

Inhibition of the Growth of a Panel of Pathogenic Bacteria by *Kunzea flavescens* C.T.White and W.D.Francis Solvent Extractions

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ABSTRACT

Introduction: *Kunzea flavescens* C.T.White & W.D.Francis is a shrub that is native to eastern Australia. Several *Kunzea* spp. have been used as traditional medicines against bacterial pathogens. Despite this, *K. flavescens* leaf extractions have not been rigorously examined for growth inhibitory properties against many bacterial pathogens. **Methods:** The antimicrobial activity of *K. flavescens* leaf solvent 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:** Methanolic, aqueous and ethyl acetate *K. flavescens* leaf extracts inhibited the growth of a wide range of bacterial species. Growth of both gram positive and gram negative bacteria was inhibited by the *K. flavescens* leaf extracts, although a higher proportion of gram positive species were susceptible. The methanolic extract was generally more potent than the aqueous or ethyl acetate extracts against all susceptible bacteria. The methanolic *K. flavescens* leaf extract was a particularly potent inhibitor of *P. mirabilis* and *K. pneumoniae* growth, with MIC values of 393 and 481 µg/mL respectively. The antibacterial activity of the methanolic *K. flavescens* leaf extract was further investigated by growth time course assays which showed significant growth inhibition in cultures of *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 *K. flavescens* leaf extracts and their growth inhibitory bioactivity against a panel of pathogenic bacteria indicate their potential in the development of novel antiseptic agents.

Key words: *Kunzea flavescens*, Myrtaceae, Autoimmune inflammatory disease, Rheumatoid arthritis, Ankylosing spondylitis, Terpenoid, Antibacterial activity, Medicinal plants.

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INTRODUCTION

Plants produce a wide variety of secondary metabolites which provide characteristic pigment, odour and flavour characteristics. In addition, these compounds may also provide the plants with protection against microbial challenge.¹ Traditional plant derived medicines have been used for thousands of years in most parts of the world and with the increase in microbial antibiotic resistance, their use in fighting bacterial pathogens is becoming the focus of intense study.^{2,3} Whilst much of the research into traditional medicinal plant use has focused on Asian⁴, African⁵ and South American⁶ plants, the therapeutic potential of the flora of Australia has also been recognised for thousands of years. The first Australians had well developed ethnopharmacological 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 there has been a recent increase in this field.

The healing properties of Australian plants of the family Myrtaceae have long been understood by Australian Aborigines. More recently, the bacterial growth inhibitory properties of many genera within the family Myrtaceae have been examined and documented. In particular, *Callistemon* spp.⁸, *Eugenia* spp.⁷, *Kunzea* spp.^{7,9}, *Leptospermum* spp.^{7,10,11} and *Syzygium* spp.¹²⁻¹⁴ have been reported to inhibit the growth of a wide panel of bacteria, including many medicinally important pathogens. The genus *Kunzea* (family Myrtaceae) consists of approximately 50 species of small to medium shrubs which are native to Australia, with 2 species also occurring in New Zealand. Perhaps the best known *Kunzea* spp. are *Kunzea ambigua* (Sm.) Druce (commonly known as tick bush), *Kunzea ericoides* (A.Rich) Joy Thomps. (commonly known as Kānuka, white tea-tree, Burgan) and *Kunzea pomifera* F.Muell. (commonly known as muntries, emu apples, native cranberries). These species have each been

reported to inhibit bacterial growth.^{7,9,15,16} However, most *Kunzea* spp. are yet to be screened for bacterial growth inhibitory activity and much work is required to examine the therapeutic potential of this genus.

