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Original Article

Solanum chippendalei Symon, *Citrus glauca* (Lindl.) Burkill, *Ficus racemosa* L. and *Melaleuca alternifolia* Cheel Inhibit Axillary and Foot Odour Producing Bacteria

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ABSTRACT

Introduction: Plant-based preparations have potential to replace synthetic antiperspirant/deodorant additives as growth inhibitors of malodour producing bacteria and may thus be useful deodorant components. However, relatively few plant species have yet been tested against the bacteria. **Materials and Methods:** Methanolic *S. chippendalei* and *C. glauca* fruit extracts, as well as *F. racemosa* and *M. alternifolia* 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:** All of the tested extracts inhibited the growth of two or more bacteria, although the MIC values measured for the *C. glauca* fruit and *F. racemosa* leaf extracts indicated only weak antibacterial activity. In contrast, the *M. alternifolia* leaf extract displayed noteworthy growth inhibitory activity against all of the malodour forming bacteria tested, with liquid dilution (LD) MIC values of 1667, 1230, 1250 and 2720 µg/mL against *C. jeikeium, C. acnes, B. linens* and *S. epidermidis* respectively. Interestingly, the *S. chippendalei* fruit extract was the strongest inhibitor of *C. jeikeium* growth (MIC=1070 µg/mL), although it was substantially less potent against the other bacterial species. All extracts were nontoxic in the *Artemia fransiscana* bioassay. **Conclusion:** The lack of toxicity of the *S. chippendalei, C. glauca, F. racemosa* and *M. alternifolia* extracts and growth inhibition of axillary and plantar malodour producing bacteria by the *S. chippendalei* fruit and *M. alternifolia* leaf extracts indicate their potential as deodorant components. Further studies are warranted to isolate and identify the active components.

Keywords: Australian plants, Body odour, Deodorant, *Corneybacterium jeikeium*, *Propionobacter acnes*, *Brevibacter linens*, *Staphylococcus epidermidis*.

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INTRODUCTION

Excessive body malodour has substantial social consequences that may result in social exclusion and mental health issues. For this reason, antiperspirants and deodorants account for one of the largest sectors of the cosmetics market. To decrease body odour formation, many products include synthetic compounds, including metal (e.g. Al) salts to decrease perspiration, as well as propylene glycol, triclosan, benzalkonium chloride, which inhibit the growth of skin bacteria and thereby decrease odour formation.[1] The regulation of antiperspirant/deodorant additives is substantially less rigorous than for pharmaceuticals and the safety of many of these additives has not yet to be completely verified. Indeed, several of these additives are hazardous to human health. Aluminium salts have been linked with several neuro-degenerative conditions (e.g. Alzheimers disease, encephalopathy)[2] and cancer,[3] and may also cause respiratory problems and induce anaphylactic shock in susceptible individuals.[2] Additionally, chronic exposure to triclosan, which is included in many deodorants as an inhibitor of bacterial growth, has been reported to cause dermal irritation and allergies in some people.[4] Natural deodorant additives that inhibit the growth of body odour producing bacteria may have less impact on human health and be more acceptable to consumers due to their natural origin. Several recent studies have searched plants for safe and effective preparations that may replace the synthetic additives, with several promising plant extracts already identified.[5-8]

Eccrine gland secretions (perspiration) contain fats and nutrients that provide energy sources for many bacteria. Bacteria in axillary region degrade proteins and lipids in the eccrine secretions to produce malodorous volatile components.[1,9] The

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most significant bacterial contributors to malodour formation in the axillary region are *Corneybacterium jeikeium, Staphylococcus* spp. and *Cutibacterium acnes* (formerly *Propionobacter acnes*). Whilst these bacteria also convert plantar sweat into volatile malodourous compounds, *Cutibacterium* spp. and *Brevibacterium linens* are the major microbial contributors to foot odour.[1] Therefore, we selected *Corneybacterium jeikeium, Cutibacterium acnes, Staphylococcus epidermidis* and *Brevibacterium linens* for screening for growth inhibitory activity as their inhibition indicates potential deodorant properties.

Despite the interest in developing natural deodorant additives from plants, relatively few Australian plants have been screened for activity against malodour forming bacteria. We selected four native Australian plants to screen in this study, based on their high antioxidant contents and/or their inhibitory activity against other bacterial species. Recent studies have reported exceptionally high antioxidant content of the fruits *Solanum chippendalei* Symon (commonly known as bush tomato; Figures 1a, 1b and 1c) and *Citrus glauca* (Lindl.) Burkill (commonly known as desert lime; Figures 1d, 1e, 1f).[10-13] Furthermore, multiple studies have examined the antibacterial properties of high antioxidant plant extracts and have reported strong inhibitory activity for several species,[14-18] including methicillin-resistant (MRSA) bacteria.[19] Recent studies have also reported substantial growth inhibitory activity for *S. chippendalei* and *C. glauca* extracts against several bacterial pathogens,[20] although they have not neen tested against malodour producing bacteria. *Ficus racemosa* L. and *Melaleuca alternifolia* Cheel were also selected to screen against the panel of malodour forming bacteria as they have previously been reported to inhibit the growth of several other pathogenic bacteria.[21,22] This study aimed to test extracts prepared from selected Australian native plants against the axillary and foot odour forming bacteria *Corneybacterium jeikeium, Cutibacterium acnes* and *Brevibacterium linens* and *Staphylococcus epidermidis* with the aim of identifying safe and effective deodorant components.

