Antimicrobial Essential Oil Combinations to Combat Foot Odour

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ABSTRACT

Foot odour (bromodosis) is an embarrassing and perplexing condition mostly caused by bacteria of the *Brevibacterium* species. Essential oils are a credible option as an affordable treatment of odour and contribute towards antimicrobial efficacy. Therefore, this study sets out to investigate the antimicrobial activity of essential oil combinations against odour-causing bacteria. The broth microdilution method was used to investigate the antimicrobial activity of 119 essential oil combinations, and the fractional inhibitory index was calculated to determine the interactive profile. Combinations that resulted in synergy in 1 : 1 ratios were further evaluated in different concentrations, and isobolograms were plotted to determine the influence of the ratio on overall activity. Numerous combinations could be identified as having synergistic interactions against the *Brevibacterium* spp. and no antagonism was observed. The combination of *Juniperus virginiana* (juniper) and *Styrax benzoin* (benzoin) demonstrated synergy against all three *Brevibacterium* spp. tested and *J. virginiana* was the essential oil responsible for the majority of the synergistic interactions. The results reported here confirm the promising potential of the majority of these oils and selected combinations in treating and controlling bromodosis.

Introduction

Foot odour (bromodosis) is a distressing disorder (both socially and medically) and is caused by the release of sulphur compounds generated by potent proteolytic enzymes produced by *Brevibacterium* [1–4]. These bacteria are Gram-positive, catalase-positive, obligate aerobic bacilli.

There appears to be a constant concern with regards to body hygiene and malodour, making the use of agents such as fragrant deodorants and antiperspirants one of the largest cosmetic sellers globally [5, 6]. The global antiperspirant and deodorant market is estimated to be an industry worth US$72.7 billion (estimates for 2018) [7]. One of the most important personal care products is deodorant. It is a product that continues to retain constant investment by companies to improve quality, and formulations include aerosols, roll-ons, and gels [7]. Deodorants are applicable as a spray to body parts and the feet, whereas antiperspirants decrease sweat. Both are aimed at inhibiting the bacteria causing malodour.

The limitations of current available treatments are that they may be inconvenient, expensive, require extensive application, and are often disconcerting due to the reoccurrence of odour after ceasing treatment [8]. Furthermore, deodorants and antiperspirants may contain antimicrobial substances; however, with the amount of antimicrobial chemicals [such as propylene glycol, triclosan, benzalkonium chloride, and metal (e.g., aluminium) salts] being added to combat these bacteria, there is a constant concern of the toxicity and potential resistance to these ingredients [9–11].

By 2014, the antibiotic industry was estimated to be worth approximately US$65.5 billion [12]. The worth of the global antibiotic market is still on the rise, especially because of the high cost of developing new drugs or finding alternatives to the ever-growing antibiotic resistance issues. A contributor to the poor availability of resistant free antibiotics is the lack of newer antibiotics for the last two decades. Investment is aimed at either developing new antibiotics or identifying alternative antibiotic treatments. Alternatives would, in fact, be preferable if one considers the high costs involved in research and development (R&D), and the rate at which resistance is developing, which is faster than the rate at which new antibiotics can even be developed. This is evident by the fact that the net worth of the antibiotic industry is dominated by generic manufacturers and only a few new patented products [13].

The global fragrance market is predicted to be worth US$ 43.6 billion by 2021. Closely following the household product sector, the second largest market share for fragrance products is personal care, and one of the key elements in fragrances is essential oils [14]. Essential oils are frequently used in dermatology, and 5% of essential oils used in dermatology are recommended for body odour [15]. This is not surprising considering the pleasant fragrance imparted by these natural products. It is not only the pleasant organoleptic properties that render essential oils appealing in treating bromodosis, but also the antimicrobial activity displayed by these essential oils. Promising activity has been observed for essential oils against body odour-causing bacteria [16]. Essential oils are, however, predominantly used in combination, yet the recommended combinations as contained in the layman's literature against foot malodour have yet to be investigated [17–28]. No reports could be found reporting antimicrobial resistance against essential oil combinations.

