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Original Article

Eucalyptus major (Maiden) Blakely Leaf Extracts Inhibit the Growth of Axillary and Foot Odour Producing Bacteria

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ABSTRACT

Introduction: *Eucalyptus* spp. extracts inhibit the growth of many bacterial pathogens. They may also inhibit the growth of malodour producing bacteria and thus be useful deodorant components, although this is yet to be tested. **Materials and Methods:** Methanolic and aqueous *Eucalyptus major* (Maiden) Blakely leaf extracts were investigated by disc diffusion and liquid dilution MIC assays against the most significant bacterial contributors to axillary and plantar malodour. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** Methanolic and aqueous *E. major* leaf extracts displayed noteworthy bacterial growth inhibitory activity against all of the malodour forming bacteria tested. The methanolic extract was a particularly potent growth inhibitor, with liquid dilution (LD) MIC values of 183, 500, 500 and 125 µg/mL against *C. jeikeium, P. acnes, B. linens* and *S. epidermidis* respectively. Similar, albeit slightly higher LD MIC values were noted for the aqueous *E. major* leaf extract (LD MICs of 625, 625, 750 and 500 µg/mL against *C. jeikeium, P. acnes, B. linens* and *S. epidermidis* respectively). The methanolic and aqueous *E. major* leaf extracts were nontoxic in the *Artemia fransiscana* bioassay. **Conclusion:** The lack of toxicity of the methanolic and aqueous *E. major* leaf extracts and their potent growth inhibition of axillary and plantar malodour producing bacteria indicate their potential as deodorant components. Further studies are warranted to isolate and identify the active components.

Keywords: Grey gum, Myrtaceae, body odour, deodorant, *Corneybacterium jeikeium*, *Propionobacter acnes*, *Brevibacter linens*, *Staphylococcus epidermidis*.

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INTRODUCTION

Multiple synthetic compounds (including propylene glycol, triclosan, benzalkonium chloride and metal (e.g. Al) salts are currently included in deodorants and antiperspirants to decrease sweating, as well inhibiting the growth of skin bacteria, with the aim of decreasing odour formation.[1] Notably, the safety of many of these additives has yet to be definitively verified and the use of several of them is considered to be hazardous to human health. Aluminium salts have been linked with a wide range of negative health effects including degenerative neurological conditions (e.g. Alzheimers disease, encephalopathy)[2] and cancer.[3] Aluminium salt additives may also cause respiratory problems and induce anaphylactic shock in susceptible individuals.[2] There are also concerns about chronic exposure to triclosan, a common bacterial growth inhibitor in many deodorants, due to reports of dermal irritation and allergies.[4] Natural alternative deodorant molecules or preparations that inhibit the growth of odour forming bacteria may be less concern for human health and may also be more acceptable to consumers due to their natural origin. Indeed, there has been a recent trend to search natural resources for safe and effective plant preparations to replace these synthetic additives and several promising plan species have been identified.[5-8]

The genus *Eucalyptus* (family Myrtaceae) consists of more than 700 species of trees and shrubs, with the majority native to Australia.[9] *Eucalyptus major* (Maiden) Bailey (commonly known as grey gum) is an Australian tree species that is endemic to the Queensland-New South Wales border region of Eastern Australia. It is a large tree that grows to approximately 20 metres. The dark green leaves (9-20 cm long by 2-4 cm wide) are curved-lance shaped and paler on the undersides. Multiple *Eucalyptus* spp. were used by the first Australians for their antiseptic properties[10] and the efficacy has been verified for several species in laboratory studies.[11] However, relatively few studies have tested the antibiotic properties of *Eucalyptus major* (Maiden) Bailey. One study reported noteworthy growth inhibitory activity for *E. major* leaf extracts against a limited panel of pathogenic bacteria,[11] although they are yet to be tested against body odour forming bacteria. Due to their ethnobotanical usage and the previously

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reported bacterial growth inhibitory activity of several *E. major* leaf extracts against a limited panel of bacteria, it is likely that they may also inhibit the growth of bacteria directly involved in axillary and foot malodour formation. This study aimed to test solvent extracts produced from *E. major* leaves against the axillary and foot odour forming bacteria *Corneybacterium jeikeium, Propionibacterium acnes* and *Brevibacterium linens* and *Staphylococcus epidermidis* with the aim of identifying safe and effective deodorant components.

MATERIALS AND METHODS

Plant source and extraction

The *Eucalyptus major* (Maiden) Bailey leaves used in this study was obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. The harvested leaves were washed in deionised water and processed within 4 hr of collection. The leaves were dried using a Sunbeam food dehydrator. The dried material was stored at -30°C until use. The plant materials were thawed and freshly ground to a coarse powder prior to extraction. Individual 1 g quantities of the ground leaves were weighed into individual tubes and 50 mL of methanol (AR grade, Ajax Fine Chemicals, Australia) or sterile deionised water was added. The leaf material was extracted in each solvent for 24 hr at 4°C with gentle shaking. The extracts were filtered through filter paper (Whatman No. 54) under vacuum, followed by drying by evaporation in a vacuum oven at 50°C. The resultant dry extracts were weighed and redissolved in 10 mL of deionised water (containing 0.5% DMSO) and passed through a 0.22 µm filter (Sarstedt) to remove particulates. The extracts were then stored at 4°C until use.

