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Original Article

Growth Inhibitory Activity of Selected Australian *Syzygium* Species against Malodour Forming Bacteria

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ABSTRACT

Background: Extracts produced from *S. australe* and *S. luehmannii* fruit and leaves are potent growth inhibitors 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. **Methods:** *S. australe* and *S. luehmannii* fruit and leaf solvent extracts were investigated by disc diffusion assays against significant bacterial contributors to axillary and plantar malodour formation. Toxicity was determined using the *Artemia franciscana* nauplii bioassay. **Results:** *S. australe* and *S. luehmannii* solvent extracts were good inhibitors of *B. linens* and *C. jeikeium* growth, with zones of inhibition up to 10 mm measured. *S. australe* extracts were generally better inhibitors of both bacterial species compared with the *S. luehmannii* extracts. Ethyl acetate extracts were particularly potent, with MIC values of 300 and 857 µg/mL for the *S. australe* fruit and leaf extracts respectively against *B. linens*, and 1000 and 311 µg/mL against *C. jeikeium*. The *S. luehmannii* fruit ethyl acetate extracts were similarly potent growth inhibitors, with MIC values of 571 and 203 µg/mL against *B. linens* and *C. jeikeium* respectively. *S. australe* aqueous and methanolic leaf extracts were also potent inhibitors of *C. jeikeium* (MIC's of 285 and 306 µg/mL respectively). All other extracts had moderate or low inhibitory activity. All of the most potent ethyl acetate extracts were nontoxic in the *Artemia franciscana* bioassay. In contrast, the methanolic and aqueous *S. australe* leaf extracts, as well as the aqueous and methanolic *S. luehmannii* fruit extracts displayed apparent toxicity. However, these results may be fallacious and instead result from the high antioxidant content of these extracts. **Conclusion:** The potent growth inhibition of axillary and plantar malodour producing bacteria by the *Syzygium* spp. extracts indicate their potential as deodorant components.

Keywords: Body odour, Deodorant, *Corynebacterium*, Myrtaceae, Riberry, Brush cherry, High antioxidant, Medicinal plants.

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INTRODUCTION

Antiperspirants and deodorants are cosmetic products used to prevent and manage undesirable body odour and/or excessive sweating. Antiperspirants are designed to decrease sweat production, whereas deodorants inhibit the formation of body odour. Antiperspirants and deodorants are frequently combined and sold as a single product. These products are applied directly to the skin as aerosols, roll-ons, in a powdered form or as a gel. These products are used by over 90% of adult consumers in the United States and are one of the most widely used cosmetic products globally.[1] Sweating results in the production fats and other wastes which can serve as a nutrient source for natural bacterial flora. The undesirable odours associated with sweat production is a direct result of enzymatic breakdown of these compounds through bacterial interaction.[2]

Several well-known bacteria are associated with the production of noxious malodours, with the prevalence and distributions of these varying between different regions of the body. Three predominating genera of bacteria are responsible for malodour production within the axillary region of the body; *Corynebacterium*, *Propionibacterium* and *Staphylococcus.*[3,4] *Corynebacterium* spp. are generally considered to be the major contributors to axillary odour. Malodour manifestation occurs through the metabolism of short branched chain fatty acids, sulfanylalkanols and steroidal compounds to produce malodorous volatile compounds.[5] Similar processes occur in the foot region. However the major bacteria responsible for odour formation in this region belong to the genera *Brevibacterium* and *Propionibacterium.*[6]

Deodorants contain chemicals formulated to minimize growth of odour forming bacteria. The efficacy of these chemicals is dependent on the microbial community present. The chemicals may include triclosan, benzalkonium chloride, glycol or metal (especially aluminium) salts.[7] However, the potential health risks associated with several of these chemical additives are poorly studied and in some instances they have been linked with serious health issues. Some antiperspirant and deodorant components may induce a range of negative side effects including respiratory problems, anaphylactic shock and degenerative neurological disorders (encephalopathy and Alzheimer's disease).[8] There are also concerns of chronic toxicity associated with prolonged exposure to triclosan, as well as allergies and dermal irritation, although the concentrations required are not typically present in deodorants.[8] The antibacterial properties of numerous plants have long been recognized by many cultures and may be alternatives to chemically-formulated deodorants.