Thus, with essential oils having potential antimicrobial activity, they are an attractive option for treating malodorous bacteria involved in bromodosis. To the R&D industry, the odour-inducing bacteria are not a priority, yet clearly by the predicted worth of the fragrance industry and the fact that personal care is the second largest contributor to this value, foot odour should be considered important. The natural origin of essential oils also makes them an appealing alternative to consumers.

This study is the first to investigate the antimicrobial activity of essential oil combinations against odour-inducing bacteria and aims to find the most promising oils to be used in combination.

Results

The minimum inhibitory concentrations (MICs) of 19 commercial essential oils, not previously investigated, are shown in ▸ **Table 1**. *Brevibacterium agri* and *Brevibacterium epidermidis* appeared to be the most susceptible to essential oil inhibition and were inhibited by 18 and 19 essential oils, respectively, at a noteworthy concentration (MIC ≤ 1.00 mg/mL). *Santalum austrocaledonicum* (sandalwood) was found to display the strongest inhibitory activity (MIC values of 0.01–0.13 mg/mL).

From the 119 combinations against each of the *Brevibacterium* spp., it can be observed (▸ **Table 2**) that 118 combinations resulted in noteworthy antimicrobial activity against *B. agri*, 117 against *B. epidermidis* (most associated with odour), and 91 against *Brevibacterium linens*, proving the latter of the three as being the most resilient against antimicrobial inhibition. The combinations based on aromatherapeutic literature are shown as shaded areas. No antagonism was observed in any of the combinations.

B. agri had four synergistic, 68 additive, and 47 indifferent interactions and *B. epidermidis* had 12 synergistic, 85 additive, and 22 indifferent interactions. *B. linens* had six synergistic, 52 additive, and 61 indifferent interactions.

The synergistic combination with the lowest MIC value of 9.00 µg/mL against *B. agri* was when *Pelargonium odoratissimum* (geranium) was combined with *S. austrocaledonicum*. The most effective synergistic combination against *B. epidermidis* was *Pelargonium graveolens* (rose geranium) with *Santalum album* (sandalwood) (MIC = 0.13 mg/mL) and against *B. linens*, it was *Litsea cubeba* (may chang) with *Cananga odorata* (ylang ylang) (MIC = 0.30 mg/mL).

The combination of *Cedrus atlantica* (cedarwood) with *Vetiveria zizanioides* (vetiver) **1** (MIC values ranging 0.19–0.23 mg/mL) and the combination of *Pogostemon patchouli* (patchouli) and *S. austrocaledonicum* (MIC values ranging 0.05–0.25 mg/mL) displayed the overall strongest inhibition against all three *Brevibacterium* spp. Four additional combinations could also be noted for strong inhibition against two of the *Brevibacterium* spp. and noteworthy activity against a third. These include *C. atlantica* with *V. zizanioides* **2**, *Coriandrum sativum* (coriander) with *S. austrocaledonicum, Foeniculum dulce* (fennel) with *S. austrocaledonicum*, and *Juniperus virginiana* (juniper) with *S. austrocaledonicum*.

The combination of *J. virginiana* and *Styrax benzoin* (benzoin) displayed synergy against each of the three *Brevibacterium* spp., with noteworthy MIC values ranging from 0.13–0.42 mg/mL and fractional inhibitory concentration index (ΣFIC) values from 0.16–0.49. This is encouraging considering the pleasant organoleptic property offered by this combination.

The varied ratio combinations were further evaluated and plotted on isobolograms. These are shown in ▸ **Figs. 1**–**4** with corresponding tables (▸ **Tables 3**–**6**) that indicate the MIC at the different ratios.

▸ **Fig. 1** shows that the combinations of *Cupressus sempervirens* with *Commiphora myrrha* (myrrh), *P. odoratissimum* with *S. austrocaledonicum*, and *Salvia sclarea* (clary sage) with *Boswellia carteri* (frankincense) against *B. agri*. The combination of *P. odoratissimum* and *S. austrocaledonicum* predominantly requires *S. austrocaledonicum* to be in a higher concentration. Synergy was observed for combinations closest to and including 1 : 1 ratios. These isobolograms demonstrate how important it is to mix the essential oils in the appropriate ratios, as varied ratios can change the interaction considerably. ▸ **Table 3** displays the MIC values of each ratio that corresponds to the combinations shown in ▸ **Fig. 1**.