Qualitative phytochemical studies

Phytochemical analysis of the *E. major* leaf extracts for the presence of cardiac glycosides, alkaloids, saponins, tannins, flavonoids, phenolic compounds, phytosterols, flavonoids and triterpenoids were achieved as previously described.[12-14]

Antibacterial screening

Test bacterial strains

All media and components were supplied by Oxoid Ltd., Australia. Reference strains of *Brevibacterium linens* (ATCC9172), *Corneybacterium jeikeium* (ATCC43734) and *Propionibacterium acnes* (ATCC6919) were purchased from American Type Culture Collection, USA. The clinical isolate strain of *Staphylococcus epidermidis* used in this study was supplied by Ms. Jane Gifkins of the School of Environment and Science, Griffith University, Australia. To culture the bacteria, *B. linens* and *S. epidermidis* were inoculated into separate flasks of nutrient broth and grown aerobically at 37°C for 24 hr. Cultures of *C. jeikeium* were grown and maintained in nutrient broth supplemented with 300 µL Tween 80/L of broth at 37°C for 24 hr. *Propionibacterium acnes* was cultured using a thioglycollate liquid media under induced anaerobic conditions through the use of anaerobic jars and AnaeroGen™ 3.5 L atmospheric generation systems (Thermo Scientific). Incubation was at 37°C for 72 hr. All stock cultures were maintained and subcultured in liquid media at 4°C.

Evaluation of antibacterial activity

Antibacterial activity screening of the *E. major* leaf extracts was achieved using a modified disc diffusion assay.[15-17] Briefly, 100 µL of each individual bacteria was grown separately in 20 mL of the appropriate broth until an approximate count of 10^8 cells/mL was reached. A volume of 100 µL of each bacterial suspension was spread onto agar plates and the extracts were tested for antibacterial activity using 6 mM sterilised filter paper discs. *Brevibacterium linens, S. epidermidis* and *P. acnes* cultures were spread onto nutrient agar plates. *Corneybacterium jeikeium* cultures were spread onto nutrient agar plates supplemented with 300 µL Tween 80/L of agar. Discs were infused with 10 µL of the individual extracts, allowed to dry and placed onto the inoculated plates. The plates were left to stand at 4°C for 2 hr before incubation. Plates inoculated with *B. linens, S. epidermidis* or *C. jeikeium* cultures were incubated aerobically at 37°C for 24 hr. Plates spread with *P. acnes* cultures were incubated under induced anaerobic conditions at 37°C for 72 hr. The diameters of the inhibition zones (ZOIs) were measured to the closest whole millimetre. Each assay was completed three times, each with internal triplicates (n=9). Mean values (±SEM) are reported in this study. Ampicillin (10 µg) and vancomycin (5 µg) discs were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter discs infused with 10 µL of distilled water (containing 0.5% DMSO) were used as negative controls.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentration for each extract was determined using two methods. A liquid dilution MIC assay was employed as it is generally considered the most sensitive bacterial growth inhibitory assay.[18] Furthermore, as microplate liquid dilution (LD) MIC assays are perhaps the most commonly used method of quantifying bacterial growth inhibition efficacy, use of this method allows for comparisons with other studies. A solid phase agar disc diffusion (DD) assay was also used in this study as this bioassay was deemed to provide a closer representation of the environment and conditions relevant to solid axillary and foot skin systems.

Microplate liquid dilution MIC assay

The MICs of the extracts were evaluated by standard methods.[19,20] Briefly, overnight bacterial cultures were added dropwise to fresh liquid broth and the turbidity was visually adjusted to produce a McFarlands number 1 standard culture. This was subsequently

diluted 1 in 50 with fresh broth, resulting in the MIC assay inoculum culture. A volume of 100 µL sterile broth was added to all wells of a 96 well plate. Test extracts or control antibiotics (100 µL) were then added to the top row of each plate and 1 in 2 serial dilutions were prepared in each column of wells by transferring 100 µL from the top well to the next well in each column, etc. A growth control (without extract) and a sterile control (without inoculum) were included on each plate. A volume of 100 µL of bacterial culture inoculum was added to all wells except the sterile control wells. Plates inoculated with *B. linens, S. epidermidis* or *C. jeikeium* cultures were incubated aerobically at 37°C for 24 hr. Plates inoculated with *P. acnes* cultures were incubated under induced anaerobic conditions at 37°C for 72 hr. p-Iodonitrotetrazolium violet (INT) was obtained from Sigma, Australia and dissolved in sterile deionised water to prepare a 0.2 mg/mL INT solution. A 40 µL volume of this solution was added into all wells and the plates were incubated for a further 6 hr at 30°C. Following incubation, the MIC was visually determined as the lowest dose at which colour development was inhibited.

Disc diffusion MIC assay

The minimum inhibitory concentrations (MIC) of the extracts was also evaluated by disc diffusion assay as previously described.[21,22] Briefly, the *E. major* leaf extracts were diluted in deionised water (containing 0.5% DMSO) and tested across a range of concentrations. Discs were infused with 10 µL of the extract dilutions, allowed to dry and placed onto inoculated plates. The assay was achieved as outlined above and graphs of the zone of inhibition versus Ln concentration were plotted. Determination of MIC values were achieved using linear regression.