MATERIALS AND METHODS

Plant source and extraction

Solanum chippendalei Symon, *Citrus glauca* (Lindl.) Burkill fruit were purchased from Taste Australia Bush Food, Australia. *Ficus racemosa* L. and *Melaleuca alternifolia* Cheel leaves were obtained from and identified by Philip Cameron, senior botanic officer, Mt Cootha Botanical Gardens, Brisbane, Australia. All plant materials were washed in deionised water and thoroughly dried using a Sunbeam food dehydrator. The dried plant material was stored at -30°C until use. Voucher samples are stored in the School of Environment and Science, Griffith University. Prior to extraction, the dried plant materials were thawed and ground to a coarse powder. Individual 1 g quantities of the each powder were weighed into individual tubes and 50 mL of methanol (AR grade, Ajax Fine Chemicals, Australia) was added and extracted for 24°C with gentle shaking. The extracts were subsequently filtered through filter paper (Whatman No. 54) under vacuum, and dried by evaporation in a vacuum oven at 50°C. The dried extracts were weighed to determine extraction yield, 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 plant extracts for the presence of cardiac glycosides, alkaloids, saponins, tannins, flavonoids, phenolic compounds, phytosterols, flavonoids and triterpenoids was achieved as previously described.[14-16]

Antibacterial screening

Test bacterial strains

All bacterial growth media was purchased from Oxoid Ltd., Australia. Reference strains of *Brevibacterium linens* (ATCC9172), *Corneybacterium jeikeium* (ATCC43734) and *Cutibacterium 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. The *B. linens* and *S. epidermidis* strains were inoculated into separate flasks containing nutrient broth and grown aerobically at 37°C for 24 hr. *Corneybacterium jeikeium* was cultured in nutrient broth supplemented with 300 µL Tween 80/L of broth at 37 °C for 24 hr. *Cutibacterium acnes* was cultured in thioglycollate liquid media in anaerobic jars and AnaeroGen™ 3.5 L atmospheric generation systems (Thermo Scientific) to maintain induced anaerobic conditions. The *C. acnes* cultures were grown at 37°C for 72 hr.

Antibacterial activity screening

The plant extracts were initially screened for antibacterial activity using a modified disc diffusion assay.[23,24] Briefly, 100 µL of each individual bacterial cultures (10^8 cells/mL) was spread onto separate agar plates to test for antibacterial activity using 5 mm sterilised filter paper discs. The *B. linens, S. epidermidis* and *C. acnes* cultures were individually spread onto nutrient agar plates. The *C. jeikeium* culture was spread onto nutrient agar plates supplemented with 300 µL Tween 80/L of agar. Filter paper discs (5mm diameter) were infused with 10 µL of the individual extracts, allowed to dry and placed onto the surface of the inoculated plates. Following 2 hr incubation at 4°C to allow bacteria to settle into the agar, the plates were inoculated with the individual cultures. The plates inoculated with *B. linens, S. epidermidis* or *C. jeikeium* cultures were incubated aerobically at 37°C for 24 hr, whilst the plates spread with *C. acnes* cultures were incubated under induced anaerobic conditions at 37°C for 72 hr. Following the incubation period, the diameters of the Zones of

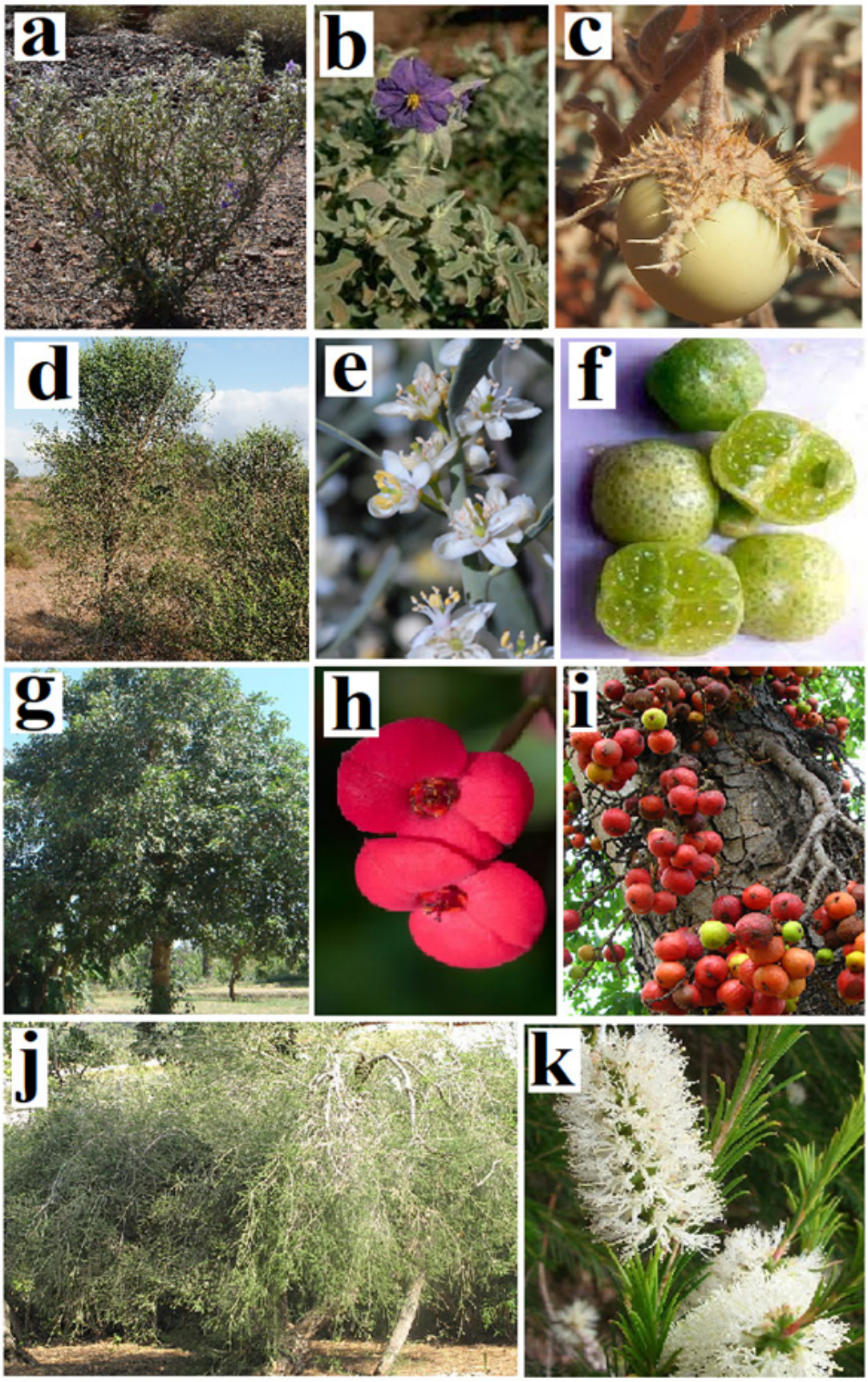

Figure 1: (a) *Solanum chippendalei* whole plant; (b) *S. chippendalei* flower and leaves; (c) *S. chippendalei* fruit, (d) *Citrus glauca* whole plant; (e) *C. glauca* flowers; (f) *C. glauca* fruit; (g) *Ficus racemosa* tree; (h) *F. racemosa* flowers; (i) *F. racemosa* fruit; (j) *Melaleuca alternifolia* tree; and (k) *M. alternifolia* flowers.