▸ **Fig. 2** (corresponds to ▸ **Table 4**) shows *J. virginiana* and *S. benzoin* essential oils in combination against the three *Brevibacterium* spp. It can also be observed that synergy results where the combination is closest to the 1 : 1 ratio. *S. benzoin,* however, is a strong common denominator for synergy, as points 5–7 and even point 8 (*B. linens*) where *S. benzoin* is in the higher ratio, synergy is demonstrated. Point 9, however, is consistently an outlier against

▶ **Table 1** The mean MIC (n = 3) values of the individual essential oils investigated against *Brevibacterium* spp.

Essential oils	*B. agri* (ATCC 51663)	*B. epidermidis* (DSM 20660)	*B. linens* (DSM 20425)
Abies balsamea (balsam)	**1.00***	**0.50**	**1.00**
Cinnamomum verum (cinnamon bark)	**0.25**	**0.25**	**0.19**
Cinnamomum zeylanicum (cinnamon leaf)	**0.25**	**0.25**	**0.50**
Cistus ladanifer (rock rose) 1	**1.00**	**0.25**	**1.00**
Cistus ladanifer (rock rose) 2	**0.38**	**0.38**	**1.00**
Cymbopogon nardus (citronella)	**0.50**	**0.25**	**0.67**
Foeniculum dulce (fennel)	**1.00**	**0.25**	**1.00**
Hypericum perforatum (St Johns wort)	2.00	**1.00**	2.00
Matricaria recutita (German chamomile)	**0.25**	**0.38**	**0.50**
Mentha spicata (spearmint)	**0.50**	**0.50**	2.00
Nardostachys jatamansi (spikenard)	**1.00**	**0.75**	3.00
Ocimum tenuiflorum (holy basil aromatics) 1	**0.50**	**0.50**	**1.00**
Ocimum tenuiflorum (holy basil SE) 2	**1.00**	**0.50**	**1.00**
Origanum vulgare (oregano)	**0.19**	**0.25**	**0.50**
Pelargonium graveolens (rose geranium)	**0.50**	**0.50**	**1.00**
Rosa damascena (rose otto) 1	**0.25**	**0.25**	**0.50**
Rosa damascena (rose otto) 2	**0.50**	**0.25**	**0.50**
Santalum austrocaledonicum (sandalwood)	**0.01**	**0.13**	**0.13**
Vetiveria zizanioides (vetiver) 2	**0.13**	**0.13**	**0.50**
Control (Ciprofloxacin)	8.3×10^3 µg/mL	2.61×10 µg/mL	6.25×10 µg/mL

*Noteworthy activity (bold)

each *Brevibacterium*. These combinations (ratio mixes 6:4, 5:5, 4:6, and 3:7) should be strongly considered for further formulation studies, especially as a neutral (men and women) deodorant due to the earthly woody and vanilla smell offered by this combination.

In ▶ **Fig. 3**, it can be observed that all but one of the synergistic interactions against *B. epidermidis* containing the *Pelargonium* spp. is due to this oil (regardless of chemotype) being in the majority. Interestingly, both *Pelargonium* spp. in combination with *Lavandula angustifolia* (lavender) reflect similar patterns where points 3–7 are synergistic. ▶ **Table 5** corresponds to the combinations shown in ▶ **Fig. 3**.

▶ **Fig. 4** (MIC of ratios shown in ▶ **Table 6**) displays *J. virginiana* in combination with different essential oils against *B. epidermidis* and *B. linens*, and in five of the six combinations, *J. virginiana* being used in the majority is shown to be responsible for the synergy.