Toxicity screening

Reference toxin for toxicity screening

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

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water (0.5% DMSO) and qualitative phytochemical screenings of the *E. major* leaf extracts.

			Methanolic extract	Aqueous extract
Mass of extracted material (mg)			315	236
Concentration of resuspended extract (mg/mL)			32	24
Qualitative Phytochemical Tests	Phenols	Total phenols	+++	+++
		Water soluble phenols	+++	+++
		Insoluble phenols	+	-
	Saponins	Froth persistence	+	++
		Emulsion test	+	+
	Cardiac glycosides	Keller-Kiliani Test	-	-
	Tri-terpenoids	Salkowski Test	-	+
	Phyto-sterols	Acetic Anhydride Test	-	-
	Alkaloids	Meyer's Test	-	-
		Wagner's Test	-	-
		Draggendoff's Test	-	-
	Flavo-noids	Kumar Test	+++	+++
	Tannins	Ferric Chloride Test	+	-
		Lead Acetate Test	-	-
	Anthra-quinones	Free	-	-
		Combined	-	-

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

Artemia franciscana nauplii toxicity screening

Toxicity was assessed using a modified *Artemia franciscana* nauplii lethality assay.[23,24] Briefly, 400 µL of seawater containing ~47 (mean 47.1, *n*=125, SD 12.8) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. A volume of 400 µL of the reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25±1°C under artificial light (1000 Lux). For each plate, a 400 µL seawater negative control was run in triplicate. The wells were assessed at regular intervals and the number of dead counted. The nauplii were deemed dead if no movement of the appendages was observed within 10 sec. After 24 hr, all nauplii were sacrificed and counted to determine the total % mortality per well. The LC_{50} with 95% confidence limits for each treatment was calculated using probit analysis.

Statistical Analysis

Data is expressed as the mean±SEM of at least three independent experiments, each with internal triplicates (*n*=9).

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Dried *E. major* leaf powder (1 g) were extracted with methanol and water resulting in 315 mg and 236 mg yields respectively (Table 1). The dried extracts were resuspended in 10 mL of deionised water (containing 1% DMSO), resulting in the concentrations presented in Table 1. Qualitative phytochemical studies showed that both leaf extracts had similar phytochemical profiles. Both contained high levels of phenolic compounds and flavonoids, as well as moderate levels of saponins and low levels of triterpenoids.

Inhibition of bacterial growth

To determine the ability of the *E. major* leaf extracts to inhibit the growth of some bacterial species associated with the production of body and foot malodour, 10 µL of each extract was screened using a disc diffusion assay. *Corneybacterium jeikeium* growth was inhibited by both the methanolic and aqueous *E. major* leaf extracts (Figure 1). Whilst both extracts were good inhibitors of *C. jeikeium* growth, the methanolic *E. major* leaf extract was a slightly better growth inhibitor than the aqueous extract (as judged by zone of inhibition), with inhibition zones of 9.2 mM, compared to 8.5 mM for the aqueous extract. Notably, whilst the ampicillin (10 µg) and vancomycin (5 µg) controls produced slightly larger zones of inhibition (10.3 mM and 11.5 mM respectively) than the extracts, they consisted of relatively high doses of pure antibiotics. In contrast, the extracts are crude mixtures, which would contain many individual compounds. Thus, it is likely that the growth inhibitory activity of individual bioactive extract component(s) is/are particularly noteworthy. As *C. jeikeium* is responsible for the formation of strongest axillary malodour and also contributes to foot odour formation, the methanolic and aqueous *E. major*