Kunzea flavescens C.T.White & W.D.Francis (Figure 1a) is a small-medium shrub which is native to eastern Australia, especially in south east Queensland where it is usually found inhabiting rocky ridges in heathland and in open woodland. The shrub grows 1-4 m tall with crowded alternate, glabrous leaves which grow 4-8 mm long. White to cream flowers (approximately 10-13 mm diameter) usually develop from September to November. Interestingly, we were unable to find confirmed reports of Aboriginal medicinal use of any part of *K. flavescens* although other *Kunzea* spp. have documented uses in treating skin diseases including eczema, dermatitis and rashes.^{1,16} Many of these ailments are caused by microbial pathogens. Oils prepared from several *Kunzea* spp. have also been reported to ease the pain of insect bites, minor burns, headaches and inflammation.¹⁷ Despite this, antibacterial studies examining the growth inhibitory properties of *K. flavescens* are lacking.

Several interesting phytochemical components have been identified in *Kunzea* spp. extracts and essential oils. In particular, several terpenoid components including α-pinene (Figure 1b), 1,8-cineole (Figure 1c), bicyclogermacrene (Figure 1d), spathulenol (Figure 1e), globulol (Figure 1f) and viridiflorol (Figure 1g) have been identified in *Kunzea* spp. extracts and essential oils.^{15,18} Interestingly, those studies also reported broad spectrum antibacterial and anti-protozoal activity for the bark extracts and all of the isolated compounds. Indeed, the extract and isolated compounds inhibited the growth of all bacteria and protozoa screened. Broad spectrum antifungal activity was also reported, albeit at doses which would indicate only moderate to low growth inhibitory

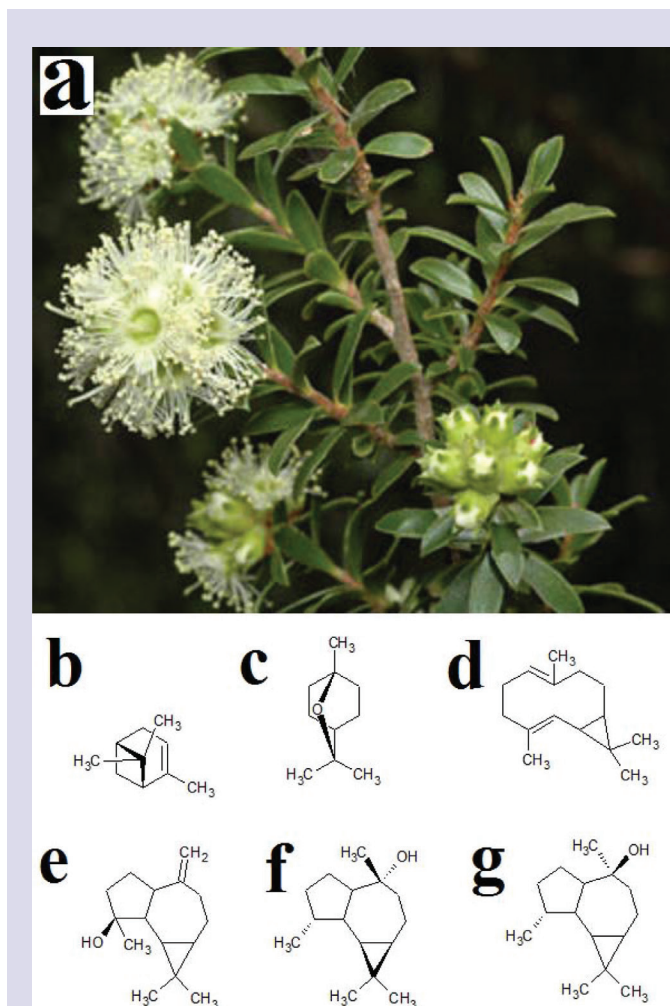


Figure 1: *K. flavescens* (a) leaves and flowers as well as the chemical structures of (b) α -pinene, (c) 1,8-cineole, (d) bicyclogermacrene, (e) spathulenol, (f) globulol and (g) viridiflorol.

activity. Despite these promising earlier studies, examination of the antibacterial properties and phytochemistry of many other *Kunzea* spp. including *K. flavescens* is lacking. The current report was undertaken to screen *K. flavescens* leaves for growth inhibitory properties against a panel of pathogenic bacteria.