Syzygium is a large genus of evergreen flowering plants (family Myrtaceae) consisting of approximately 500 species.[9] Various species occur throughout the tropical and subtropical regions of South-East Asia, Australia and Africa. Many species produce edible fruits and berries with high antioxidant contents. In the commercially most important species *Syzygium aromaticum* (clove), the unopened flower bud is used as a spice. This plant also has uses in traditional medicine as an anaesthetic and for its antibacterial activity.[10] Numerous studies have reported on the ability of *S. aromaticum* oils to inhibit the growth of panels of bacteria[11] and fungi.[12] Indian species (*Syzygium lineare* and *Syzygium cumini*),[9] South East Asian species (*Syzygium jambos*),[13] and African species (*Syzygium cordatum*)[14, 15] also have potent antimicrobial activity. In contrast, much of our knowledge about the antiseptic properties of Australian *Syzygium* spp. is anecdotal, although Australian Aborigines are known to have used some species as medicinal agents.[9,16]

Despite their reported therapeutic uses, most recent interest in the Australian *Syzygium* spp. has focused on their high antioxidant capacities[17] and the potential correlation between antioxidants and chronic disease.[18] High antioxidant levels have also been shown to act as a preventative

against the development of some degenerative diseases including cancer,[19] cardiovascular diseases,[28] neural degeneration,[21] diabetes and obesity.[22] Furthermore, recent studies have also reported antibacterial activity in extracts from several other high antioxidant Australian plants.[23-26] Particularly noteworthy, the bacterial growth inhibitory properties of several Australia *Syzygium* spp. have been reported against a broad panel of bacteria.[16, 27, 28] In particular, high antioxidant *Syzygium australe* (J.C.Wendl. Ex Link) B. Hyland (bush cherry) and *Syzygium luehmannii* (F.Muell.) L.A.S.Johnson (riberry) extracts have been highlighted as potent bacterial growth inhibitors.[9,16] Despite the reported broad spectrum antibacterial activity of *S. australe* and *S. luehmannii* extracts, they are yet to be evaluated for their ability to inhibit the growth of odour forming bacteria. The current study was undertaken to test the ability of *S. australe* and *S. luehmannii* fruit and leaf extracts to inhibit the growth of bacteria associated with axillary and plantar malodour formation.

MATERIALS AND METHODS

Plant source and extraction

Syzygium australe (J.C.Wendl. Ex Link) B. Hyland and *Syzygium luehmannii* (F.Muell.) L.A.S. Johnson fruit and leaves were collected from verified trees in suburban regions of Brisbane, Australia. Voucher samples of plant specimens are deposited at the School of Natural Sciences, Griffith University. The materials were comprehensively desiccated in a Sunbeam food dehydrator and the dried materials were stored at -30°C. Prior to usage, the materials were thawed and ground into a coarse powder. Individual 1 g masses of the material were then weighed into separate tubes and 50 mL of deionised water, methanol, chloroform, hexane or ethyl acetate were added. All solvents used were analytical-reagent grade and were obtained from Ajax Chemicals, Australia. The ground plant materials were separately extracted in each solvent for 24 hours at 4°C with gentle shaking. The extracts were subsequently filtered through Whatman No. 54 filter paper under vacuum, followed by drying by rotary evaporation in an Eppendorf concentrator 5301. The resultant dried extracts were weighed and suspended in 10 mL sterilized deionised water containing 1 % DMSO.

Qualitative phytochemical studies

Phytochemical analysis of the extracts was completed as previously described[29,30] and used to determine the presence of phenolic compounds, anthraquinones, phytosterols, cardiac glycosides, tannins, saponins, flavonoids, alkaloids, and triterpenoids.

Antioxidant capacity

The antioxidant capacity of each sample was assessed using a modified DPPH free radical scavenging method.[31] Ascorbic acid (0-25 µg per well) was used as a reference and the absorbances were measured and recorded at 515 nm. All tests were completed alongside controls on each plate and all were performed 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 bacterial strains

All media and components was supplied by Oxoid Ltd., Australia. Reference strains of *Brevibacterium linens* (ATCC 9172) and *Corynebacterium jeikeium* (ATCC 43734) were acquired from American Type Culture Collection, USA. To culture the bacteria, *B. linens* were inoculated into separate flasks of nutrient broth and grown aerobically at 37 °C for 24 h. 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 h. All stock cultures were subcultured and maintained in liquid media at 4°C.

