	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-
Hexane	55	5.5	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-

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

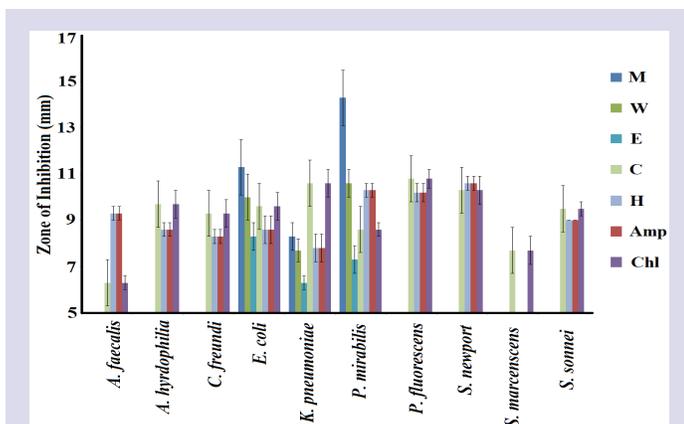


Figure 2: Growth inhibitory activity of *C. oliveri* leaf extracts against the gram negative bacterial species measured as zones of inhibition (mm) \pm SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 μ g) control;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

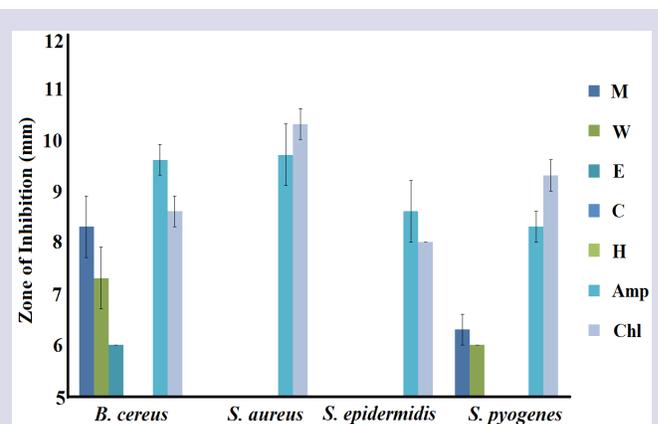


Figure 3: Growth inhibitory activity of *C. oliveri* leaf extracts against the gram positive bacterial species measured as zones of inhibition (mm) \pm SEM. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 μ g) control; Chl = chloramphenicol (2 μ g) control. All determinations were in at least triplicate and the results are expressed as mean zones of inhibition (mm) \pm SEM.

not detected. Other techniques are required to further examine the nature of these nonpolar components.

Antimicrobial activity

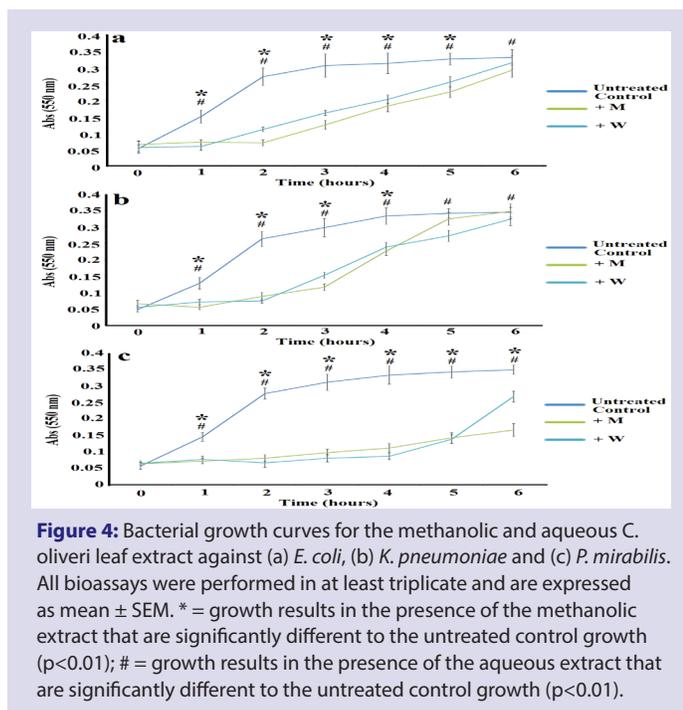
To determine the growth inhibitory activity of the *C. oliveri* leaf extracts against the panel of pathogenic bacteria, aliquots (10 μ L) of each extract were screened in the disc diffusion assay. The *C. oliveri* leaf extracts were

potent inhibitors of several gram negative bacterial species (Figure 2). Of the 10 gram negative bacterial strains tested, 3 (30 %) were inhibited by all *C. oliveri* leaf extracts. *E. coli*, *K. pneumoniae* and *P. mirabilis* were highly susceptible to the *C. oliveri* extracts. Indeed, the growth of these bacteria was inhibited by all of the *C. oliveri* extracts. The higher polarity methanolic and aqueous extracts were the most potent bacterial growth inhibitors against most susceptible bacterial species compared