MATERIALS AND METHODS

Plant collection and extraction

Kunzea flavescens C.T.White & W.D.Francis leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. The leaf samples were dried in a Sunbeam food dehydrator and stored at -30°C . Prior to use, the dried leaves were freshly ground to a coarse powder and 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 hours 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 *K. flavescens* leaf extracts for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.¹⁹⁻²¹

Antibacterial screening

Test microorganisms

All media was supplied by Oxoid Ltd., Australia. Reference strains of *Klebsiella pneumoniae* (ATCC31488), *Escherichia coli* (ATCC 25922) and *Proteus mirabilis* (ATCC21721) were purchased from American Tissue Culture Collection, USA. Clinical isolate microbial strains of *Aeromonas hydrophila*, *Alcaligenes faecalis*, *Bacillus cereus*, *Citrobacter freundii*, *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.²²⁻²⁴ 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.^{25, 26} Briefly, the *K. flavescens* leaf 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.²⁷ Briefly, 3 mL of *Klebsiella pneumoniae* (ATCC31488) and *Proteus mirabilis* (ATCC21721) in nutrient broth were added individually to 27 mL nutrient broth containing 3 mL of 10 mg/mL methanolic and aqueous plant extract to give a final concentration of 1000 $\mu\text{g/mL}$ 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 except without the extract. All assays were performed in triplicate.

Toxicity screening

Reference toxin for toxicity screening

Potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$) (AR grade, Chem-Supply, Australia) was prepared as a 4 mg/mL solution in distilled water and was serially

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

Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Phytosteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
M	206	20.6	+++	+++	+++	-	++	-	+	+	+	+++	+++	-	-
W	184	18.4	+++	+++	+++	-	+	-	++	+	+	+++	+++	-	-
E	133	13.3	+++	+++	++	-	-	-	++	+	+	+++	++	-	-
C	196	19.6	-	-	-	-	-	-	-	-	-	-	-	-	-
H	122	12.2	-	-	-	-	-	-	-	-	-	-	-	-	-

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; H = hexane extract.

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.²⁸⁻³⁰ Briefly, 400 μ L of seawater containing approximately 54 (mean 54.3, $n = 75$, SD 12.6) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400 μ L 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 μ L 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 were 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 *K. flavescens* leaf with solvents of varying polarity yielded dried extracts ranging from 122mg (hexane extract) to 206 mg (methanolic extract) (Table 1). The aqueous (184 mg) and chloroform extracts (196 mg) also yielded 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 higher polarity methanol, water and ethyl acetate solvents extracted the greatest diversity and highest levels of phytochemicals. Each contained high levels of phenolics, flavonoids and tannins, as well as moderate levels of phytosterols and 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 not detected and other techniques are required to further examine the nature of these nonpolar components.

Antimicrobial activity

To determine the growth inhibitory activity of the *K. flavescens* leaf extracts against the panel of pathogenic bacteria, aliquots (10 μ L) of each extract were screened in the disc diffusion assay. The methanolic, aqueous and ethyl acetate *K. flavescens* leaf extracts inhibited 3 of the 10 gram negative bacterial species screened (Figure 2). The methanolic *K. flavescens* leaf extract was a more potent growth inhibitor than the aqueous or ethyl acetate extracts against all susceptible bacterial species (as assessed by the sizes of the zones of inhibition). The methanolic extract was a particularly potent inhibitor of *P. mirabilis* growth, with zones of inhibition of 12.3 ± 0.9 mm. This inhibition was particularly noteworthy compared to the inhibition by the ampicillin control (10 μ g; inhibition zones of approximately 8.3 ± 0.3 mm). The aqueous and ethyl acetate *K. flavescens* leaf extracts were also good *P. mirabilis* growth inhibitors, with inhibition zones of 9.5 ± 0.5 mm and 8.0 ± 0.5 mm respectively. The chloroform extract also inhibited *P. mirabilis* growth, albeit with substantially smaller inhibition zones. The hexane extract was devoid of growth inhibitory activity.

