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Published

2021

Journal Title

Pharmacognosy Communications

Version

Version of Record (VoR)

DOI

https://doi.org/10.5530/pc.2021.2.17

Pharmacogn. Commn. 2021;11(2):81-87.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogcommn.org

Original Article

Kunzea ambigua (Sm.) Druce and *Kunzea flavescens* C.T. White and W.D. Francis Essential Oils Inhibit the Growth of Some Bacterial Triggers of Inflammatory Diseases

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ABSTRACT

Introduction: *Kunzea ambigua* (Sm.) Druce and *Kunzea flavescens* C.T. White and W.D. Francis are endemic Australian plants. Decoctions, infusions and essential oils produced from the leaves were used traditionally to treat a variety of bacterial diseases. Despite this, these species have not been rigorously examined for antibacterial properties against many pathogens. **Methods:** The antimicrobial activity of *K. ambigua* and *K. flavescens* essential oils and a *K. ambigua* hydrosol was investigated by disc diffusion and liquid dilution MIC assays against a panel of pathogenic bacteria. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *K. ambigua* and *K. flavescens* essential oils displayed noteworthy growth inhibitory activity against *A. baylyi, K. pneumonia, P. mirabilis* and *P. aeruginosa* (MIC values substantially <1000µg/mL). Indeed, MIC values as low as 33µg/mL were noted against *P. aeruginosa*. Noteworthy growth inhibitory activity was also noted for the *K. ambigua* hydrosol against *A. baylyi* and *P. aeruginosa*. All extracts were determined to be non-toxic in the *Artemia franciscana* nauplii bioassay, indicating their safety for internal use as well as for topical uses. **Conclusion:** The lack of toxicity of the *Kunzea* spp. 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: Myrtaceae, Tick bush, White Kunzea, Rheumatoid arthritis, Ankylosing spondylitis, Multiple sclerosis, Australian plants, Antibacterial activity.

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DOI: 10.5530/pc.2021.2.17

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] African[5] and South American[6] plants, the therapeutic potential of the flora of Australia has been recognised for many 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 recently there has been increased study in this field.

The development of new antibiotic therapies is particularly urgent. The recent establishment of bacterial pathogens that are either extremely (XDR) or totally resistant (TDR) to common clinically used antibiotics[8] has resulted in the need to develop new and effective antibiotic chemotherapies. There are now limited therapeutic options for many diseases caused by bacterial pathogens and the situation is expected to worsen in the future as bacteria exchange resistance genes. Indeed, 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.[9] For reasons reviewed elsewhere,[8] 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.[10-24]

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.,[25] *Eugenia* spp.,[7] *Kunzea* spp.,[7,26] *Leptospermum* spp.[7,27,28] and *Syzygium* spp.[29-31] 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; Figure 1a), *Kunzea ericoides* (A.Rich) Joy Thomps. (commonly known as Kānuka, white tea-tree, Burgan), *Kunzea pomifera* F. Muell. (commonly known as muntries, emu apples, native cranberries) and *Kunzea flavescens* C.T. White and W.D. Francis (commonly known as white *Kunzea*; Figure 1b). These species have each been reported to inhibit bacterial growth.[7,26,32-34] However, most studies have tested *Kunzea* spp. solvent extracts and only a few have screened essential oils against human pathogens.[32-34]

Several interesting phytochemical components have been identified in *Kunzea* spp. extracts and essential oils. In particular, several terpenoid components including α-pinene (Figure 1c), 1,8-cineole (Figure 1d), bicyclogermacrene (Figure 1e), spathulenjol (Figure 1f), globulol (Figure 1g) and viridflorol (Figure 1h) have been identified in *Kunzea* spp. extracts and essential oils.[33,35,36] Interestingly, those studies also reported broad spectrum antibacterial and anti-protozoal activity for the