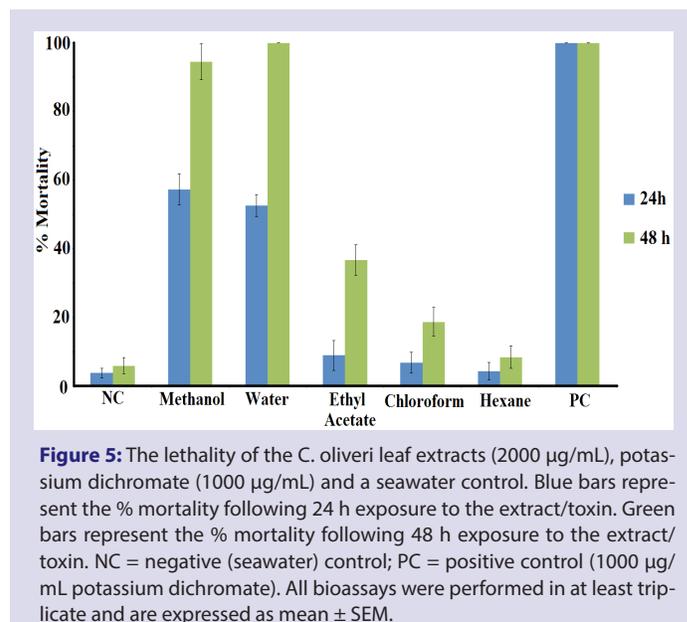
Table 2: Minimum bacterial growth inhibitory concentration ($\mu\text{g/mL}$) of the *C. oliveri* leaf extracts against susceptible bacterial species.

Bacterial species	<i>C. oliveri</i> Extract				
	Methanol	Water	Ethyl Acetate	Chloroform	Hexane
Gram negative					
<i>A. faecalis</i>	-	-	-	2873	1467
<i>A. hydrophilia</i>	-	-	-	2076	1650
<i>C. freundii</i>	-	-	-	2250	1809
<i>E. coli</i>	215	427	330	1255	1438
<i>K.pneumoniae</i>	686	706	1286	1023	1562
<i>P. mirabilis</i>	127	525	688	987	1066
<i>P. fluorescens</i>	-	-	-	1738	1354
<i>S. newport</i>	-	-	-	1924	1285
<i>S. marcescens</i>	-	-	-	4050	-
<i>S. sonnei</i>	-	-	-	1436	1387
Gram positive					
<i>B. cereus</i>	613	984	1500	-	-
<i>S. aureus</i>	-	-	-	-	-
<i>S. epidermidis</i>	-	-	-	-	-
<i>S. pyogenes</i>	1680	2263	-	-	-

Numbers indicate the mean MIC values of triplicate determinations. - indicates no inhibition.



with the lower polarity extracts (as assessed by the sizes of the zones of inhibition). The methanolic *C. oliveri* extract strongly inhibited *E. coli* and *P. mirabilis* growth with 11.3 ± 1.2 mm and 14.3 ± 1.2 mm zones of inhibition respectively. The aqueous extract was similarly potent with 10 ± 1 mm and 10.6 ± 0.6 mm zones of inhibition against *E. coli* and *P. mirabilis* respectively. This inhibition was particularly noteworthy compared to the inhibition by the ampicillin ($10 \mu\text{g}$: inhibition zones of 8.6 ± 0.6 mm and 10.3 ± 0.3 mm) and chloramphenicol controls ($2 \mu\text{g}$: inhibition



zones of 8.6 ± 0.3 mm and 9.6 ± 0.6 mm respectively).

The growth of some gram positive bacteria was also inhibited by the *C. oliveri* leaf extracts (Figure 3). The growth of 2 of the 4 gram positive bacteria species screened (50 %) was inhibited by the *C. oliveri* leaf extracts. However, in general, only the methanolic and aqueous *C. oliveri* leaf extracts inhibited the growth of the gram positive bacteria. Furthermore, the inhibition of gram positive bacterial growth appeared substantially less potent than was the inhibition of gram negative growth (as deter-

Table 3: LC₅₀ (95% confidence interval) for *Artemia* nauplii exposed to the *C. oliveri* leaf extracts and the reference toxin potassium dichromate.