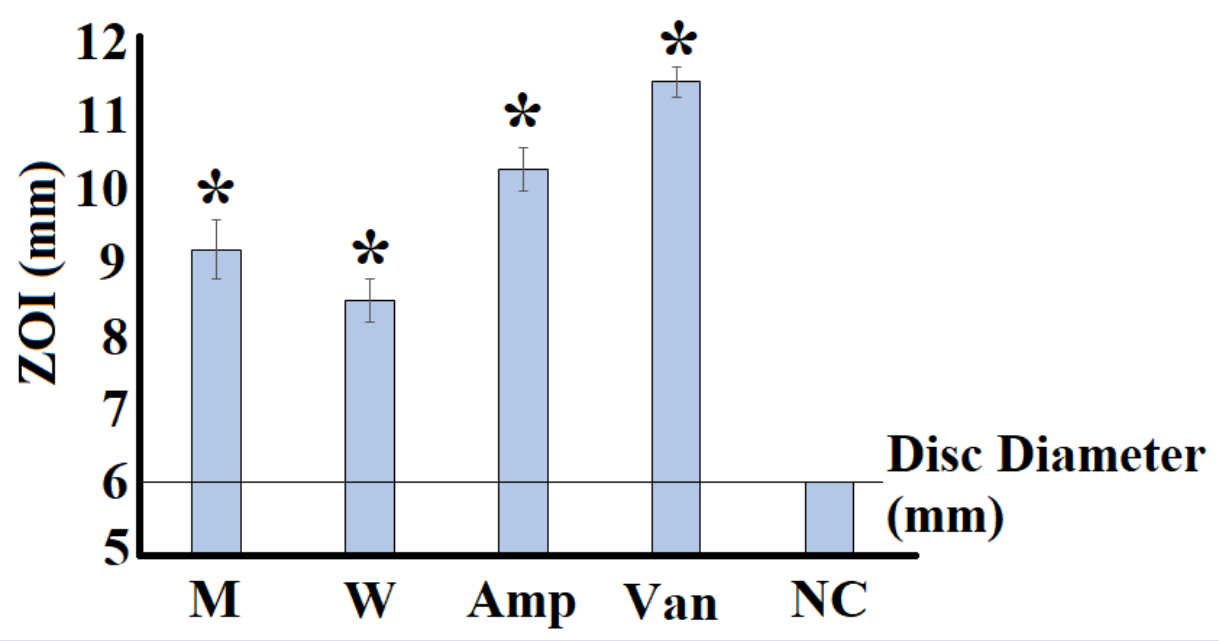

Figure 1: Growth inhibitory activity of the *E. major* leaf extracts against *Corneybacterium jeikeium* (ATCC 43734) measured as zones of inhibition (mm). M=methanolic extract; W=aqueous extract; Amp=ampicillin (10 µg); Van=vancomycin (5 µg); NC=negative control (0.5% DMSO). Results are expressed as mean zones of inhibition±SEM. * indicates results that are significantly different to the negative control ($p<0.05$).

leaf extracts have potential for use in odour mitigating personal hygiene products and further study is warranted.

Inhibition of *C. jeikeium* growth may result in less competition for other bacteria and the growth of other malodour producing bacteria may therefore increase. Thus, it would be beneficial for personal hygiene products aimed at reducing body odour to also inhibit the growth of the other odour forming bacteria. As *Staphylococcus* spp. and *Propionibacterium* spp. contribute significantly to axillary odour formation and *Propionibacterium* spp. and *Brevibacterium* spp. produce malodorous volatile compounds from foot sweat, an effective deodorant would also need to inhibit the growth of those bacteria. Notably, the methanolic and aqueous *E. major* leaf extracts also significantly inhibited the growth of *P. acnes* (Figure 2). Indeed, the both *E. major* leaf extracts produced ZOIs of approximately 8 mM (compared to approximately 10.6 and 11.2 mM for the ampicillin and vancomycin controls respectively).

Brevibacterium spp. are also major contributors to foot malodour.[1] Thus, the ability of the *E. major* leaf extracts to inhibit *B. linens* growth was also tested in this study (Figure 3). A similar growth inhibitory profile was seen to the other bacteria, with ZOIs of 8.4 and 7.6 mM measured for the methanolic and aqueous *E. major* leaf extracts respectively. Notably, these ZOIs were comparable to the ZOIs measured for the pure ampicillin and vancomycin controls, indicating that these extracts may also be useful in inhibiting the growth of this bacterium.

Notably, the growth of *S. epidermidis* was also highly susceptible to the methanolic and aqueous *E. major* leaf extracts (Figure 4). Consistent with the trend noted for the growth inhibition of the other bacteria screened, the methanolic extract (ZOI=9.7 mM) was a better inhibitor of *S. epidermidis* growth than the aqueous extract (ZOI=8.8 mM). The inhibition of *S. epidermidis* growth by the *E. major* leaf extracts was particularly noteworthy compared to the 7.6 mM growth inhibition of the ampicillin (10 µg) control and similar to the inhibition by the vancomycin control (ZOI=9.2 mM).

Quantification of minimum inhibitory concentration (MIC)

The relative level of antimicrobial activity was further evaluated by determining the MIC values (Table 2) for each extract against the bacteria which were shown to be susceptible in the disc diffusion screening assays. The results of the disc diffusion (DD) and liquid dilution (LD) MIC screening assays correlated relatively well, with similar MIC values generally recorded for both assay methods. Noteworthy antibacterial activity (LD MIC values substantially <1000 µg/mL) were calculated for both the methanolic and aqueous extracts against all of the bacteria tested. *Staphylococcus epidermidis* was the most susceptible bacteria to both extracts, with LD MIC values of 125 and 500 µg/mL respectively. The *E. major* leaf extracts were also potent inhibitors of *C. jeikeium* growth, with LD MIC values of 183 and 625 µg/mL for the methanolic and aqueous extracts respectively. Similar,