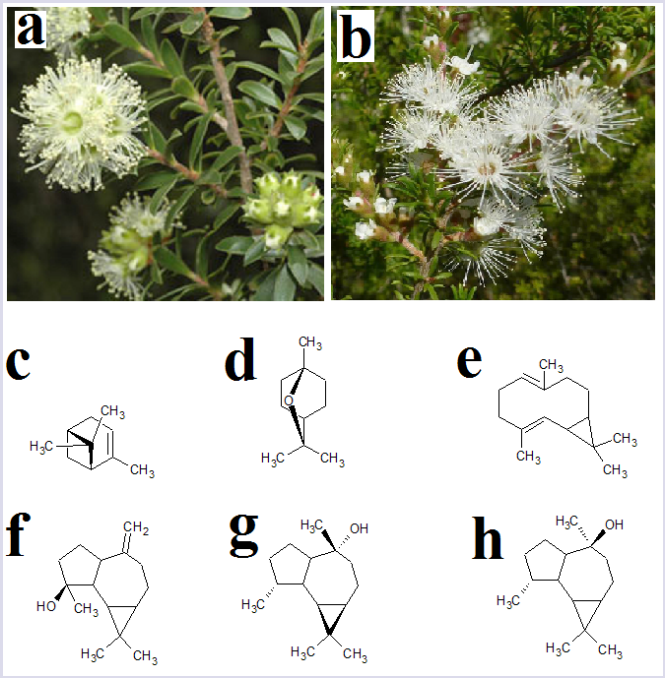

Figure 1: (a) *K. flavescens* and (b) *K. ambigua* leaves and flowers, as well as the chemical structures of (c) α-pinene, (d) 1,8-cineole, (e) bicyclogermacrene, (f) spathulenjol, (g) globulol and (h) viridflorol.

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 activity. Despite these promising earlier studies, examination of the antibacterial properties and phytochemistry of many other *Kunzea* spp. (and of the essential oils produced from them) is lacking. The current report was undertaken to screen *K. ambigua* and *K. flavescens* essential oils for growth inhibitory properties against a panel of pathogenic bacteria, and compare the activities to those of a commercial hydrosol.

MATERIALS AND METHODS

Kunzea ambigua (Sm.) Druce and *Kunzea flavescens* C.T. White and W.D. Francis preparations

Kunzea ambigua (Sm.) Druce and *Kunzea flavescens* C.T.White and W.D. Francis essential oils was obtained from Biodistributors, Australia. *K. ambigua* hydrosol was purchased from Aphrodite Australia. Five millilitre volumes of each sample were extensively dried by freeze drying for 48h until no decrease in mass was noted on repeated measurements. All samples were subsequently emulsified in 5mL of sterile deionised water containing 1% DMSO and stored at 4°C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *Kunzea* spp. essential oils and hydrosol for the presence of saponins, phenolic compounds, flavonoids, phytosteroids, triterpenoids, cardiac glycosides, anthraquinones, tannins and alkaloids was conducted by previously described assays.[26-28]

Antioxidant capacity

The antioxidant capacity of each sample was assessed using the DPPH free radical scavenging method[37] with modifications. Briefly, DPPH solution was prepared fresh each day as a 400μM solution by dissolving DPPH (Sigma) in AR grade methanol (Ajax, Australia). A 2mL aliquot of each sample was evaporated and the residue resuspended in 2 mL of methanol. Each sample was added to a 96 well plate in 5, 10, 25, 50, 75μL volumes in triplicate. Methanol was added to each well to give a volume of 225μL. A volume of 75μL of the fresh DPPH solution was added to each well to give a total reaction volume of 300μL. Ascorbic acid was prepared fresh and examined across the range 0-25μg per well as a reference and the absorbance's were recorded at 515nm. All tests and controls were tested in triplicate. The antioxidant capacity based on DPPH free radical scavenging ability was determined for each extract and expressed as μg ascorbic acid equivalents per gram of original plant material extracted.

Antibacterial screening

Test micro-organisms

All media was purchased from Oxoid Ltd., Australia. The reference strains of *A. baylyi* (ATCC21721), *Klebsiella pneumoniae* (ATCC31488), *Proteus mirabilis* (ATCC21721), *Proteus vulgaris* (ATCC21719) and *Pseudomonas aeruginosa* (ATCC39324) were purchased from American Tissue Culture Collection (ATCC), USA. Clinical isolate microbial strains of *Alcaligenes feacalis*, *Bacillus cereus, Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli, Klebsiella pneumonia*, *Proteus mirabilis* and *Pseudomonas aeruginosa* 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 *Kunzea* spp. essential oils and hydrosol was determined using a modified disc diffusion assay.[22,38] 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 5mm 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 2h before incubation at 37°C for 24h. 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 for each extract was determined using liquid dilution MIC assays and solid phase agar disc diffusion assays.