Evaluation of antibacterial activity

Antibacterial activity screening of the *S. australe* and *S. luehmannii* extracts was assessed using a modified disc diffusion assay [32, 33]. Briefly, 100 µL of each individual isolate 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 nutrient agar plates and the extracts were tested for antibacterial activity using 6 mm sterilised filter paper discs. *B. linens* cultures was spread onto nutrient agar plates and *C. jeikeium* cultures 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 h before incubation. Plates inoculated with *B. linens* or *C. jeikeium* cultures were incubated aerobically at 37 °C for 24 h. The diameters of the inhibition zones were measured to the closest whole millimetre. Each assay was completed in at least triplicate. Mean values (± SEM) are reported in this study. 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 were used as a negative control.

Minimum inhibitory concentration (MIC) determination

The minimum inhibitory concentrations (MIC) of each plant extract was determined as previously described.[34,35] Briefly, each individual plant extract was diluted in deionised water and tested across a decreasing concentration gradient. Discs were impregnated with 10 µL of the extract dilutions, allowed to dry and placed onto plates inoculated with *B. linens* or *C. jeikeium*. The assay was performed in triplicate as outlined above and graphs of the zone of inhibition versus concentration were plotted. Linear ln regression was utilized to determine MIC values.

Toxicity screening

Reference toxin for toxicity screening

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

Artemia franciscana nauplii toxicity screening

Toxicity of all extracts were determined using an adapted *Artemia franciscana* nauplii lethality assay.[36, 37] Briefly, 400 µL of seawater containing ~43 (mean 43.2, n = 155, SD 14.5) *A. franciscana* nauplii were added to wells of a 48 well plate and immediately used in the bioassay. Volumes of 400 µL of reference toxin or the diluted plant extracts were transferred to the wells and incubated at 25 ± 1 °C under artificial light (1000 Lux). Negative controls (400 µL seawater) and all test treatments were run in triplicate for each plate. The wells were observed at regular intervals and the number of dead were counted. The nauplii were classified as dead if no movement 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 is expressed as the mean ± SEM of at least three independent experiments.

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water, qualitative phytochemical screenings and antioxidant capacities of the *Syzygium* spp. extracts.

Plant	Extract	Mass of Dried Extract (mg)	Concentration of Resuspended Extract (mg/mL)	Antioxidant Capacity (mg Ascorbic Acid Equivalency)	Total Phenolics	Water Soluble Phenolics	Water Insoluble Phenolics	Cardiac Glycosides	Saponins	Triterpenes	Polysteroids	Alkaloids (Mayer Test)	Alkaloids (Wagner Test)	Flavonoids	Tannins	Free Anthraquinones	Combined Anthraquinones
S. australe	**FW**	240	24	40.7	+++	+++	++	-	+	-	-	-	-	+++	+	-	-
	FM	360	36	55.2	+++	+++	+++	-	-	+	-	-	-	+++	+	-	-
	FE	110	11	9.2	++	-	++	-	-	-	-	-	-	+	-	-	-
	LW	180	18	25.3	+++	+++	++	-	+	+	-	-	-	++	+	-	-
	LM	360	36	40.2	+++	+++	+++	-	+	+	-	-	-	+++	+	-	-
	LE	110	11	2.58	++	-	++	-	-	-	-	-	-	++	-	-	-
S. luehmannii	**FW**	120	12	59.2	+++	+++	++	-	+	+	-	-	-	+++	+	-	-
	FM	560	56	94.6	+++	+++	+++	-	+	+	-	-	-	+++	+	-	-
	FE	130	13	1.5	++	-	++	-	-	-	-	-	-	+	-	-	-

+ indicates a minor response; ++ indicates a moderate response; +++ indicates a major response. FW = aqueous fruit extract; FM = methanolic fruit extract; FE = ethyl acetate fruit extract; LW = aqueous leaf extract; LM = methanolic leaf extract; LE = ethyl acetate leaf extract. Antioxidant capacity was determined by DPPH reduction and is expressed as mg ascorbic acid equivalents per g of original plant material extracted.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

Extraction of the dried *Syzygium* spp. fruit and leaf materials (1 g) with various solvents yielded dried plant extracts ranging from 110 mg to 360 mg (*S. australe* fruit and leaf extracts) and 120 mg to 560 mg (*S. luehmannii* fruit extracts) (Table 1). Methanolic extracts provided significantly greater yields of extracted material relative to the aqueous and ethyl acetate counterparts, which provided only low to moderate yields. The dried extracts were resuspended in 10 mL of deionised water (containing 1 % DMSO), resulting in the concentrations presented in Table 1.