K. pneumoniae was also highly susceptible to the *K. flavescens* leaf

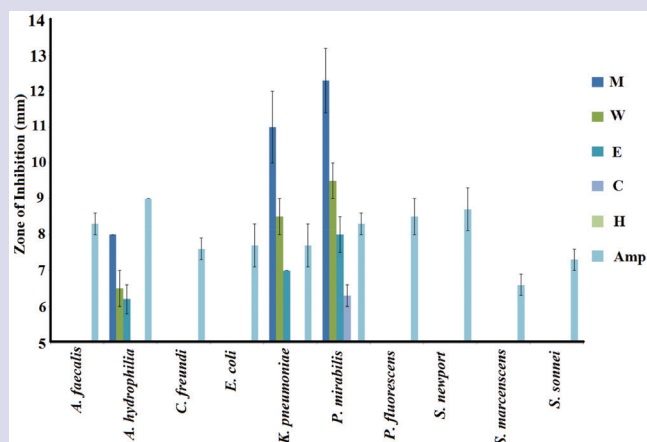


Figure 2: Growth inhibitory activity of *K. flavescens* leaf extracts against the gram negative bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 µg) control. All determinations were performed in triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.

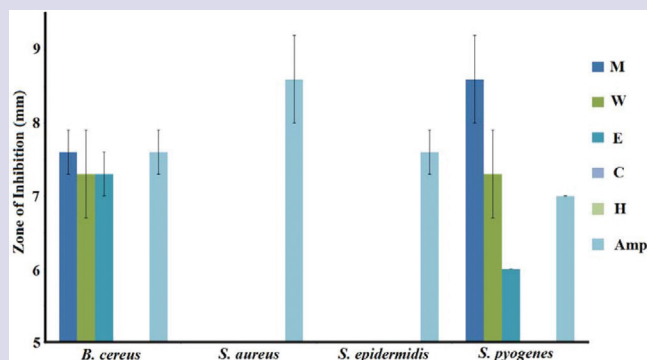


Figure 3: Growth inhibitory activity of *K. flavescens* leaf extracts against the gram positive bacterial species. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; Amp = ampicillin (10 µg) control. All determinations were performed in triplicate and the results are expressed as mean zones of inhibition (mm) ± SEM.

extracts. As reported for *P. mirabilis* growth inhibition, the methanolic extract was substantially more potent than the other extracts, with 11.0 ± 1.0 mm zones of inhibition. Growth inhibition by the aqueous extract (8.5 ± 0.5 mm) was also indicative of good growth inhibition. In contrast, the inhibition by the ethyl acetate extract (7.0 mm) was indicative of low to moderate growth inhibitory activity. Whilst *A. hydrophilia* was also susceptible to inhibition by the *K. flavescens* leaf extracts, the zones of inhibition were substantially smaller than for the other bacteria. Gram positive bacteria were also inhibited by the *K. flavescens* leaf extracts. Indeed, 2 of the 4 gram positive bacteria (50 %: *B. cereus*, *S. pyogenes*) were inhibited, with zones of inhibition consistent with low to moderate inhibition. Zones of 7.6 ± 0.3 mm and 8.6 ± 0.6 mm were determined for the methanolic *K. flavescens* leaf extract against *B. cereus* and *S. pyogenes* respectively. The aqueous and ethyl acetate extracts also

inhibited these gram positive bacteria, although generally with substantially less potency (as judged by the size of the inhibition zone).

The antimicrobial efficacy was further quantified by determining the MIC values for each extract against the susceptible microbial species. The methanolic, aqueous and ethyl acetate *K. flavescens* 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 *K. flavescens* leaf extracts, with MIC values <400 µg/mL (<4 µg infused into the disc). The aqueous and ethyl acetate *K. flavescens* leaf extracts were also good *P. mirabilis* growth inhibitor, with MIC values <850 µg/mL. As *P. mirabilis* infection is a common cause of urinary tract infections and has also been identified as a trigger of rheumatoid arthritis [31, 32], the methanolic, aqueous and ethyl acetate *K. flavescens* leaf extracts have potential for the prevention of these diseases in genetically susceptible individuals. Similarly, *K. pneumoniae* and *S. pyogenes* were also susceptible to growth inhibition by the methanolic, aqueous and ethyl acetate *K. flavescens* leaf extracts. As *K. pneumoniae* can trigger ankylosing spondylitis [33, 34] and *S. pyogenes* can trigger rheumatic heart disease [35, 36] in genetically susceptible individuals, these extracts also have potential to prevent and treat these diseases.