Microplate liquid dilution MIC assay

A standard liquid dilution MIC assay[39] was used to evaluate the bacterial growth inhibitory activity of the essential oils, hydrosol and conventional antibiotic. Briefly, log phase bacterial cultures were diluted to produce a McFarlands inoculation culture. A 100μL volume of sterilized nutrient broth was dispensed into all wells of a 96 well micro-titre plate. A volume of 100μL of the plant extracts or conventional antibiotics was subsequently dispensed into separate wells of the top row of the plate. A negative control (nutrient broth), sterile control (broth without bacteria) and a sample-free culture control (to ensure the media was capable of supporting microbial growth) were also included on all plates. Each test sample or control was serially diluted down each column on the plate by doubling dilution. The assay culture inoculum (100μL, containing

approximately 1x10^6 colony forming units (CFU)/mL) was then added to all wells except the sterile control wells and incubated overnight at 37°C. p-Iodonitrotetrazolium violet (INT, Sigma-Aldrich, Australia) was dissolved in sterile deionised water to a concentration of 200µg/mL. A 40µL volume of the INT solution was added into all wells and the plate was incubated for a further 6h at 37°C. The MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc diffusion MIC assay

The minimum inhibitory concentration (MIC) of each extract was also quantified by disc diffusion assay.[40,41] Graphs of the zone of inhibition (ZOI) versus ln concentration were plotted and MIC values were calculated by linear regression.

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.[11,13] Briefly, 400µL of seawater containing approximately 43 (mean 42.6 ± 10.6; n = 125) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used for bioassay. A volume of 400µL of diluted plant preparations or the reference toxin were transferred to the wells and incubated at 25±1°C under artificial light (1000 Lux). A 400µL seawater negative control was run in triplicate for each plate. All treatments were performed in at least triplicate. The wells were checked at regular intervals and the number of dead counted. The nauplii were considered dead if no movement of the appendages was detected within 10 sec. After 24h, 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 ± 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

Extract/hydrosol yields and qualitative phytochemical screening

Freeze drying the *Kunzea* spp. essential oils and hydrosol resulted in masses of dried material ranging from 2780mg (*K. ambigua* hydrosol) to 2980mg (*K. ambigua* essential oil) (Table 1). The dried extracts were resuspended in 5mL of deionised water (containing 1% DMSO), resulting in the extract concentrations shown in Table 1. Qualitative phytochemical studies showed that only low levels of polar polyphenolic compounds were detected in the essential oils and hydrosol. Due to their non-polar 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. Other techniques are required to further examine the nature of these non-polar components. Moderate to low levels of triterpenoids were also detected in the essential oils of both *Kunzea* species. Interestingly, we were unable to detect antioxidant content levels for either of the essential oils, and only a low antioxidant capacity was measured for the *K. ambigua* hydrosol.

Antimicrobial activity

To determine the growth inhibitory activity of the *Kunzea* spp. essential oils and hydrosol against the panel of pathogenic bacteria, aliquots (10 µL) of each extract were screened in the disc diffusion assay. Noteworthy growth inhibitory activity was seen against several bacterial species (Figure 2). The essential oils were generally better inhibitors of bacterial growth, and the *K. ambigua* essential oil was generally better than the *K. flavescens* essential oil (as judged by ZOI). *K. ambigua* essential oil was a particularly good inhibitor of *A. baylyi*, with a ZOI of 8.3±0.4mm recorded against the reference strain. Notably, only *E. aerogines* was resistant to each of the essential oils and the hydrosol.

The relative level of antimicrobial activity was further evaluated by determining the MIC values (Table 2) against the bacterial pathogens. The *K. ambigua* and *K. flavescens* essential oils were particularly effective at inhibiting the growth of *A. baylyi, K. pneumonia, P. mirabilis* and *P. aeruginosa* (MIC values substantially <1000µg/mL). Indeed, MIC values as low as 33µg/mL were noted against *P. aeruginosa*. Noteworthy growth inhibitory activity was also noted for the *K. ambigua* hydrosol against *A. baylyi* and *P. aeruginosa*. These growth inhibitory activities are especially interesting as *P. aeruginosa* is resistant against many antibiotics. Indeed, in our study, both the reference and clinical strains of this bacterium were completely resistant to ampicillin. The relatively high MIC value (2.5µg/mL) indicates that this bacterium is also resistant to chloramphenicol. *A. baylyi*, *K. pneumoniae* and *P. mirabilis* were similarly resistant to both antibiotic controls. Therefore, the *Kunzea* spp. preparations may be useful in treating infections caused by these bacteria. Previous studies have reported that *P. mirabilis* and *K. pneumoniae* can induce rheumatoid arthritis and ankylosing spondylitis respectively in genetically susceptible individuals.[12,13,15] Additionally, *A. baylyi* and *P. aeruginosa* can induce multiple sclerosis in genetically susceptible people.[42] Thus, the *Kunzea* spp. essential oils and hydrosol have potential in the prevention and treatment of these diseases, as well as other diseases caused by these bacteria. All other bacterial pathogens were either completely resistant to the *Kunzea* spp. essential oils and hydrosol,