Inhibition (ZOIs) were measured to the closest whole millimetres. Each extract was assessed in three independent experiments, each with internal triplicates (n=9) and expressed as mean ZOIs (±SEM). Ampicillin (10 µg) and vancomycin (5 µg) discs were obtained from Oxoid Ltd., Australia and used as positive controls to compare antibacterial activity. Filter paper discs infused with 10 µL of distilled water (containing 0.5% DMSO) were included on each agar plate as negative controls.

Minimum inhibitory concentration determination

The minimum inhibitory concentration (MIC) of each extract was determined using two methods: The liquid dilution MIC assay was used as it is generally considered the most sensitive bacterial growth inhibitory assay and because it is one of the most commonly used method of quantifying bacterial growth inhibition efficacy, allowing for comparisons with other studies. MIC values were also determined using solid phase agar disc diffusion (DD) assays as this bioassay provides 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.[25,26] Briefly, McFarlands 0.5 standard cultures were freshly prepared and for use in the assay. A 100 µL volume of sterile nutrient broth was added to all wells of a 96 well plate. The plant extracts or control antibiotics (100 µL) were individually added to the top row of each plate and diluted using 1 in 2 serial dilutions 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. The bacterial culture inoculum to be tested (100 µL) was then added to all wells on the plate except the sterile control wells. The plates that were inoculated with *B. linens, S. epidermidis* or *C. jeikeium* were incubated aerobically at 37°C for 24 hr, whilst the plates inoculated with *P. acnes* cultures were incubated under induced anaerobic conditions at 37°C for 72 hr. *p*-Iodonitrotetrazolium violet (INT) was purchased from Sigma, Australia and dissolved in sterile deionised water to prepare a 0.2 mg/mL 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 to allow for colour development. The MIC of each test was visually determined as the lowest concentration inhibited that inhibited colour development.

Disc diffusion MIC assay

The MICs of the plant extracts were also evaluated using standard disc diffusion assays across a range of concentrations.[23,24] The assay was achieved as outlined above for the bacterial susceptibility screening and graphs of the zone of inhibition versus Ln concentration were plotted. Determination of MIC values were achieved using linear regression.

Artemia franciscana nauplii toxicity evaluation

The toxicity of the plant extracts was assessed using a modified *Artemia franciscana* nauplii lethality assay.[27-29] Briefly, 400 µL of seawater containing ~56 (mean 55.8, SEM 7.3, n=288) *A. franciscana* nauplii were added to all wells of a 48 well plate and 400 µL of the reference toxin potassium dichromate (AR grade, Chem-Supply, Australia) at an assay concentration of 1000 µg/mL, or the diluted plant extracts were transferred to the wells and incubated at 25±1°C under artificial light (1000 Lux). Additionally, 400 µL of artificial seawater was added in triplicate on each plate as a negative control. The wells were assessed at regular intervals and the number of dead were counted. The nauplii were deemed dead if no movement of the appendages was observed within 10 sec. After 24 hr, all nauplii were sacrificed by adding a drop of acetic acid and the nauplii were 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) unless otherwise stated.

RESULTS

Liquid extraction yields and qualitative phytochemical screening

1 g masses of the dried plant powders were extracted individually with methanol, resulting in 235, 184, 202 and 250 mg yields for the *S. chippendalei, C. glauca, F. racemosa* and *M. alternifolia* extracts respectively (Table 1). The dried extracts were resuspended in 10 mL of deionised water (containing 0.5% DMSO), resulting in the concentrations presented in Table 1. Qualitative phytochemical studies showed that all of the tested extracts were relatively rich in flavonoids and contained lower levels of triterpenoids. The *F. racemosa* and *M. alternifolia* extracts also contained low to moderate amounts of tannins and saponins. All other phytochemical classes were either absent in the extracts, or were below the detection threshold in the qualitative phytochemical assays.

Inhibition of bacterial growth

As an initial screen of the ability to inhibit the growth of some bacteria associated with body and foot malodour, 10 µL volumes of each extract were screened by disc diffusion assays. *Corneybacterium jeikeium* growth was inhibited by the *Schippendalei, C. glauca* and *M. alternifolia* extracts (Figure 2). In contrast, the *F. racemosa* extract was completely ineffective against *C. jeikeium* growth. The *S. chippendalei* and *M. alternifolia* extracts showed the strongest apparent *C. jeikeium* growth inhibitory activity, with ZOIs of 7.7 and 7.4 mm respectively. In contrast, the ampicillin (10 µg) and vancomycin (5 µg) control antibiotics

produced larger ZOIs (8.8 and 10.2 mm respectively). However, it is noteworthy that the control, antibiotic discs contained relatively high doses of pure antibiotics, whilst the extracts are crude mixtures, which would contain many individual compounds, of which the antibacterial components may only account for a small % of the extract. Thus, the growth inhibitory activity of bioactive extract component(s) may be substantially greater than the apparent activity seen in these assays. As *C. jeikeium* has the greatest contribution to axillary malodour of the tested bacteria (and a minor contributor to foot odour), the methanolic and aqueous *S. chippendalei* and *M. alternifolia* extracts may be useful as deodorant components for mitigating body odour formation, and further study is warranted.

As inhibiting the growth of *C. jeikeium* growth would decrease competition with other skin bacteria, their growth may increase and have a greater contribution to body malodour formation. Indeed, *Staphylococcus* spp. and *Cutibacterium* spp. are also significant causes of axillary odour formation. Additionally, *Cutibacterium* spp. and *Brevibacterium* spp. are significant contributors to plantar malodour formation. Notably, all of the plant extracts tested in this study inhibited the growth of *C. acnes* (Figure 3), although the *M. alternifolia* extract had the greatest apparent inhibitory activity, producing ZOIs of approximately 8.2 mm (compared to approximately 9.4 and 10.4 mm for the ampicillin and vancomycin controls respectively).