Extract	LC ₅₀ (µg/mL)	
	24 h	48 h
Methanol	1487 ± 127	783 ± 22
Water	1626 ± 185	1136 ± 125
Ethyl acetate	CND	CND
Chloroform	CND	CND
Hexane	CND	CND
Potassium dichromate	88 ± 5	82 ± 4

Results represent the mean ± SEM of triplicate determinations. CND indicates that a LC₅₀ could not be determined as the mortality did not exceed 50 % at any concentration tested.

mined by zone of inhibition.

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against the microbial species which were determined to be susceptible. The methanolic, aqueous and ethyl acetate *C. oliveri* leaf extracts were potent growth inhibitors of several bacterial species (as judged by MIC; Table 2). *P. mirabilis* was the most susceptible bacteria to the *C. oliveri* leaf extracts, with MIC values as low as 127 µg/mL for the methanolic extract (approximately 1 µg infused into the disc) recorded for against this bacteria. The MIC values determined for the aqueous and ethyl acetate extract against *P. mirabilis* also indicate potent growth inhibition (525 and 688 µg/mL respectively). As *P. mirabilis* infection is a common cause of urinary tract infections and has also been identified as a trigger of rheumatoid arthritis,^{22,23} the aqueous and methanolic *C. oliveri* extracts have potential for the prevention and treatment of these diseases in genetically susceptible individuals. Furthermore, the aqueous, methanolic and ethyl acetate *C. oliveri* leaf extracts were also potent *E. coli* and *K. pneumoniae* growth inhibitors, with MIC values generally in the 200-700 µg/mL range. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,^{24,25} these extracts may also be useful in the prevention and treatment of this disease. The aqueous and methanolic *C. oliveri* leaf extracts were also good *B. cereus* growth inhibitors (MIC <700 µg/mL). Moderate to low growth inhibition (or no inhibition) was noted for all other extract/bacterium combinations.

Bacterial growth time course assay

The antibacterial activity of the *C. oliveri* extracts was further investigated in the most susceptible bacterial species (*E. coli*, *K. pneumoniae*, *P. mirabilis*) by bacterial growth time course assays in the presence and absence of the extract. Only the effect of the methanolic extract on the bacterial growth time courses was evaluated as this extract was generally more potent than the other *C. oliveri* extracts. The starting concentration of the extract used in these assays was 1000 µg/mL. The methanolic *C. oliveri* extract significantly inhibited *E. coli* (Figure 4a), *K. pneumoniae* (Figure 4b) and *P. mirabilis* (Figure 4c) growth within 1 h, indicating a rapid antimicrobial action. Whilst both *E. coli* and *K. pneumoniae* growth was inhibited for at least the first 4 hours of the time course, the bacteria were generally able to overcome this inhibition by 6h, with the recorded turbidity not significantly different to that of the untreated control. This indicates that the growth inhibition of these bacteria was bacteriostatic for the methanolic *C. oliveri* extract at the concentrations tested against these bacteria. In contrast, inhibition of *P. mirabilis* by the methanolic *C. oliveri* extract was substantially more profound, with growth still significantly inhibited by the end of the 6 h time course study. Indeed, the turbidity in the presence of the methanolic extract at 6 h was not greatly increased from the starting turbidity. In contrast, the aqueous extract did

not completely inhibit *P. mirabilis* growth and levels approaching those seen in the untreated control were evident by 6h. This may indicate that the aqueous extract is bacteriostatic, whilst the methanolic *C. oliveri* has bactericidal activity against *P. mirabilis* at the dose tested.

Quantification of toxicity

The toxicity of the *C. oliveri* extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 µg/mL (Figure 5). The aqueous and methanolic extracts induced >50 % mortality at 24 h and were thus deemed to be toxic. All other extracts induced low levels of mortality at 24 h, similar to the % mortality seen for the seawater control. By 48 h, the aqueous and methanolic extracts had induced approximately 100% mortality. Whilst mortality induction by the ethyl acetate and chloroform extracts was significantly higher than that in the untreated control following 48 h exposure, the levels were still <50 % and thus were deemed to be nontoxic. In contrast, the potassium dichromate positive control induced mortality within 4 h (results not shown), with 100 % mortality induction seen by 24 h.