Bacterial growth time course assay

The antibacterial activity of the *K. flavescens* leaf extracts was further investigated in reference bacterial strains by bacterial growth time course assays in the presence and absence of each extract. Only the effect of the methanolic and aqueous extract on the bacterial growth time course were evaluated as these extracts were generally the most potent of the other *K. flavescens* leaf extracts. Furthermore, the time course studies only examined the effect of the extracts on *K. pneumoniae* and *P. mirabilis* as these were the most susceptible to inhibition by the *K. flavescens* leaf extracts. The starting concentration of the extract used in these assays was 1000 µg/mL. The methanolic and aqueous *K. flavescens* extracts significantly inhibited *K. pneumoniae* (Figure 4a) and *P. mirabilis* (Figure 4b) growth within 1 h, indicating a rapid antimicrobial action. Furthermore, the inhibition of both *P. mirabilis* and *K. pneumoniae* by the methanolic and aqueous *K. flavescens* leaf extracts was still significantly inhibited by the end of the 6 h time course study. This may indicate that these extracts have bactericidal activity against *P. mirabilis* and *K. pneumoniae* at the dose tested. Indeed, the turbidity at 6 h was not greatly increased from the starting turbidity.

Quantification of toxicity

The toxicity of the *K. flavescens* leaf extracts was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000 µg/mL (Figure 5). All 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 begun to induce mortality significantly higher than that in the untreated control. As all of the extracts induced <50 % toxicity at 24 h, all were deemed to be nontoxic. Extracts with an $LC_{50} >1000$ µg/mL towards *Artemia* nauplii have previously been defined as being nontoxic [30]. In contrast, the potassium dichromate positive control induced mortality within 4 h (results not shown), with 100 % mortality induction seen by 24 h.

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 *K. flavescens* leaf extracts against a panel of pathogenic bacteria, and on their toxicity. Both gram positive

Table 2: Minimum bacterial growth inhibitory concentration ($\mu\text{g/mL}$) of the *K. flavescens* extracts.

	Bacteria	M	W	E	C	H
Gram negative bacteria	<i>A. faecalis</i>	-	-	-	-	-
	<i>A. hydrophilia</i>	1637	2320	1875	-	-
	<i>C. freundii</i>	-	-	-	-	-
	<i>E. coli</i>	-	-	-	-	-
	<i>K. pneumoniae</i>	481	887	946	-	-
	<i>P. mirabilis</i>	393	804	837	3652	-
	<i>P. fluorescens</i>	-	-	-	-	-
	<i>S. newport</i>	-	-	-	-	-
	<i>S. marcescens</i>	-	-	-	-	-
	<i>S. sonnei</i>	-	-	-	-	-
Gram positive bacteria	<i>B. cereus</i>	1718	1562	1490	-	-
	<i>S. aureus</i>	-	-	-	-	-
	<i>S. epidermidis</i>	-	-	-	-	-
	<i>S. pyogenes</i>	1205	1950	1885	-	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no inhibition. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract.