Several other bacteria, including *Brevibacterium* spp., use plantar sweat to produce the volatile compounds responsible for foot malodour.[1] Thus, the ability of the selected plant extracts to inhibit the growth of *B. linens* was also screened in this study (Figure 4). A similar growth inhibitory profile was seen as noted for the other bacteria, with ZOIs of 7.3, 7.8, 6.7 and 7.9 mm measured for the *S. chippendalei, C. glauca, F. racemosa* and *M. alternifolia* extracts respectively. Notably, these ZOIs were comparable to the ZOIs measured for the pure ampicillin and vancomycin controls (6.4 and 7.9 mm respectively), indicating that all of these extracts may be useful in inhibiting the growth of this bacterium.

Table 1: The mass of dried extracted material, the concentration after resuspension in deionised water (0.5% DMSO) and qualitative phytochemical screenings of the selected plants.

	Mass dried extract (mg)	Extract concentration (mg/mL)	Qualitative phytochemical tests						
			Cardiac glycosides	Saponins	Triterpenoids	Phytosterols	Alkaloids	Flavonoids	Tannins
SC	235	23.5	-	-	+	-	-	++	-
CG	184	18.4	-	-	+	-	-	+++	-
FR	202	20.2	-	-	+	-	-	++	+
MA	250	25	-	++	++	-	-	++	++

SC=*S chippendalei* extract; CG=*C. glauca* extract; FR=*F. racemosa* extract; MA=*M. alternifolia* extract; +++ indicates a large response; ++ indicates a moderate response; + indicates a minor response; - indicates no response in the assay.

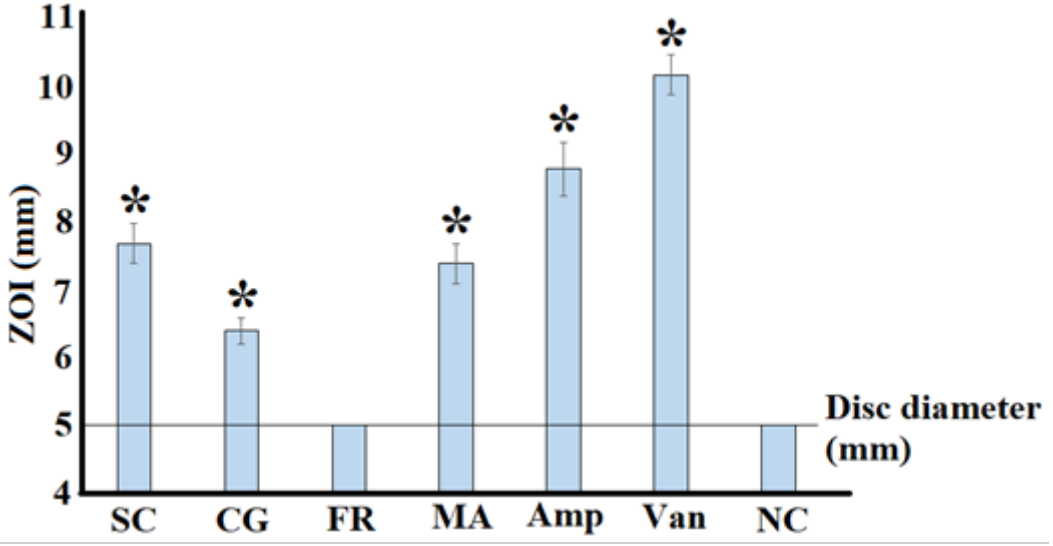

Figure 2: Growth inhibitory activity of the plant extracts against *Corneybacterium jeikeium* (ATCC 43734) measured as zones of inhibition (mm). SC=*S. chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf extract; Amp=ampicillin (10 µg); 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).

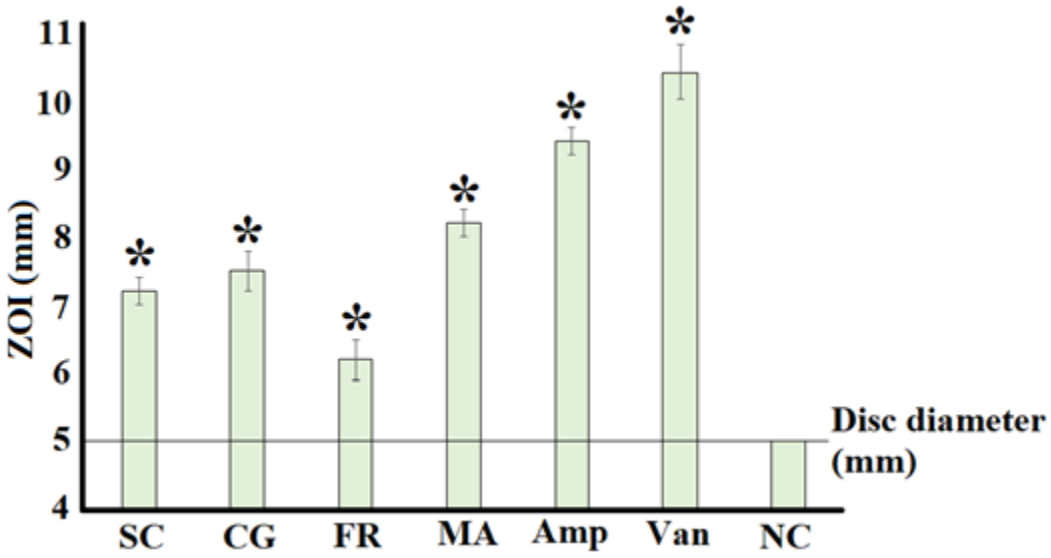

Figure 3: Growth inhibitory activity of the plant extracts against *Cutibacterium acnes* (ATCC 6919) measured as zones of inhibition (mm). SC=*S. chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf extract; Amp=ampicillin (10 µg); Van=vancomycin (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).