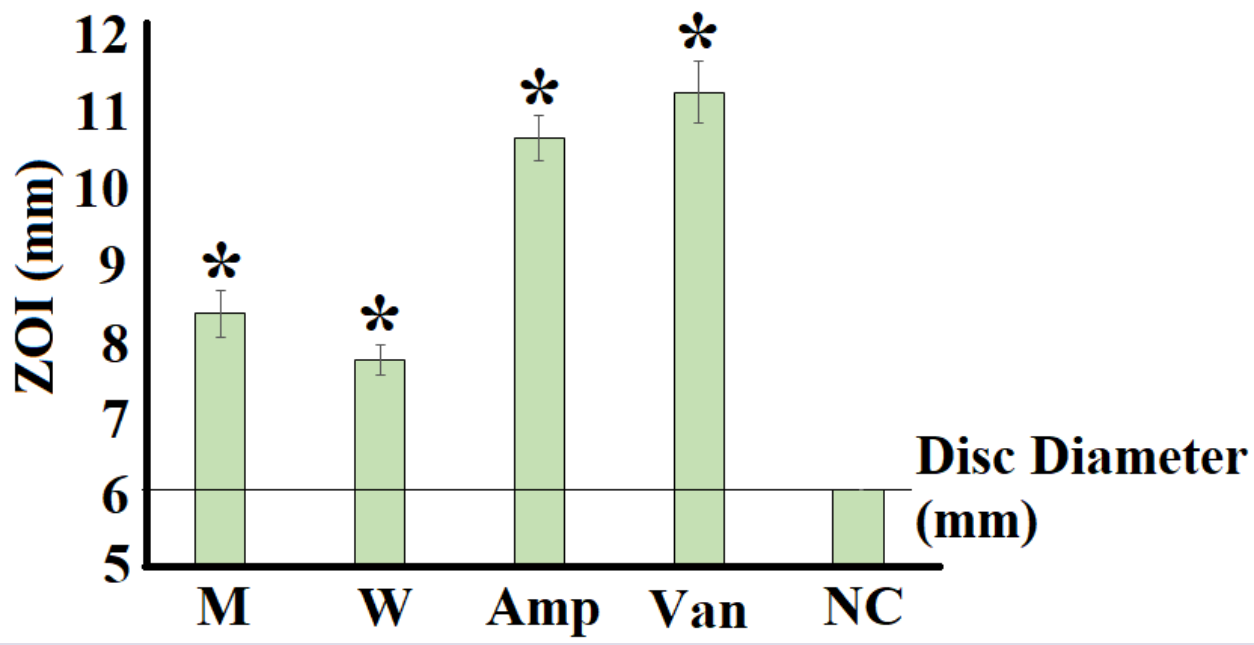

Figure 2: Growth inhibitory activity of the *E. major* leaf extracts against *Propionibacterium acnes* (ATCC 6919) measured as zones of inhibition (mm). M=Methanolic extract; W=Aqueous extract; Amp=Ampicillin (10 µg); Van=Vancomycin (5 µg); NC=Negative control (0.5% DMSO). Results are expressed as mean zones of inhibition±SEM. * indicates results that are significantly different to the negative control (p<0.05).

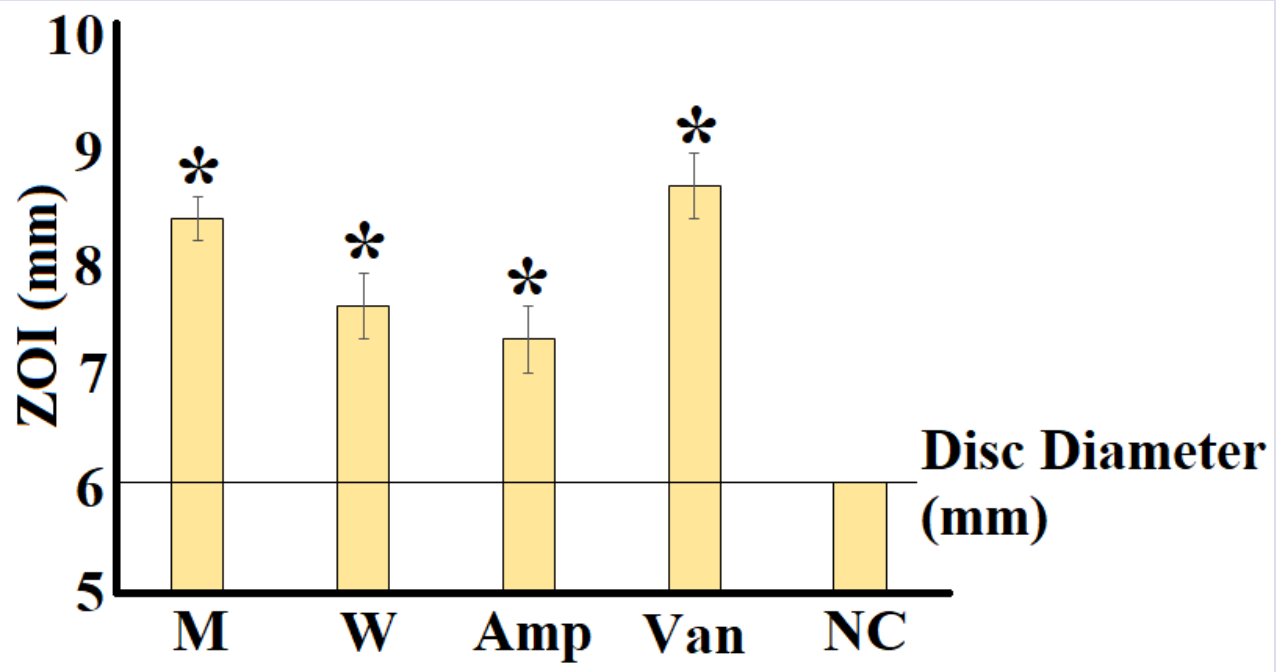

Figure 3: Growth inhibitory activity of the *E. major* leaf extracts against measured *Brevibacterium linens* (ATCC9172) as zones of inhibition (mm). M=Methanolic extract; W=Aqueous extract; Amp=Ampicillin (10 µg); Van=Vancomycin (5 µg); NC=Negative control (0.5% DMSO). Results are expressed as mean zones of inhibition±SEM. * indicates results that are significantly different to the negative control (p<0.05).

albeit slightly higher LD MIC values were determined for the *E. major* leaf extracts against *P. acnes* (LD MICs of 500 and 625 µg/mL for the methanolic and aqueous extracts respectively) and *B. linens* (LD MICs of 500 and 750 µg/mL for the methanolic and aqueous extracts respectively). Therefore, both the methanolic and aqueous *E. major* leaf extracts have noteworthy potential to ameliorate axillary malodour via the inhibition of *C. jeikeium, P. acnes, B. linens* and *S. epidermidis* growth.