To further quantify 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 3). For comparison, serial dilutions of potassium dichromate were also tested. All extracts were determined to be nontoxic, with LC₅₀ values substantially greater than 1000 µg/mL following 24 h exposure. Extracts with an LC₅₀ of greater than 1000 µg/mL towards *Artemia* nauplii have previously been defined as being nontoxic.²¹

DISCUSSION

Plant derived remedies are becoming increasingly sought after in the treatment of a myriad of diseases and disorders due both to their perception of greater safety than synthetic drugs, and the failure of current drug regimens to effectively treat many diseases. Our study reports on the growth inhibitory properties of *C. oliveri* leaf extracts against a panel of pathogenic bacteria, and on their toxicity. The gram positive and gram negative bacteria tested in this study demonstrated similar susceptibilities towards the *C. oliveri* leaf extracts. In contrast, many previous studies with other plant species report a greater susceptibility of many bacterial species towards solvent extracts for South American,²⁶ African²⁷ and Australian plant extracts.^{28,29}

Our study examined the ability of *C. oliveri* leaf extracts to inhibit the growth of a panel of medically important bacterial pathogens. The methanolic and aqueous extracts were identified as being particularly potent inhibitors of *P. mirabilis* with MIC values of 127 and 525 µg/mL respectively. The ethyl acetate extract and chloroform extracts were also good *P. mirabilis* growth inhibitors, albeit with higher MIC values (688, 987 and 1066 µg/mL respectively). As *P. mirabilis* can trigger rheumatoid

arthritis in genetically susceptible individuals,^{22,23} these extracts have potential for the development of rheumatoid arthritis inhibitory therapies. The methanolic and aqueous extract also displayed potent *K. pneumoniae* growth inhibitory properties, with MIC values 686 and 706 µg/mL respectively. The ethyl acetate, chloroform and hexane extracts were also moderate-good *K. pneumoniae* growth inhibitors with MIC values <1500 µg/mL. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,^{30,31} this extract may also be useful in the prevention and treatment of ankylosing spondylitis.

Whilst not as potent, the methanolic and aqueous *C. oliveri* extracts were moderate inhibitors of *S. pyogenes* growth (MICs of 1680 and 2263 µg/mL respectively). *C. oliveri* leaf extracts also inhibited the growth of several bacterial species associated with food poisoning. The methanolic, aqueous and ethyl acetate extracts were particularly potent inhibitors of growth of *E. coli* growth, with MIC values of 215, 427 and 330 µg/mL respectively. The chloroform and hexane extracts were also moderate *E. coli* growth inhibitors, albeit with higher MIC values (1255 and 1438 µg/mL respectively). Similarly, the methanolic and aqueous *C. oliveri* leaf extracts were potent inhibitors of *B. cereus* growth (MIC <1000 µg/mL), and the ethyl acetate extract was a moderate growth inhibitor (MIC = 1500 µg/mL). Therefore, the *C. oliveri* leaf extracts have therapeutic potential in the treatment of food poisoning, diarrhoea and dysentery.

Whilst a detailed investigation of the phytochemistry of the *C. oliveri* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Some commonalities were noted: the most potent aqueous and methanolic extracts all contained relatively high levels of total phenolics and flavonoids. It is likely that these and other phytochemical classes may contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies also indicated that triterpenoids, phytosterols and saponins were present in the *C. oliveri* leaf extracts. Many studies have reported potent antibacterial activities for a wide variety of these compounds.⁸ Further phytochemical evaluation studies and bioactivity driven isolation of active components is required to further evaluate the mechanism of bacterial growth inhibition.

The findings reported here also demonstrate that all of the *C. oliveri* leaf extracts were nontoxic towards *Artemia franciscana* nauplii, with LC₅₀ values substantially > 1000 µg/mL. Extracts with LC₅₀ values > 1000 µg/mL towards *Artemia* nauplii are defined as being nontoxic.²¹ Whilst our preliminary toxicity studies indicate that these extracts may be safe for therapeutic use, studies using human cell lines are required to further evaluate the safety of these extracts. Furthermore, whilst these studies have demonstrated the potential of the *C. oliveri* leaf extracts in the development of future antibiotic chemotherapeutics for the prevention and treatment of urinary tract infections, autoimmune diseases (particularly rheumatoid arthritis and ankylosing spondylitis), more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSIONS

The results of this study demonstrate the potential of the *C. oliveri* leaf extracts as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates that they are safe for internal as well as topical treatment. Further studies aimed at the purification and identification of bioactive components are required to examine the mechanisms of action of these agents.

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CONFLICTS 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.

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PICTORIAL ABSTRACT



SUMMARY

- *C. oliveri* leaf extracts displayed broad spectrum antibacterial activity against gram positive and gram negative bacteria.
- Methanolic, aqueous and ethyl acetate extracts were potent inhibitors of *P. mirabilis* growth (MIC values as low as 127 µg/mL).
- *E. coli*, *K. pneumoniae* and *B. cereus* were also particularly susceptible (MICs substantially <1000 µg/mL).
- All *C. oliveri* leaf extracts were nontoxic in the *Artemia* nauplii bioassay.

ABOUT AUTHORS



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



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

Ms Lindiwe Mpala completed at 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.