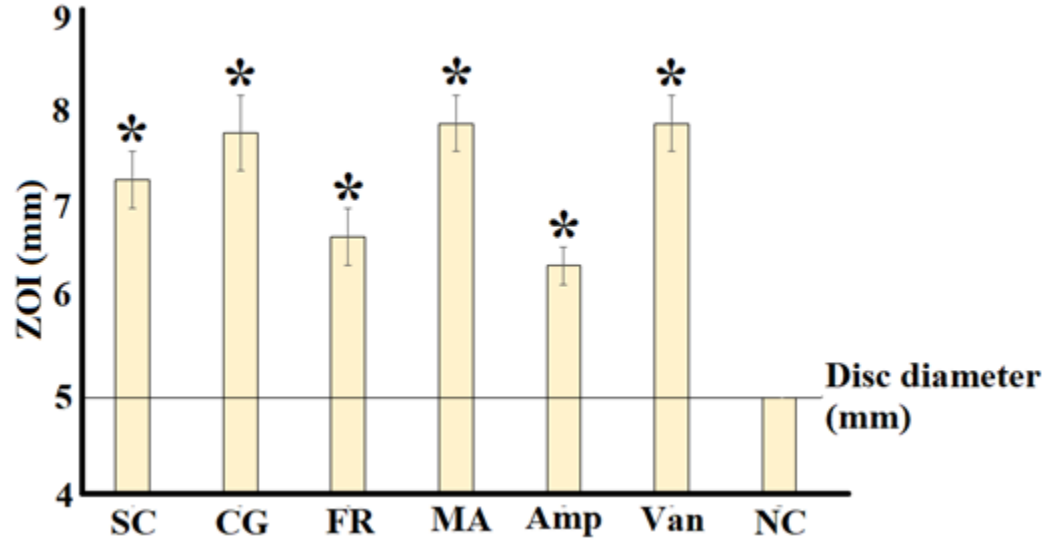

Figure 4: Growth inhibitory activity of the plant extracts against measured *Brevibacterium linens* (ATCC9172) as zones of inhibition (mm). SC=*S. chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf extract; Amp=ampicillin (10 µg); Van=vancomycin (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).

The growth of *S. epidermidis* was also susceptible to the *S. chippendalei, C. glauca, F. racemosa* and *M. alternifolia* extracts, albeit with relatively small ZOIs that indicate only low to moderate inhibitory activity (Figure 5). Consistent with the trend noted for the growth inhibition of each of the other bacteria screened, the *M. alternifolia* extract (ZOI=7.4 mm) was the best bacterial growth inhibitor. This compares favourably to the inhibition of *S. epidermidis* growth by the ampicillin (SOI=7.2 mm) and vancomycin controls (ZOI=8.5 mm), indicating that this extract may be particularly useful for body odour mitigation.

Quantification of Minimum Inhibitory Concentration (MIC)

The relative level of antimicrobial activity was further evaluated by determining the MIC values (Table 2) for all extracts against each bacteria. The results of the Disc Diffusion (DD) and liquid dilution (LD) MIC screening assays correlated relatively well, with similar MIC values generally recorded for most assays. The *M. alternifolia* extract was generally the best inhibitor of bacterial growth, with LD MIC values of 1667, 1230, 1250 and 2720 µg/mL against *C. jeikeium, C. acnes, B. linens* and *S. epidermidis*

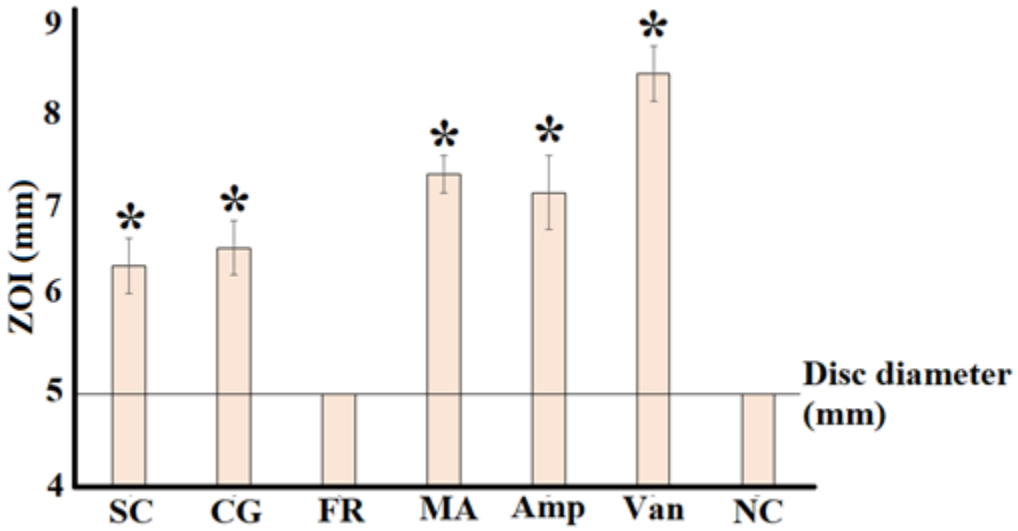

Figure 5: Growth inhibitory activity of the plant extracts against *Staphylococcus epidermidis* (clinical isolate) measured as zones of inhibition (mm). SC=*S. chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf extract; Amp=ampicillin (10 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, C. acnes, B. linens* and *S. epidermidis*, growth (µg/mL) of the plant extracts.

Extract	MIC (µg/mL)							
	C. jeikeium		*C. acnes*		*B. linens*		*S. epidermidis*	
	DD	LD	DD	LD	DD	LD	DD	LD
SC	2500	1070	4167	2790	2083	1850	>5000	>5000
CG	>5000	4167	4585	1810	4167	1520	>5000	>5000
FR	-	-	>5000	>5000	3190	1833	-	-
MA	1980	1667	1667	1230	1250	1250	3750	2720

DD=disc diffusion; LD=liquid dilution; SC=*S chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf extract; Numbers indicate the mean DD MIC and LD MIC values of triplicate determinations. - indicates no inhibition at any concentration tested.

respectively. However, the *S. chippendalei* extract was the best inhibitor of *C. jeikeium* growth (LD MIC=1070 µg/mL). As *C. jeikeium* is the major contributor to axillary odour formation, this extract may be particularly useful for odour mitigation. In contrast, the *S. chippendalei* extract was a substantially less potent inhibitor of the other malodour forming bacteria screened, with MIC values ≥1850 µg/mL against all other species. Substantially higher MIC values were recorded for *C. glauca* and *F. racemosa* (>4000 µg/mL) against most of the bacterial species tested, indicating that these extracts would have limited beneficial effects as deodorant additives.