Quantification of toxicity

All extracts were initially screened in the *Artemia* nauplii assay at 2000 µg/mL (Figure 5). Additionally, potassium dichromate was also tested in the bioassay as a reference toxin. The reference toxin was rapid in its onset of mortality, promoting nauplii death within the first 3h of exposure, with 100% mortality evident within 5 hr (unpublished results). In contrast, the methanolic and aqueous *E. major* leaf extracts both induced substantially less than 50% mortality (36 and 23% respectively) following 24 hr exposure. As 24 hr LC_{50} values>1000 µg/mL have previously been defined as nontoxic in this assay,[23,24] the methanolic and aqueous *E. major* leaf extracts were deemed to be nontoxic and their LC_{50} values were not determined.

DISCUSSION

Axillary odour and bromodosis (foot odour) have social consequences and may lead to social exclusion and mental health issues. Excessive sweating, which allows for odour formation, may also cause considerable damage to clothing and footwear items. Sweating produces fats and nutrients, which provide ideal growth conditions for many bacteria. *Corneybacterium* spp. possess enzymes to degrade protein and lipid sweat components to produce strongly malodorous volatile components.[25] *Staphylococcus* spp. possess similar (albeit less active) enzymes and are therefore capable of producing similar volatile compounds and thus also contribute to axillary malodour.[25] *Propionbacterium* spp. and *Brevibacterium* spp. (and to a lesser extent, *Corneybacterium* spp. and *Staphylococcus* spp.) are the main bacteria responsible for foot malodour production.[1] Body malodour formation may be controlled either by:

- Using chemical antiperspirants to reduce sweating, thereby depriving odour producing bacteria of the fuels to produce volatile malodorous compounds. Aluminium salts are commonly used in antiperspirant formulations to block eccrine sweat glands, despite being associated with numerous health issues.
- Direct inhibition/reduction of the bacteria that cause axillary and/or plantar malodours through the use of formulations, which most frequently contain propylene glycol, triclosan, benzalkonium chloride or metal (e.g. Al) salts. There are also serious concerns with the safety of several of these additives for long-term exposure.
- The use of masking agents (e.g. perfumes) to mask the malodour.

There is concern about the safety of many of the additives to antiperspirant/deodorants. Exposure to aluminium salts has

been linked with a wide range of negative health effects including degenerative neurological conditions (e.g. Alzheimers disease, encephalopathy)[6] and cancer.[3] Chronic exposure, as is the case with long term daily use in body odour management, may also result in structural and functional degradation of eccrine sweat ducts and the loss of secretory functionality.[26] Similarly, there is concern about including triclosan (and other deodorant compounds) in commercial deodorants as they have also been linked to a myriad of serious health problems.[4] Furthermore, triclosan persists for a relatively short period and is rapidly inactivated.[27] Therefore, there is a need to develop more effective antimicrobial deodorant components. As antiperspirants and deodorants are used at least daily by large section of the population, the potential for chronic and additive effects also needs to be considered and safer products are needed. Plant formulations are ideal candidates for the development of new deodorant compounds. Many plant extracts and oils have been used traditionally for hundreds (or even thousands) of years to inhibit bacterial growth and in some case their efficacy has been verified by rigorous scientific examination. Additionally, the use of natural alternatives to inhibit the growth of malodour forming bacteria may be more acceptable to consumers due to their natural origin and consumer perception of safety. This study examined the growth inhibitory properties of methanolic and aqueous *E. major* leaf extracts against several bacterial species associated with axillary and plantar malodour formation. *Eucalyptus major* leaf extracts was selected for this study as previous studies have reported good activity for plants of this genus against several human pathogens.[28-30] Additionally, *E. major* leaves are safe, readily available and are relatively inexpensive, making them attractive options for commercial formulations.

Our study confirmed the potential of *E. major* leaf extracts for inhibiting the growth of axillary and foot malodour producing bacteria. The methanolic leaf extract was the most promising growth inhibitor of all bacterial species. As *Corneybacterium* spp. have been reported to produce the most unpleasant and strongest