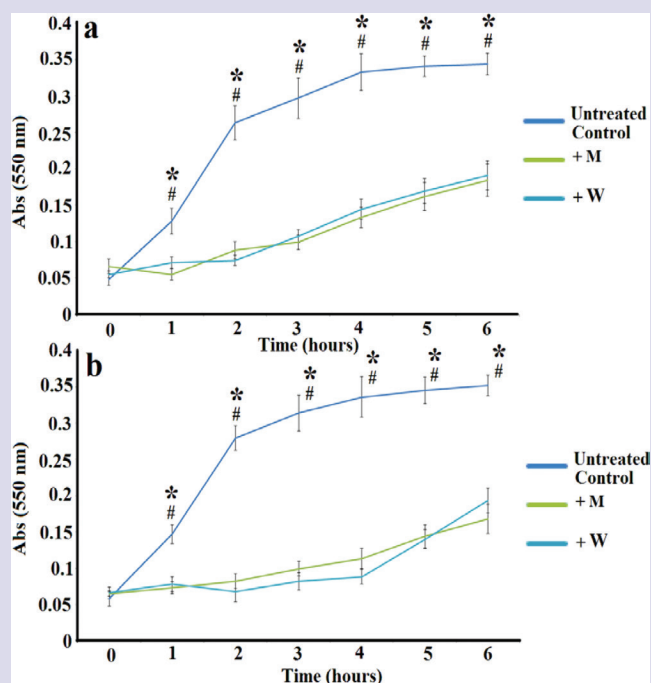


Figure 4: Bacterial growth curves for the methanolic and aqueous *K. flavescens* leaf extracts against (a) *K. pneumoniae* (ATCC31488) and (b) *P. mirabilis* (ATCC21721). All bioassays were performed in at least triplicate and are expressed as mean \pm SEM. * = results that are significantly different between the between the growth in the presence of the methanolic extract and the untreated control growth ($p < 0.01$); # = results that are significantly different between the between the growth in the presence of the aqueous extract and the untreated control growth ($p < 0.01$).

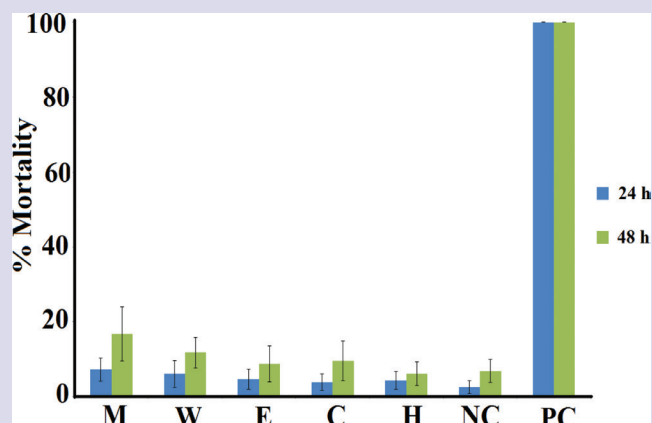


Figure 5: The lethality of the *K. flavescens* leaf extracts (2000 $\mu\text{g/mL}$), potassium dichromate (1000 $\mu\text{g/mL}$) and a seawater control. M = methanolic extract; W = aqueous extract; E = ethyl acetate extract; C = chloroform extract; H = hexane extract; NC = negative (seawater) control; PC = positive control (1000 $\mu\text{g/mL}$ potassium dichromate). All bioassays were performed in triplicate and are expressed as mean \pm SEM.

and gram negative bacteria were susceptible towards the *K. flavescens* leaf extracts although gram positive bacteria were slightly more susceptible (as judged by the % bacterial species which were inhibited). This is in agreement with many previous studies which have also reported a greater susceptibility of gram positive than gram negative bacterial species towards solvent extracts for South American,³⁷ African³⁸ and Australian plant extracts.^{39, 40}

The methanolic *K. flavescens* extract was identified as being a particularly

potent inhibitor of *P. mirabilis* and *K. pneumoniae* growth (MIC values of 393 and 481 µg/mL against *P. mirabilis* and *K. pneumoniae* respectively). As *P. mirabilis* can trigger rheumatoid arthritis in genetically susceptible individuals,^{31,32} these extracts have potential for the development of rheumatoid arthritis inhibitory therapies. As *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals,^{33,34} this extract may also be useful in the prevention of ankylosing spondylitis. Similarly, *S. pyogenes* is a trigger rheumatic heart disease.^{35,36} Thus, the *K. flavescens* leaf extracts have potential in the prevention and treatment of multiple autoimmune inflammatory diseases.