Quantification of toxicity

All extracts were initially screened in the *Artemia nauplii* assay at 2000 µg/mL (Figure 6). Potassium dichromate (1000 µg/mL) was also included in the bioassay as a control toxin (positive control). Exposure of the nauplii to the potassium dichromate control rapidly induced nauplii death, with 100% mortality achieved within 5 hr (unpublished results). In contrast, all of the tested extracts induced substantially less than 50% mortality following 24 hr exposure. As 24 hr LC_{50} values >1000 µg/mL have previously been defined as nontoxic in this assay,[27-29] all of the plant extracts tested were deemed to be nontoxic and their LC_{50} values were not determined.

DISCUSSION

Axillary and plantar malodour may lead to social exclusion and mental health issues. Additionally, excessive sweating (which provides nutrients for the odour forming to produce volatile compounds) may also damage clothing and footwear. Antiperspirants and deodorants that are used to mitigate body malodour formation generally function via one or more of the following mechanisms:

• Reducing sweat production by blocking eccrine glands. This deprives axillary odour-producing bacteria of the proteins and lipids that they require to produce malodorous volatile compounds. Antiperspirant formulations often include aluminium salts for this purpose, despite prolonged exposure to aluminium being associated with several serious health issues.[2,3]

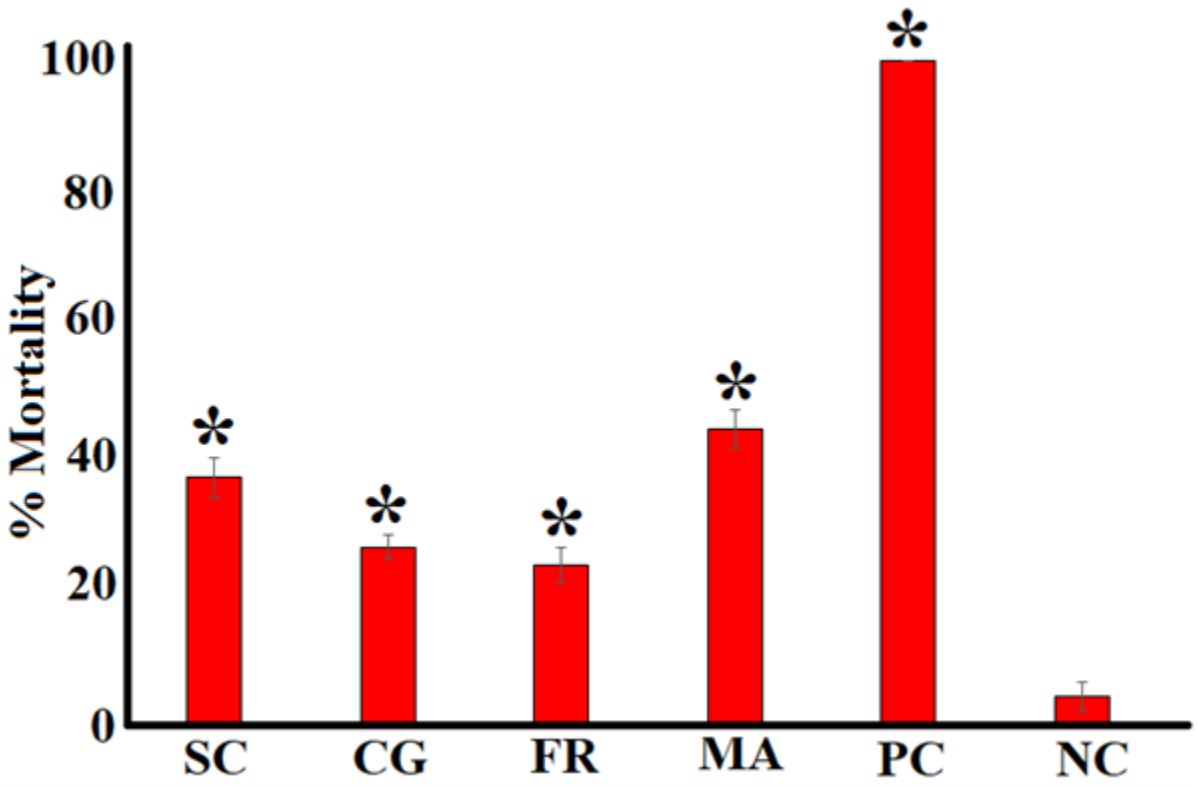

Figure 6: The lethality of the plant extracts (2000 µg/mL) and the potassium dichromate (1000 µg/mL) and seawater controls towards *Artemia franciscana* nauplii after 24 hr exposure. SC=*S. chippendalei* fruit extract; CG=*C. glauca* fruit extract; FR=*F. racemosa* leaf extract; MA=*M. alternifolia* leaf 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.

• Directly inhibiting the growth and/or metabolism of the axillary and/or plantar malodour-producing bacteria via antibacterial additives (e.g. propylene glycol, triclosan, benzalkonium chloride). There are also concerns about prolonged exposure to several of these additives.

• Masking the smell of the malodour using strong/pleasant aromas (e.g. perfumes).

Of particular concern, antiperspirants and/or deodorants are generally used at least once a day by many people, and may be used several times within a 24 hr period. As the clearance rates for some of these additives is relatively long, their levels may build up over a period of time, thereby increasing the exposure concentration substantially above the amount applied with each usage. For example, triclosan has an elimination half-life of approximately 11 hr.[30,31] Therefore, for individuals that apply deodorant several times a day, the levels in the bloodstream may increase over time and the risk of toxicity may therefore increase. This is concerning as several deodorant additives (including triclosan) have also been linked to a serious health problems.[4] Furthermore, these studies generally only consider acute toxicity effects. Chronic exposure may induce other toxic effects that are otherwise not identified in acute toxicity assays. As antiperspirants/deodorants are used frequently (at least daily by most users), the potential for chronic and additive effects also needs to be considered and the development of novel safe and effective products is required.