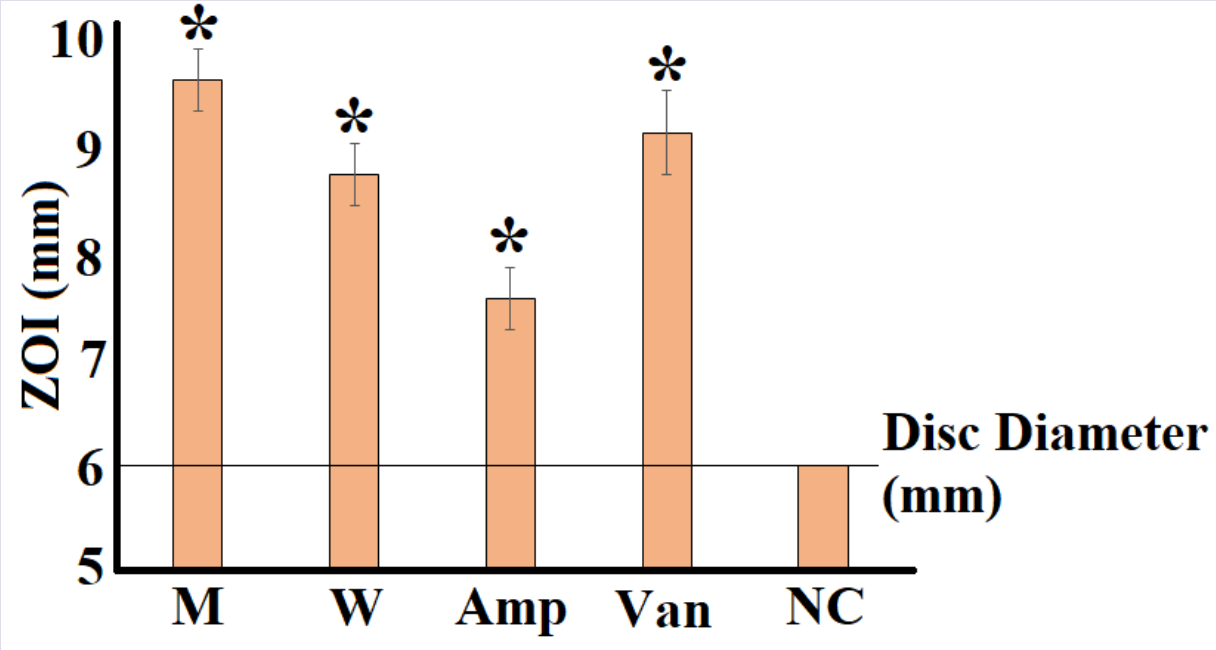

Figure 4: Growth inhibitory activity of the *E. major* leaf extracts against *Staphylococcus epidermidis* (clinical isolate) measured as zones of inhibition (mm). M=Methanolic extract; W=Aqueous extract; Amp=Ampicillin (10 µg); Van=Vancomycin (5 µg); NC=Negative control (0.5% DMSO). Results are expressed as mean zones of inhibition±SEM. * indicates results that are significantly different to the negative control (p<0.05).

Table 2: Disc diffusion and liquid dilution MICs against *C. jeikeium, P. acnes, B. linens* and *S. epidermidis*, growth (µg/mL) of the *E. major* leaf extracts.

Extract	MIC (µg/mL)							
	C. jeikeium		*P. acnes*		*B. linens*		*S. epidermidis*	
	DD	LD	DD	LD	DD	LD	DD	LD
M	250	183	500	500	1000	500	250	125
W	1238	625	2250	625	1846	750	1000	500

DD: Disc diffusion; LD: Liquid dilution; M=Methanolic extract; W=Aqueous extract; Numbers indicate the mean DD MIC and LD MIC values of triplicate determinations. - indicates no inhibition at any concentration tested.

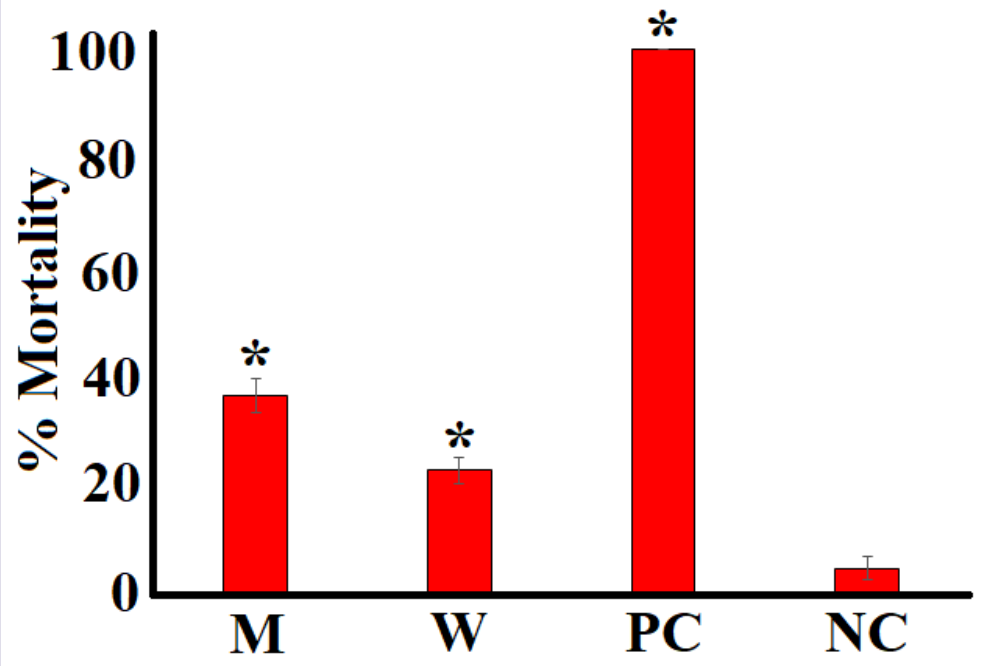

Figure 5: The lethality of the *E. major* leaf extracts (2000 µg/mL) and the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana nauplii* after 24 hr exposure. M=Methanolic extract; W=Aqueous extract; PC=Potassium dichromate control; NC=Negative (seawater) control. * indicates results that are significantly different to the negative control ($p<0.05$). Results are expressed as mean% mortality±SEM.