Antioxidant content

Antioxidant capacity for the plant extracts (Table 1) ranged from 1.5 mg (*S. luehmannii* fruit ethyl acetate extract) to a high of 55.2 mg ascorbic acid equivalence per gram of dried plant material extracted (*S. australe* fruit methanolic extract). The antioxidant capacities for the aqueous and methanolic extracts of each plant part were generally similar for both species and were substantially higher than the corresponding ethyl acetate extracts.

Antimicrobial activity

Brevibacterium linens

B. linens growth was inhibited by all of the *Syzygium* spp. extracts screened (100 %) (Figure 1). The *S. australe* extracts were generally more potent inhibitors of growth than the *S. luehmannii* extracts (as judged by zone of inhibition), with inhibition zones as high as 9.0 ± 0.6 mm (*S. australe* leaf methanolic extract). This compares favourably with the vancomycin (5 µg) control, with an inhibitory zone of 12.3 ± 0.6 mm. Indeed, all *Syzygium* spp. fruit and leaf extracts displayed inhibition zones ≥ 7 mm except for the aqueous *S. luehmannii* fruit extract.

Corynebacterium jeikeium

Bacterial growth was inhibited by 8 of 9 extracts screened (89 %) (Figure 1). The *S. australe* extracts were again the more potent inhibitors of bacterial growth than were the *S. luehmannii* extracts (as judged by zone of inhibition), with inhibitory zones as large as 10.0 ± 0.6 mm (*S. australe* fruit aqueous extract) and 9.6 ± 0.3 mm (*S. australe* fruit methanolic extract). In contrast, *S. luehmannii* extracts were less effective at inhibiting *C. jeikeium* growth, with smaller zones of inhibition of 6.3 ± 0.3 mm (*S. luehmannii* fruit methanolic extract) and 8.0 ± 1.2 mm (*S. luehmannii* fruit ethyl acetate extract) respectively.

Minimal inhibitory concentration determinations

The antimicrobial efficacy of the extracts was further quantified through the determination of MIC values (Table 2). The ethyl acetate fruit and

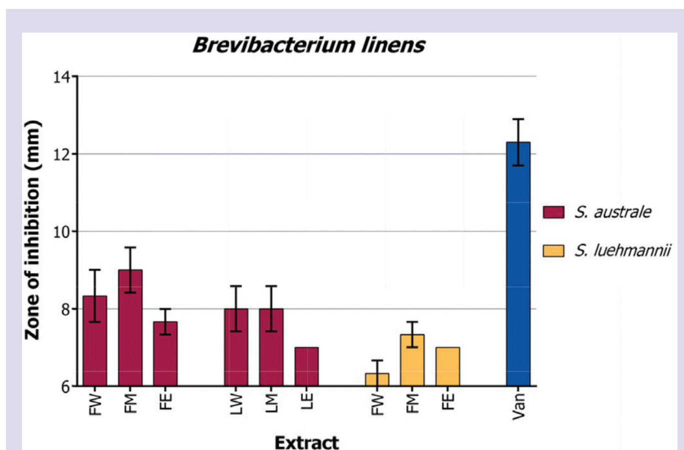

Figure 1: Growth inhibitory activity of *Syzygium* spp. plant extracts against the *B. linens* isolate measured as zones of inhibition (mm). FW = aqueous fruit extract; FM = methanolic fruit extract; FE = ethyl acetate fruit extract; LW = aqueous leaf extract; LM = methanolic leaf extract; LE = ethyl acetate leaf extract; Van = vancomycin (5 µg). Results are expressed as mean zones of inhibition ± SEM.