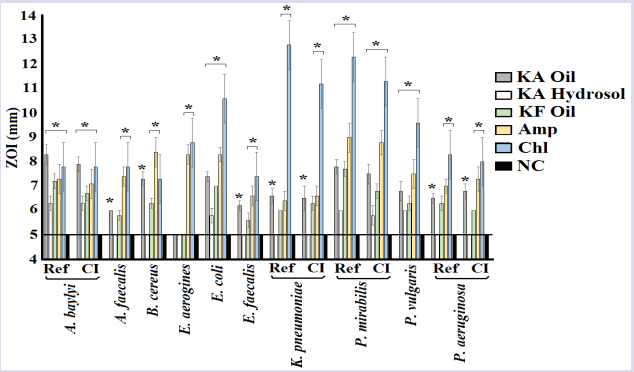

Figure 2: Antibacterial activity of *Kunzea* spp. essential oils and extracts, as well as ampicillin and chloramphenicol controls (10 µg) measured as zones of inhibition (mm) against bacterial pathogens. Results are expressed as mean ± SEM of at least triplicate determinations. Ref = reference strains; CI = clinical isolate strains; KA = *K. ambigua*; KF = *K. flavescens*; Amp = ampicillin' Chl = chloramphenicol; NC = negative control. * = results that are significantly different to the negative control. 5mm line indicates the diameter of the disc. * indicates results that are significantly different to the untreated control (p<0.01).

or displayed only low inhibitory activity (as judged by MIC values).

Interestingly, major differences were noted between the efficacy of the essential oils and the hydrosol between the disc diffusion and liquid dilution assays. The disc diffusion assay is reliant on the movement of compounds through an aqueous gel. Low polarity compounds do not readily diffuse through agar gels and low or fallacious results may be recorded using this method to quantify the activity of essential oils. No such limitation to diffusion occurs for liquid dilution assays and the results obtained from those assays may be considered more accurate for non-polar compounds and solutions.

Quantification of toxicity

The toxicity of the *Kunzea* spp. essential oils and hydrosol was initially tested in the *Artemia franciscana* nauplii bioassay at a concentration of 2000µg/mL (Figure 3). The *K. ambigua* essential oil induced 100% mortality in the *Artemia* nauplii following 24h exposure, indicating some toxicity toxic. In contrast, the *K. flavescens* essential oil induced only approximately 20% mortality and the *K. ambigua* hydrosol did not induce mortality significantly different to that seen for the seawater control. As samples with LC_{50} values <1000µg/mL towards *Artemia* nauplii are defined as being toxic,[11,13,43] both the *K. flavescens* essential oil and the *K. ambigua* hydrosol were deemed to be non-toxic. In contrast, the potassium dichromate positive control induced mortality within 4h (results not shown), with 100 % mortality induction seen by 24h.

To further quantify the effect of *K. ambigua* essential oil concentration on the induction of mortality, it was serially diluted in artificial seawater to test across a range of concentrations in the *Artemia* nauplii bioassay (Table 2). For comparison, serial dilutions of potassium dichromate were also tested. As an LC_{50} value of 1680µg/mL was noted for the *K. ambigua* essential oil (i.e. substantially greater than 1000µg/mL), it was also deemed to be non-toxic.

Table 1: The mass of dried plant material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant contents of *the Kunzea* spp. essential oils and hydrosol.

		KA Oil	KA Hydrosol	KF oil
	Mass of dried preparation (mg)	2980	1780	2820
	Concentration of stock preparation (mg/mL)	298	178	282
	Total phenolics	+	+	+
Phenolics	Water soluble phenolics	-	-	-
	Water insoluble phenolics	+	+	+
	Cardiac glycosides	-	-	-
	Saponins	-	-	-
	Triterpenes	++	-	+
	Phytosterols	-	-	-
Alkaloids	Meyer test	-	-	-
	Wagner test	-	-	-
	Flavonoids	-	-	+
	Tannins	-	-	-
Anthraquinones	Free	-	-	-
	Combined	-	-	-
	Antioxidant capacity	BDT	1.34	BDT

+++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay. AA = ascorbic acid; BDT = below detection threshold. Antioxidant capacity determined by DPPH reduction (expressed as mg AA equivalence per g plant material extracted).