malodours,[31] the strong inhibition of *C. jeikeium* growth by the methanolic and aqueous *E. major* leaf extracts (LD MICs of 183 and 625 µg/mL) was particularly encouraging. Studies using extracts from other plants have reported comparable or considerably higher MIC values as signifying potent inhibitory activity. Inhibitory activity against *S. epidermidis* has been most extensively reported. Extracts produced from the Asian medicinal and edible plant *Caesalpinia minosoides* were reported to be "potent inhibitors" of *S. epidermidis* growth with an MIC value of 3130 ppm (equivalent to 3130 µg/mL).[32] In contrast, MICs of 125 and 500 µg/mL was determined for the methanolic and aqueous *E. major* leaf extracts in our studies. This represents approximately 25 and 6-fold increases in potency for the methanolic and aqueous extracts respectively compared to the *Caesalpinia minosoides* study. Similarly, *Cassia alata*,[33] *Barleria lupulina* and *Psidium guajava*[34] were reported to be moderate inhibitors of *S. epidermidis* growth, with MIC values equivalent to 2500-5000 µg/mL. *Hibiscus sabdariffa* and Eupatorium odoratum were reported to be potent *S. epidermidis* growth inhibitors, each with MICs equivalent to 625 µg/mL.[34] Indeed, on the basis of its *S. epidermidis* growth inhibitory properties, a *H. sabdariffa* deodorant formulation was the basis of for a US patent application.[35] The MIC value of the methanolic *E. major* leaf extract reported in our study had better potency compared to the *H. sabdariffa* formulation. Thus, the addition of methanolic *E. major* leaf extracts to deodorant preparations has commercial potential and should be explored further.

Examination of the growth inhibition of other malodour producing bacteria by herbal formulations has been less extensively reported. *Rubia tinctorium* (commonly known as madder) crude extracts were reported to be strong inhibitors of *Corneybacterium xerosis* growth.[36] However, that study is of limited value as a single, high dose of the extract was screened (approximately 500 µg/disc). Furthermore, MIC values were not determined, making a comparison with other studies impossible. Similarly, *Anethum graveolens* essential oils inhibited *Corneybacterium* spp. Growth.[37] However, MIC values were also not reported in that study, making a comparison difficult. Furthermore, that study utilised disc diffusion assays to test the growth inhibitory activity of the oil. Whilst disc diffusion assays are suitable for study of extracts, they are not recommended when testing oils for antibacterial activity due to the insolubility of the oils in the aqueous gel matrix. More recently, several studies have screened other plant extracts against *C. jeikeium* and have reported noteworthy growth inhibitory activity. Particularly good inhibitory activity was reported for *Terminalia ferdinandiana* Exell. extracts,[5] as well as other Australian, Indian and *African terminalia* spp.,[8] with MIC values of approximately 200 µg/mL against *C. jeikeium*. Similarly, low MIC values (200-1000 µg/mL) were reported for several *Australian syzygium* spp.,[5] whilst higher (but still noteworthy) MIC values (~1500 µg/mL) were reported for *Acronychia acidula* F. Muell. fruit extracts.[7]

Notably, the methanolic and aqueous *E. major* leaf extracts tested herein were both nontoxic towards *Artemia nauplii* and are thus

likely to be safe to use as for topical application as deodorants. However, further toxicity studies using human cell lines (and subsequent *in vivo* studies) are required to confirm the safety of these extracts before they are accepted as natural deodorant alternatives. Furthermore, whilst our study reported the *E. major* leaf extracts to be nontoxic, it is noteworthy that all of these studies have examined acute toxicity. Pharmacodynamic and pharmacokinetic studies are required to determine the ability of the extract components to cross the skin barrier, their duration in the blood stream prior to clearance and the urinary excretory products. As deodorants are applied frequently, such studies are required for any formulation to ensure that their components do not accumulate and cause chronic toxicity.

CONCLUSION

The results of this study demonstrate the potential of the *E. major* leaf extracts as natural antibacterial components for deodorant formulation. These extracts were potent growth inhibitors of all of the malodour forming bacteria tested. Furthermore, the lack of toxicity of the extract indicates its suitability for topical use.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

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

SUMMARY

• Methanolic and aqueous *Eucalyptus major* leaf extracts were screened for the ability to block the growth of a panel of axillary and plantar odour producing bacteria.

• The methanolic extracts were particularly good inhibitors of bacterial growth, (MIC values 180-500 µg/mL against *C. jeikeium, P. acnes, B. linens* and *S. epidermidis*).

• The aqueous was also a good growth inhibitor of all bacteria (MIC values 500-750 µg/mL).

• The toxicity of the *E. major* extracts was verified using the *Artemia nauplii* toxicity bioassay.

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