Plant-based formulations are good candidates for new antibacterial deodorant additives as many plant preparations have well established traditional uses to inhibit bacterial growth. Antibacterial activity has also already been verified for many plant preparations against other bacterial species. Additionally, the incorporation of natural products into deodorants to inhibit malodour forming bacteria growth may be more acceptable to consumers due to the perception of safety of natural additives. This study examined the growth inhibitory properties of methanolic extracts prepared from *S. chippendalei* and *C. glauca* fruit, as well as from *F. racemosa* and *M. alternifolia* leaves against several bacterial species associated with body and foot odour formation. Our study determined that all of the extracts screened inhibited the growth of most or all of the bacteria tested. However, the *C. glauca* fruit and *F. racemosa* leaf extracts generally had poor antibacterial activity, with MIC values >4000 µg/mL against several bacteria. Therefore, it is unlikely that the addition of these extracts to deodorants would be beneficial in odour mitigation. In contrast, the *S. chippendalei* fruit and *M. alternifolia* leaf extracts had noteworthy activity against several bacteria. The *M. alternifolia* leaf extract was the most promising growth inhibitor of most of the bacterial species screened, although the *S. chippendalei* fruit extract displayed the most potent inhibition

of *C. jeikeium* growth. As *C. jeikeium* produce the strongest and most unpleasant malodours,[32] the noteworthy inhibition of this bacterium by the *S. chippendalei* fruit and *M. alternifolia* leaf extracts highlight their potential as deodorant additives.

Previous studies examining the inhibitory activity of extracts produced from other plants have reported comparable or considerably higher MIC values and have defined them as 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).[33] Similarly, *Cassia alata*,[34] *Barleria lupulina* and *Psidium guajava*[35] 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.[35] 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.[36] As the *S. chippendalei* fruit and *M. alternifolia* leaf extracts have similar or greater inhibitory activity to several of these previous studies, the addition of these extracts to deodorants has commercial potential and further study is warranted.

Growth inhibition studies against other malodour forming bacteria by plant preparations has been substantially less extensively studied. A previous study reported *Corneybacterium xerosis* growth inhibition by *Rubia tinctorium* (commonly known as madder) extract at a high dose (approximately 500 µg/disc). Unfortunately, MIC values were not determined in that study, making a comparison with other studies impossible.[37] Similarly, *Anethum graveolens* essential oils inhibit *Corneybacterium* spp. growth, although MIC were also not reported in that study.[38] Furthermore, that study examined the growth inhibitory activity of the oil using disc diffusion assays, although disc diffusion assays are not appropriate for testing the antibacterial activity of essential oils due to the insolubility of many of the oil components in the aqueous agar gel. Several recent studies have also reported particularly good inhibition of *C. jeikeium* growth by several other plant preparations, including several Australian and Indian *Terminalia* spp. extracts.[5,8] with MIC values of approximately 200 µg/mL against *C. jeikeium*. Similarly, low MIC values (200-1000 µg/mL) were also reported for several Australian *Syzygium* spp.[5]

Notably, all of the extracts tested in this study were determined to be nontoxic in the *Artemia* nauplii toxicity assay and thus are likely to be safe for topical application as deodorants. However, toxicity studies using relevant human cell lines (e.g. human dermal fibroblasts) as well as *in vivo* testing, are required to confirm the safety of these extracts. Notably, whilst our studies determined that all of the tested extracts were nontoxic, the *Artemia* nauplii bioassay (and cell line toxicity assays) only provide an assessment of acute toxicity. As antiperspirant/deodorant products are applied frequently and some components have relatively long clearance times, chronic toxicity should also be considered. Future studies examining the pharmacodynamic and pharmacokinetic properties of the extract components are required. In particular, future studies are needed to assess the ability of the extract components to cross the skin barrier, their duration in the blood stream prior to clearance, and the metabolic products of those compounds.

CONCLUSION

The results of this study demonstrate the potential of the *S. chippendalei* fruit and *M. alternifolia* leaf extracts as natural antibacterial components that have potential to be included in deodorant formulation. The *M. alternifolia* leaf extract was particularly promising, with noteworthy MIC values recorded against all of the bacterial species 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 *Solanum chippendalei, Citrus glauca* (fruit), and *Ficus racemosa* and *Melaleuca alternifolia* (leaf) extracts were screened for growth inhibitory activity against body odour producing bacteria.
- The *M. alternifolia* leaf extract had noteworthy bacterial growth inhibitory activity (against C. *jeikeium, C. acnes, B. linens* and *S. epidermidis* (MIC values 1230-2720 µg/mL).
- All other extracts also inhibited the growth of most bacteria, although generally with higher MIC values.
- All of the extracts were nontoxic in the *Artemia* nauplii toxicity bioassay.