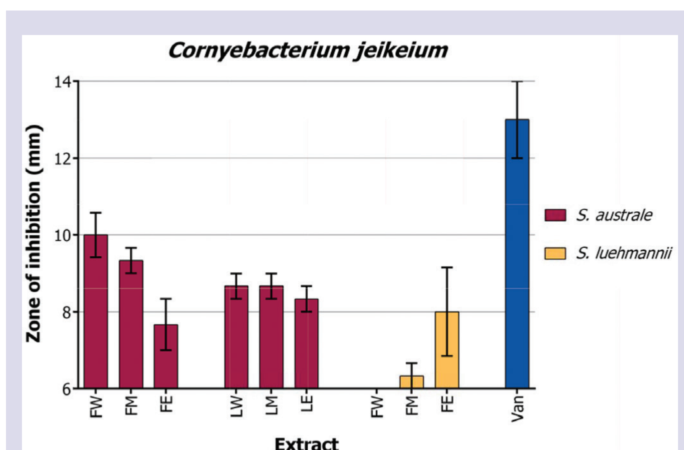

Figure 2: Growth inhibitory activity of *Syzygium* spp. plant extracts against the *C. jeikeium* isolate measured as zones of inhibition (mm). FW = aqueous fruit extract; FM = methanolic fruit extract; FE = ethyl acetate fruit extract; LW = aqueous leaf extract; LM = methanolic leaf extract; LE = ethyl acetate leaf extract; Van = vancomycin (5 µg). Results are expressed as mean zones of inhibition ± SEM.

leaf extracts of both *Syzygium* spp. were particularly effective at inhibiting *B. linens* and *C. jeikeium* growth, with MIC values generally substantially <1000 µg/mL (<10 µg infused into the disc). Similarly, the aqueous and methanolic *S. australe* leaf extracts were also good bacterial growth inhibitors, particularly against *C. jeikeium,* with MIC values of 285 and 306 µg/mL respectively (~3 µg infused into the disc) . These results compare well with the growth inhibitory activity of the vancomycin controls which were tested at 5 µg. All other extracts were of moderate or low efficacy in the assay.

Quantification of toxicity

The extracts were initially screened in the *Artemia* nauplii lethality assay at 2000 µg/mL (Figure 3). Potassium dichromate was used as a reference

Table 2: Minimum inhibitory concentration (µg/mL) of the *Syzygium* spp. extracts against *B. linens*, *C. jeikeium* and LC_{50} values (µg/mL) in the *Artemia* nauplii bioassay.

Plant	Extract	MIC (*B. linens*)	MIC (*C. jeikeium*)	LC_{50}
S. australe	FW	1,286	1,760	3,310
	FM	3,852	4,545	1,879
	FE	300	1,000	-
	LW	1,067	285	244
	LM	1,486	306	294
	LE	857	311	-
S. luehmannii	FW	>10,000	-	478
	FM	>10,000	>10,000	414
	FE	571	203	-
Controls	PC	ND	ND	186
	SW	ND	ND	-

Numbers indicate the mean MIC and LC_{50} values of triplicate determinations. - indicates no inhibition. FW = aqueous fruit extract; FM = methanolic fruit extract; FE = ethyl acetate fruit extract; LW = aqueous leaf extract; LM = methanolic leaf extract; LE = ethyl acetate leaf extract; PC = potassium dichromate control; SW = negative (seawater) control. ND = the indicated test was not performed.

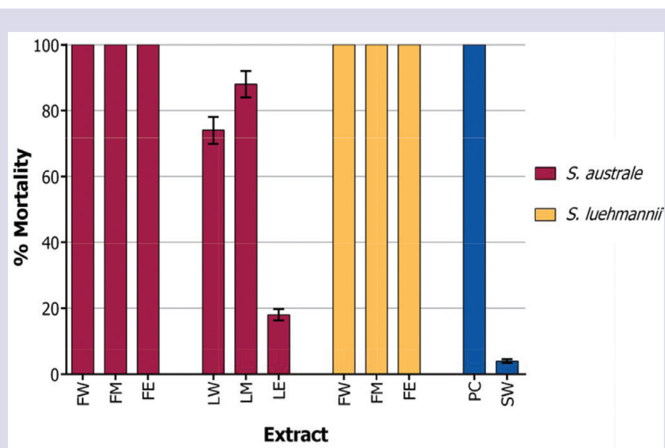

Figure 3: The lethality of the Australian plant extracts (2000 µg/mL) and the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana* nauplii after 24 hours exposure. FW = aqueous fruit extract; FM = methanolic fruit extract; FE = ethyl acetate fruit extract; LW = aqueous leaf extract; LM = methanolic leaf extract; LE = ethyl acetate leaf extract; PC = potassium dichromate control; SW = negative (seawater) control. Results are expressed as mean % mortality ± SEM.