Whilst a detailed investigation of the phytochemistry of the *K. flavescens* leaf extracts was beyond the scope of our study, qualitative screening studies were used to determine the classes of compounds present. Several commonalities were noted: the most potent aqueous, methanolic and ethyl acetate extracts all contained relatively high levels of tannins, phenolics and flavonoids. Many studies have reported potent growth inhibitory activities for a number of tannin compounds. Gallotannins have been reported to inhibit the growth of a broad spectrum of bacterial species⁴¹ through a variety of mechanisms including binding cell surface molecules including lipoteichoic acid and proline-rich cell surface proteins,^{42,43} and by inhibiting glucosyltransferase enzymes.⁴⁴ Ellagitannins are also highly potent inhibitors of bacterial growth, with MIC values as low as 62.5 µg/mL.^{41,43} Ellagitannins have also been reported to function via several antibiotic mechanisms including interaction with cytoplasmic oxidoreductases and by disrupting bacterial cell walls.^{41,43} Thus, it is likely that *K. flavescens* leaf tannins may contribute to the inhibition of bacterial growth reported in our study.

It is likely that other phytochemical classes also contribute to the growth inhibitory properties of these extracts. Our qualitative phytochemical screening studies indicate that polyphenolics and flavonoids were present in relatively high levels in the *K. flavescens* leaf extracts. Many studies have reported potent antibacterial activities for a wide variety of polyphenolic compounds, including many flavonoids.⁴⁵ 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 *K. flavescens* 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.³⁰ 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 *K. flavescens* 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) and some skin diseases, 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 *K. flavescens* 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 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

DMSO: Dimethyl sulfoxide

LC₅₀: The concentration required to achieve 50 % mortality

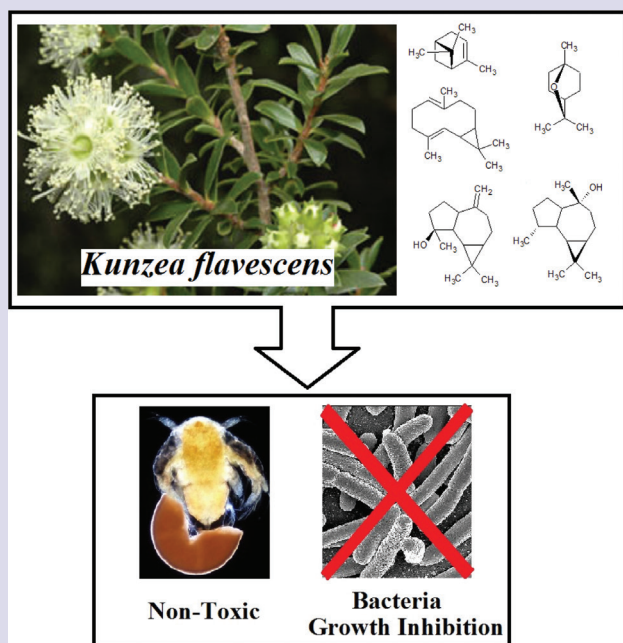
MIC: minimum inhibitory concentration

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PICTORIAL ABSTRACT



SUMMARY

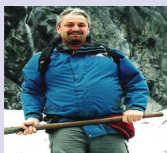
- *K. flavescens* leaf extracts inhibited the growth of both gram positive and gram negative bacteria.
- The methanolic, aqueous and ethyl acetate extracts were potent inhibitors of *P. mirabilis* growth, with MICs 375-850 µg/mL.
- The same extracts were also potent inhibitors of *K. pneumoniae* growth, with MICs 480-950 µg/mL.
- The methanolic, aqueous and ethyl acetate extracts were also moderate inhibitors of *A. hydrophilia*, *B. cereus* and *S. pyogenes* growth.
- All *K. flavescens* leaf extracts were nontoxic.

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.

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.



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.