REFERENCES

1. Kanlayavattanakul M, Lourith N. Body malodours and their topical treatment agents. International Journal of Cosmetic Science 2011;33:298-311.
2. Exley C. Does antiperspirant use increase the risk of aluminium-related disease, including Alzheimer's disease? Molecular Medicine Today 1998;4(3):107-9.
3. Darbre PD, Pugazhendi D, Mannello F. Aluminium and human breast diseases. Journal of Inorganic Biochemistry 2011;105:1484-8.
4. Bhargava HN, Leonard PA. Triclosan: applications and safety. American Journal of Infection Control 1996;24(3):209-18.
5. McManus K, Wood A, Wright MH, et al. *Terminalia ferdinandiana* Exell. extracts inhibit the growth of body odour forming bacteria. International Journal of Cosmetic Science 2017;39(5):500-10.
6. Wood A, McManus KDB, Wright MH, *et al.* Growth inhibitory activity of selected Australian *Syzygium* species against malodour forming bacteria. Pharmacognosy Communications 2017;7(3):129-36.
7. Wright MH, Wood A, Greene T, *et al.* Growth inhibitory activity of *Acronychia acidula* F. Muell. fruit extracts towards malodour-forming bacteria. Pharmacognosy Communications 2020;10(2):95-101
8. Cock IE, Wright MH, Matthews B, *et al.* Bioactive compounds sourced from *Terminalia* spp. in bacterial malodour prevention: an effective alternative to chemical additives. International Journal of Cosmetic Science 2019;41(5):496508. DOI: 10.1111/ics.12567
9. Shelley WB, Hurley HJ, Nicholas AC. Axillary odor: experimental study of the role of bacteria, apocrine sweat, and deodorants. Archives of Dermatology and Syphilology 1953;68:430-46.
10. Cock IE, Cheesman MJ. Plants of the genus *Syzygium* (Myrtaceae): A review on ethnobotany, medicinal properties and phytochemistry. In Bioactive Compounds of Medicinal Plants: Properties and Potential for Human Health 2018, Apple Academic Press, New Jersey, USA: 35-84.
11. Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. Nutrition and Cancer. 2018;70(4):579-93.
12. Jamieson N, Sirdaarta J, Cock IE. The anti-proliferative properties of Australian plants with high antioxidant capacities against cancer cell lines. Pharmacognosy Communications 2014;4(4):71-82.
13. Wright MH, Shalom J, Matthews B, et al. *Terminalia ferdinandiana* Exell. extracts inhibit *Shewanella* spp. growth and prevent fish spoilage. Food Microbiology 2019;78:114-22.
14. Sirdaarta J, Matthews B, Cock IE. Kakadu plum fruit extracts inhibit growth of the bacterial triggers of rheumatoid arthritis: Identification of stilbene and tannin components. Journal of Functional Foods 2015;17:610-20. DOI: 10.1016/j.jff.2015.06.019
15. Courtney R, Sirdaarta J, Matthews B, *et al.* Tannin components and inhibitory activity of Kakadu plum leaf extracts against microbial triggers of autoimmune inflammatory diseases. Pharmacognosy Journal 2015;7(1):18-31. DOI: 10.5530/pj.2015.7.2
16. Murhekar S, Wright MH, Greene AC, *et al.* Inhibition of *Shewanella* spp. growth by *Syzygium australe* and *Syzygium luehmannii* extracts: Natural methods for the prevention of fish spoilage. Journal of Food Science and Technology 2017;54(10):3314-26.
17. Winnett V, Sirdaarta J, White A, *et al.* Inhibition of *Klebsiella pneumoniae* growth by selected Australian plants: Natural approaches for the prevention and management of ankylosing spondylitis. Inflammopharmacology 2017;25(2):223-35.
18. Omer E, Elshamy AI, Nassar M, et al. *Plantago squarrosa* Murray extracts inhibit the growth of some bacterial triggers of autoimmune diseases: GC-MS analysis of an inhibitory extract. Inflammopharmacology 2019;27(2):373-85.
19. Cheesman MJ, White A, Matthews B, et al. *Terminalia ferdinandiana* fruit and leaf extracts inhibit methicillin-resistant *Staphylococcus aureus* growth. Planta Medica 2019;85(16):1253-62.
20. Cock IE, Maen A. Inhibitory activity of high antioxidant Australian native fruits against the bacterial triggers of selected autoimmune diseases. Pharmacognosy Communications 2015;5(1):48-59.
21. Mpala LN, Chikowe GR, Cock IE. Growth inhibitory properties of extracts prepared from selected *Leptospermum* and *Melaleuca* species against a panel of pathogenic bacteria. Pharmacognosy Communications 2016;6(4):215-24.
22. Mpala LN, Chikowe GR, Cock IE. *Ficus racemosa* L. leaf extracts lack antibacterial activity and are toxic *in vitro*. Pharmacognosy Communications 2020;10(1):52-6.
23. Hutchings A, Cock IE. An interactive antimicrobial activity of *Embelica officinalis* Gaertn. fruit extracts and conventional antibiotics against some bacterial triggers of autoimmune inflammatory diseases. Pharmacognosy Journal 2018;10(4):654-62.
24. Ilanko A, Cock IE. The interactive antimicrobial activity of conventional antibiotics and *Petalostigma* spp. extracts against bacterial triggers of some autoimmune inflammatory diseases. Pharmacognosy Journal 2019;11(2):292-309.
25. Tiwana G, Cock IE, White A, *et al.* Use of specific combinations of the triphala plant component extracts to potentiate the inhibition of gastrointestinal bacterial growth. Journal of Ethnopharmacology 2020;260:112937. DOI: 10.1016/j.jep.2020.112937
26. Ilanko P, McDonnell PA, van Vuuren S, *et al.* Interactive antibacterial profile of *Moringa oleifera* Lam. extracts and conventional antibiotics against bacterial triggers of some autoimmune inflammatory diseases. South African Journal of Botany 2019;124:420-35.
27. Cock IE, Ruebhart DR. Comparison of the brine shrimp nauplii bioassay and the ToxScreen-II test for the detection of toxicity associated with *Aloe vera* (*Aloe barbadensis* Miller) leaf extract. Pharmacognosy Research 2009;1(2):98-101.
28. Ruebhart DR, Wickramasinghe W, Cock IE. Protective efficacy of the antioxidants vitamin E and Trolox against *Microcystis aeruginosa* and microcystin-LR in *Artemia franciscana* nauplii. Journal of Toxicology and Environmental Health, Part A 2009;72(24):1567-75.
29. Sirdaarta J, Cock IE. Vitamin E and Trolox™ reduce toxicity of *Aloe barbadensis* Miller juice in *Artemia franciscana* nauplii but individually are toxic at high concentrations. Internet Journal of Toxicology 2008;5(1):5830.
30. Queckenberg C, Meins J, Wachall B, *et al.* Absorption, pharmacokinetics, and safety of triclosan after dermal administration. Antimicrobial Agents and Chemotherapy 2010;54(1):570-2.
31. Nakae T, Gomyo H, Sasaki I, *et al.* New antiaxillary odour deodorant made with antimicrobial Ag-zeolite (silver-exchanged zeolite). International Journal of Cosmetic Science 2006;28:200-309.
32. James AG, Casey J, Hyliands D, *et al.* Fatty acid metabolism by cutaneous bacteria and its role in axillary malodour. World Journal of Microbiology and Biotechnology 2004;20(8):787-93.
33. Chanwitheesuk A, Teerawutgulrag A, Kilburn JD, *et al.* Antimicrobial gallic acid from *Caesalpinia mimosoides* Lamk. Food Chemistry 2007;100(3):1044-8.
34. Yadav JP, Arya V, Yadav S, et al. *Cassia occidentalis* L.: a review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia 2010;81(4):223-30.
35. Chomnawang MT, Surassmo S, Wongsariya K, *et al.* Antibacterial activity of Thai medicinal plants against methicillin-resistant *Staphylococcus aureus*. Fitoterapia 2009;80(2):102-4.
36. Bockmuhl D, Hohne HM, Jassoy C, *et al.* Probiotically active plant extracts. US patent 11/336,164;2006.
37. Gőlcü, A, Dolaz M, Dığrak M, *et al.* The biological activity of dyer's madder (*Rubia tinctorium* L.). In 1st International Congress of the Chemistry of Natural Products 2002;255-8.
38. Singh G, Kapoor IP, Pandey SK, *et al.* Studies on essential oils: part 10; antibacterial activity of volatile oils of some spices. Phytotherapy Research 2002;16(7):680-2.

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