toxin in the bioassay and was rapid in its onset of mortality, inducing nauplii death within the first 3 h of exposure and 100 % mortality was evident within 4-5 h (unpublished results). With the exception of the ethyl acetate leaf extracts of both species, all extracts showed > 50 % mortality rates at 24 h (Figure 3). To further quantify the effects of toxin concentration on *Artemia* nauplii mortality, the extracts were serially

diluted in artificial seawater to test across a series of concentrations in the *Artemia* nauplii bioassay. The 24 h LC_{50} values of the *Syzygium* spp. extracts towards *A. franciscana* are presented in Table 2. No LC_{50} values are reported for the fruit or leaf ethyl acetate extracts of either species as < 50 % mortality was seen in all tested concentrations. Extracts with an LC_{50} > 1000 µg/ml towards *Artemia* nauplii have been defined as being nontoxic.[38] The *S. australe* fruit methanolic and aqueous extracts both also had LC_{50} values substantially > 1000 µg/mL and were therefore deemed to be non-toxic. The methanolic and aqueous extracts of *S. australe* leaf and of *S. luehmannii* fruit displayed LC_{50} values substantially < 1000 µg/mL and were therefore considered to be toxic. This was a surprising result as *S. australe* and *S. luehmannii* fruit are considered nutritious, high antioxidant foods. However, it is noteworthy that the bioassay organism (*Artemia franciscana*) is sensitive to extreme pH ranges. Our study reported that all the extracts with LC_{50} values < 1000 µg/mL had relatively high antioxidant capacities. Furthermore, previous studies[35] reported that the high antioxidant capacities of these *Syzygium* spp. is largely due to their high ascorbic acid contents. Therefore, it is likely that the apparent toxicity reported in this study may be fallacious and a result of the extracts high ascorbic acid contents.

DISCUSSION

Body malodour (encompassing both axillary and foot odour) is directly related to the production and content of an individual's sweat. Sweating provides fats and nutrients which provide ideal growth conditions for many bacteria. *Corynebacterium* spp. possess enzymes to degrade protein and lipid sweat components to produce strongly malodorous volatile components.[39] *Staphylococcus* spp. possess similar (albeit less active) enzymes and are therefore capable of producing similar volatile compounds and thus also contribute to axillary malodour.[39] *Brevibacterium* spp. and *Propionibacterium* spp. (and to a lesser extent, *Corynebacterium* spp. and *Staphylococcus* spp.) are the main bacteria responsible for foot malodour production.[40] Current deodorant compositions seek to control axillary and plantar malodour in several ways:

- Inhibition/reduction of the bacteria which cause axillary and/or plantar malodours. Triclosan is currently the most common bacterial growth inhibitor in commercial deodorants. However, chronic triclosan exposure has been linked to a myriad of serious health problems.[41] Furthermore, triclosan persists for a relatively short period and is rapidly inactivated.[42] Therefore, there is a need to develop more effective antimicrobial deodorant components and recent studies have examined plant extracts for this purpose.[4]
- Antiperspirants reduce sweating, usually by mechanical obstruction of eccrine sweat ducts.[43] Aluminium salts are the most commonly used compound in antiperspirant formulations. However, there are concerns about chronic exposure to these chemicals and even acute exposure has been linked with a wide range of negative health effects including degenerative neurological conditions (e.g. Alzheimer's disease, encephalopathy)[44] and cancer.[45]
- Masking the malodour with other, more palatable aromas.

Due to the health concerns associated with some of the current antiperspirant/deodorant components, the development of safer formulations is required. These products are used at least daily and often more frequently by large section of the population. Thus the potential for chronic and additive effects on deodorant compounds need to be taken into account when formulating new products. Formulating products with greater functionality would allow for lower doses and thus lower health risks. The antibacterial compound triclosan is present in some products at up to 1% of the formulation (although 0.1-0.15 % is more common).[46] Triclosan is readily absorbed through the skin and conjugated forms have been reported to persist in the plasma for several days.[47] Indeed, the time required to clear 50% of a single triclosan dose in humans has been reported to be 96 h.[48] As deodorants are applied frequently and often as repeated applications, the implications and possibility for systemic accumulation and chronic toxicity are obvious.