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 *Kunzea* spp. essential oils and a hydrosol against a panel of pathogenic bacteria, and on their toxicity. The *K. ambigua* and *K. flavescens* essential oils were particularly good inhibitors of *A. baylyi* and *P. aeruginosa* growth (MIC values 33-500µg/mL). As these bacteria can trigger multiple sclerosis in genetically susceptible people,[42] the *Kunzea* spp. essential oils have potential in the prevention and treatment of this disease, as well as other diseases caused by *A. baylyi* and *P. aeruginosa* infections. The *Kunzea* spp. essential oils were also good inhibitors of *P. mirabilis* and *K. pneumoniae* growth (MIC values 124-750µg/mL). *P. mirabilis* can trigger rheumatoid arthritis in genetically susceptible individuals[15] and *K. pneumoniae* can trigger ankylosing spondylitis in genetically susceptible individuals.[12,15] Therefore, these essential oils also have potential for the development of rheumatoid arthritis and ankylosing spondylitis inhibitory therapies. Thus, the *Kunzea* spp. essential oils have potential in the prevention and treatment of multiple autoimmune inflammatory diseases.

The *K. ambigua* hydrosol was also a good inhibitor of *A. baylyi* and

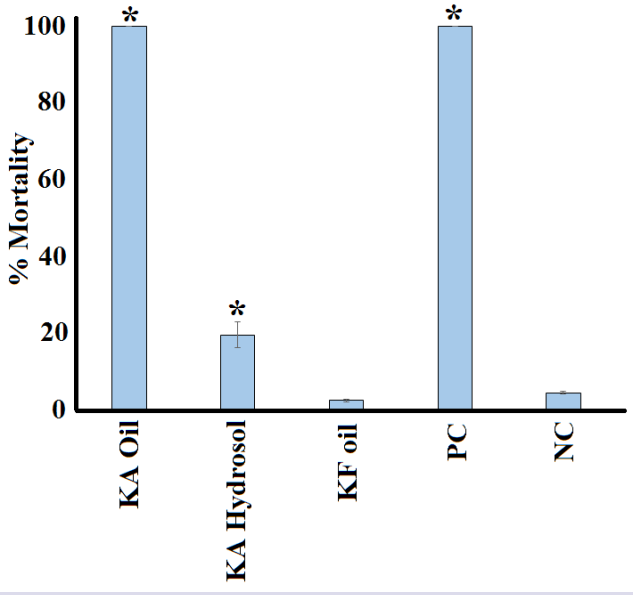

Figure 2: The lethality of the *Kunzea* spp. essential oils and extracts extracts (2000µg/mL), potassium dichromate (1000µg/mL) and a seawater control following 24h exposure. KA = *K. ambigua*; KF = *K. flavescens*; NC = negative (seawater) control; PC = positive control (1000µg/mL potassium dichromate). Results are expressed as mean ± SEM of at least triplicate determinations. * indicates results that are significantly different to the untreated control (p<0.01).

Table 2: Minimum inhibitory concentrations (µg/mL) of *Kunzea* spp. essential oils and extracts against bacterial pathogens.

		KA Oil	KA Hydrosol	KF Oil	Amp	Chl	NC
A. baylyi (R)	DD MIC	1783	820	580	ND	ND	ND
	LD MIC	500	157	157	-	2.5	
A. baylyi (CI)	DD MIC	1555	765	748	ND	ND	ND
	LD MIC	500	157	330	-	2.5	-
A. faecalis	DD MIC	>5000	-	>5000	ND	ND	ND
	LD MIC	1250	-	1250	2.5	2.5	-
B. cereus	DD MIC	>5000	-	>5000	ND	ND	ND
	LD MIC	1850	-	2560	1.25	2.5	-
E. aerogines	DD MIC	-	-	-	ND	ND	ND
	LD MIC	-	-	-	1.25	1.25	-
E. coli	DD MIC	>5000	>5000	>5000	ND	ND	ND
	LD MIC	2560	>5000	3280	1.25	1.25	-
E. faecalis	DD MIC	>5000	-	>5000	ND	ND	ND
	LD MIC	1760	-	>5000	-	2.5	-
K. pneumoniae (R)	DD MIC	1587	-	476	ND	ND	ND
	LD MIC	500	-	157	-	1.3	-
K. pneumoniae (CI)	DD MIC	1760	-	388	ND	ND	ND
	LD MIC	500	-	124	-	1.25	-
P. mirabilis (R)	DD MIC	832	1276	628	ND	ND	ND
	LD MIC	500	1150	157	1.3	0.6	-
P. mirabilis (CI)	DD MIC	746	1083	922	ND	ND	ND
	LD MIC	500	924	750	1.3	0.6	-
P. vulgaris	DD MIC	1876	>5000	2590	ND	ND	ND
	LD MIC	1250	>5000	2200	2.5	1.3	-
P. aeruginosa (R)	DD MIC	>5000	-	>5000	ND	ND	ND
	LD MIC	33	350	56	-	2.5	-
P. aeruginosa (CI)	DD MIC	>5000	-	>5000	ND	ND	ND
	LD MIC	64	700	128	-	2.5	-
Toxicity	*Artemia nauplii*	1680	-	-	85*		-