There is a need to develop safer, more effective new deodorant compositions which address these concerns. Antibacterial plant formulations are ideal candidates for potential deodorant compounds. Plant extracts and oils have been used in a variety of traditional healing systems to inhibit the growth of bacterial pathogens. In many case their efficacy has been verified by rigorous scientific examination against those bacteria. It is possible that some of these may also inhibit the growth of malodour forming bacteria. Furthermore, natural components are desirable as deodorants as they may be more acceptable to consumers due to their natural origin and consumer perception of safety. This study examined the growth inhibitory properties of a *S. australe* and *S. luehmannii* leaf and fruit extracts against two of the major bacterial species associated with axillary and plantar malodour formation. *S. australe* and *S. luehmannii* were selected for this study for several reasons. These species have been used by the first Australians as antiseptics for thousands of years.[16] Throughout this long history of use, there has been a complete lack of toxicity reporting, attesting to the safety of these products for both consumption and topical application. Furthermore, recent laboratory studies have reported potent growth inhibitory activity for *S. australe* and *S. luehmannii* fruit.[27, 35, 49] and leaf extracts[35,50] against extensive microbial panels. The same studies also reported low toxicity for all extracts, further supporting their safety.

Our study confirmed the potential of *S. australe* and *S. luehmannii* fruit and leaf extracts for inhibiting the growth of axillary and foot malodour producing bacteria. The *S. australe* ethyl acetate leaf and fruit extracts and the *S. luehmannii* ethyl acetate fruit extract were the most promising growth inhibitors against both bacterial species. As *Corynebacterium* spp. have been reported to produce the most unpleasant and strongest malodours,[4] the potent inhibition of *C. jeikeium* by these extracts was particularly encouraging. Despite *Corynebacterium* spp. being the most significant contributors to body malodour formation, the inhibitory activity against *S. epidermidis* has been most extensively reported. Extracts produced from the Asian medicinal and edible plant *Caesalpinia mimosoides* were reported to be inhibitors of *S. epidermidis* growth with an MIC value of 3130ppm (equivalent to 3130 µg/mL).[51] Similarly, *Cassia alata*[52], *Barleria lupulina* and *Psidium guajava*[53] were 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.[53] On the basis of its *S. epidermidis* growth inhibitory properties, a *H. sabdariffa* deodorant formulation was the basis for a US patent application.[54]

Examination of the growth inhibition of other malodour producing bacteria by herbal formulations has been less extensively reported. *Rubia tinctorum* (commonly known as madder) crude extracts were reported to be strong inhibitors of *Corynebacterium xerosis* growth.[55] 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 *Corynebacterium* spp. Growth.[56] However, MIC values were not reported within this study, making a comparison difficult. Furthermore, that study utilised a disc diffusion assay to test the growth inhibitory activity of the oil. Whilst disc diffusion assays are suited to the 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. Another study reported potent inhibition of *C. xerosis* and *S. epidermidis* growth by a supercritical *Humulus*

lupulus (commonly known as hops) extract.[57] The authors reported 15 mm zones of inhibition for a 2 mg/mL *H. lupulus* extract using a disc diffusion assay. It is difficult to compare the MIC quantification results reported in that study to those in our study due to the use of different assay methods. However, a direct comparison of the disc diffusion studies is possible. Our study screened substantially lower doses in the disc diffusion assay compared to the *H. lupulus* study, yet recorded similar inhibition zones. Thus, it is likely that the potency of the methanolic *S. australe* aqueous, methanolic and ethyl acetate leaf extracts and the ethyl acetate *S. luehmannii* extract compare favourably with the *H. lupulus* extract.

This study focussed on the antibacterial deodorant properties of the *S. australe* and *S. luehmannii* extracts. However, other biological properties not examined in our study may also provide further protection against malodour production. Products that inhibit perspiration would also limit the secretion of the protein and lipid sweat components required as energy sources for bacterial growth. Thus, if these extracts were subsequently found to also have antiperspirant activity (in conjunction with the deodorant activity), they may be especially useful for a deodorant formulation as they would have dual functions.