Numbers indicate the mean MIC values of triplicate determinations expressed in µg/mL. KA = *K. ambigua*; KF = *K. flavescens;* DD = disc diffusion; LD = liquid dilution; ND = MIC values were not determined as only a single dose was screened; - indicates no inhibition or toxicity at any concentration tested; * = potassium dichromate was used as the positive control. Bold text indicates noteworthy MIC values.

P. aeruginosa, and displayed moderate inhibitory activity towards *P. mirabilis* and *K. pneumoniae*. Therefore, the *K. ambigua* hydrosol also has potential in the prevention and treatment of rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis. Notably, all other bacteria screened were either completely resistant to the *Kunzea* spp. essential oils and hydrosol, or displayed only low susceptibility. Interestingly, all of the lower susceptibility species are common causes of food poisoning or food spoilage. Therefore, it is likely these preparations would be ineffective against food poisoning.

The findings reported here also demonstrate that all of the *Kunzea* spp. essential oils and hydrosol were non-toxic towards *Artemia franciscana* nauplii, with LC_{50} values substantially >1000µg/mL. 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 *Kunzea* spp. essential oils and hydrosol in the development of future antibiotic chemotherapeutics for the prevention and treatment of urinary tract infections, autoimmune diseases (particularly rheumatoid arthritis, ankylosing spondylitis and multiple sclerosis), more work is required to isolate the inhibitory components and determine the mechanism of inhibition.

CONCLUSION

The results of this study demonstrate the potential of the *Kunzea* spp. essential oils and hydrosol as inhibitors of pathogenic bacteria growth. Furthermore, their lack of toxicity indicates than 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.

ACKNOWLEDGEMENT

The authors are grateful to Michelle Mendell and Jane Gifkins of Griffith University for providing the clinical bacterial strains used in this study. Financial support for this work was provided by the Environmental Futures Research Institute and the School of Environment and Science, Griffith University, Australia.

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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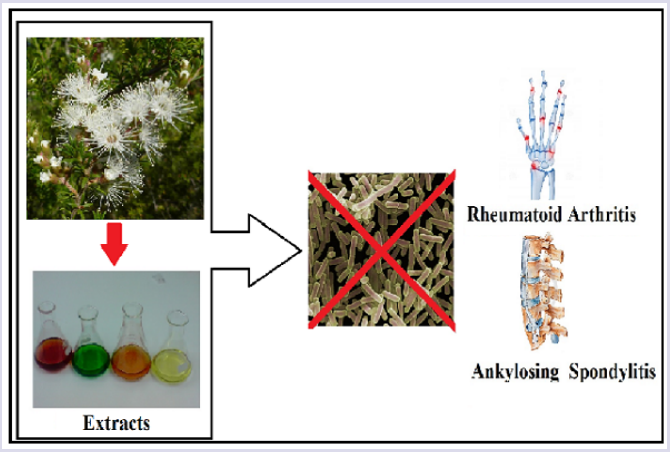
PICTORIAL ABSTRACT



SUMMARY

- *Kunzea* spp. essential oils and a hydrosol were screened for antibacterial activity against a panel of bacterial pathogens.
- *K. ambigua* and *K. flavescens* essential oils displayed broad spectrum antibacterial activity.
- The essential oils were particularly good inhibitors of *A. baylyi, K. pneumoniae, P. mirabilis* and *P. aeruginosa* growth with MIC values as low as 33µg/mL.
- The *K. ambigua* hydrosol was substantially less potent than the corresponding essential oil against all bacteria.
- All *Kunzea* spp. essential oils and hydrosols were non-toxic in the *Artemia nauplii* bioassay.

ABOUT AUTHORS

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.