The majority of the *Syzygium* spp. extracts examined in our study displayed significant toxicity (<1000 μg/mL) in the *Artemia* nauplii bioassay. Indeed, the aqueous and methanolic *S. australe* leaf extracts and the aqueous and methanolic *S. luehmannii* fruit extracts had LC_{50} values <500 μg/mL, indicating moderate to high toxicity. This toxicity would impact on the usefulness of these extracts as deodorant additives. Similar extracts prepared using these species have previously been reported to have high ascorbic acid levels in other studies.[17] Whilst these ascorbic acid levels may have beneficial therapeutic effects, they may also be responsible (at least in part) for the toxicity reported here. Whilst *A. franciscana* have generally been reported to be a robust and hardy organism for toxicity screening, they are susceptible to pH changes.[58] The levels of ascorbic acid previously reported in *S. australe* and *S. luehmannii* extracts[17] would be expected to have a significant impact on the pH of the seawater in the tests and this change may be responsible for the mortality induction reported in our study. Indeed, studies in our laboratory have shown that testing pure ascorbic acid in the concentrations previously reported to be in these extracts results in mortality similar to that reported in our study (unpublished results). Pharmacodynamics and pharmacokinetics studies are also 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.

CONCLUSIONS

The results of this study demonstrate the potential of the *S. australe* and *S. luehmannii* methanolic leaf extracts as a natural antibacterial components for deodorant formulation. These extracts were potent growth inhibitors of the major malodour forming bacterial species *B. linens* and *C. jeikeium*.

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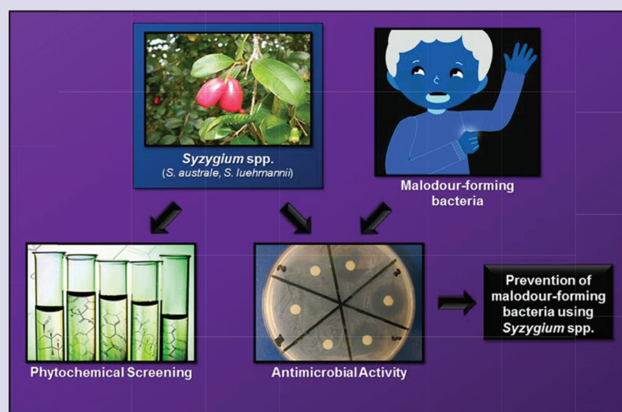
PICTORIAL ABSTRACT



SUMMARY

- Ethyl acetate *S. australe* leaf and fruit and *S. luehmannii* fruit extracts were potent inhibitors of *B. linens* and *C. jeikeium* growth.
- MIC values of 300 and 1000 µg/mL were determined for *S. australe* fruit ethyl acetate extract against *B. linens* and *C. jeikeium* respectively.
- Ethyl acetate extracts of *S. australe* leaf (857 and 311 µg/mL against *B. linens* and *C. jeikeium* respectively) and *S. luehmannii* fruit (571 and 203 µg/mL against *B. linens* and *C. jeikeium*) were similarly potent.
- *S. australe* aqueous and methanolic leaf extracts were also potent inhibitors of *C. jeikeium* (MIC's of 285 and 306 µg/mL respectively).
- All other extracts had moderate or low inhibitory activity.
- All ethyl acetate extracts were nontoxic in the *Artemia franciscana* bioassay

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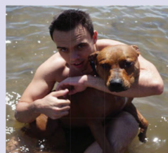
Mr. Aiden Wood completed his Biomolecular Science (Hons.) degree at the end of 2015. His thesis was investigated the potential application of Crispr/Cas9 to model inherited forms of Parkinson's disease. Currently he is investigating the antibacterial potential of Australian native plants traditionally used in Aboriginal medicine. He is proficient in a wide range of molecular and microbial techniques.

Mr Kane McManus obtained his Bachelor of Science with Honours in 2015 for his work investigating bacterial-metal interactions of contaminated suburban creeks. Since completing this project, his research has largely focused on the commercial aspects of native Australian plants, specifically the utilization of natural antibacterial agents in the prevention of malodour-forming bacteria. Kane is currently working within the medical field while he assesses his Ph.D. options both domestically and abroad.



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Dr Ian Edwin 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 scientific publications in a variety of peer reviewed journals.