Discussion

The antimicrobial activity of the majority of the essential oils have been previously reported against the three *Brevibacterium* species [16]. A selection of oils (▶ **Table 1**) not previously studied have been added for a concise overview of antimicrobial activity. *S. austrocaledonicum* displayed stronger antimicrobial activity against odour bacteria than *S. album* (0.25–0.31 mg/mL), possibly due to the higher α-santalol content [16]. *P. odoratissimum* (geranium) and *P. graveolens* (rose geranium) from this study were similar in activity [16]. *Cinnamomum zeylanicum* showed stronger antimicrobial inhibition against *B. agri* and *B. epidermidis* (0.25–0.50 mg/mL) compared to a previous study (0.50–1.50 mg/mL) [16], most likely due to the higher concentration of eugenol. *Cinnamomum verum*, containing cinnamaldehyde, allowed for a much higher antimicrobial activity to both of the *C. zeylanicum* samples (0.19–0.25 mg/mL compared to 0.25–0.50 mg/mL). The *V. zizanioides* (vetiver) sample in this study is comparable to the sample used by Orchard et al. [16]. *Matricaria recutita* (German chamomile) was shown to be the superior of the tested chamomile species with MIC values of 0.25–0.50 mg/mL compared to that of *Anthemis nobilis* (Roman chamomile) (MIC 1.00–2.00 mg/mL) [16]. The two *Rosa damascena* (Rose otto) samples predominantly displayed equal inhibitory potential (0.25–0.50 mg/mL). This shows a potential for this oil, not only because of the antimicrobial activity, but also due to the additional pleasant organoleptic properties.

The high antimicrobial activities in combination together with the synergistic interactions are encouraging findings considering that, although previously believed to be apathogenic, *Brevibacterium* spp. have been reported as being involved in opportunistic infections in immunocompromised patients [29–34]. The essential oils occuring most frequently in the most noteworthy combinations across all the *Brevibacterium* were the *Santalum* spp., *P. patchouli*, and *Pelargonium* spp. Importantly, these are also essential oils used in the fragrance industry.

▸ **Table 2** The mean MIC (n = 3) and ΣFIC values of the essential oil combinations investigated against odour-inducing bacteria.

Essential oil combinations		Mean MIC (mg/mL) (n = 3) and ΣFIC											
		B. agri (ATCC 51663)				*B. epidermidis* (DSM 20660)				*B. linens* (DSM 20425)			
Essential oil 1	**Essential oil 2**	**MIC1***	**MIC2***	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**
Achillea millefolium (yarrow)	*Citrus sinensis* (orange)	1.00	1.00	0.75§	0.75	1.00	0.75	0.50	0.58	1.50	1.00	2.00	1.67
Cedrus atlantica (cedarwood)	*Citrus bergamia* (bergamot)‡	0.50	1.00	1.00	1.50	1.00	0.50	1.00	1.50	0.25	2.00	1.00	2.25
	Boswellia carteri (frankincense)	0.50	1.00	1.00	1.50	1.00	0.50	0.50	0.75	0.25	0.75	0.75	2.00
	Juniperus virginiana (juniper)	0.50	2.00	1.00	1.25	1.00	1.00	0.50	***0.50†***	0.25	1.00	0.75	1.88
	Rosmarinus officinalis (rosemary)	0.50	1.00	0.75	1.13	1.00	1.00	0.75	0.75	0.25	1.00	1.00	2.50
	Vetiveria zizanioides (vetiver) 1	0.50	0.05	0.19	2.06	1.00	0.19	0.19	0.59	0.25	0.19	0.23	1.04
	Vetiveria zizanioides (vetiver) 2	0.50	0.13	0.19	0.91	1.00	0.13	0.19	0.81	0.25	0.50	0.75	2.25
Citrus aurantium var. *amara* leaf (petitgrain)	*Lavandula angustifolia* (lavender)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	3.00	3.00
	Rosmarinus officinalis (rosemary)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Citrus bergamia (bergamot)	*Cupressus sempervirens* (cypress)	1.00	0.75	1.00	1.17	0.50	0.50	0.50	1.00	2.00	2.00	1.25	0.63
	Lavandula angustifolia (lavender)	1.00	1.00	1.00	1.00	0.50	1.00	1.50	2.25	2.00	1.00	3.00	2.25
Commiphora myrrha (myrrh)	*Citrus aurantifolia* (lime)	0.38	1.00	0.50	0.91	0.25	0.50	0.25	0.75	0.38	1.00	0.50	0.91
	Allium sativum (garlic)	1.00	0.13	0.13	0.54	0.25	0.75	0.25	0.67	0.38	0.50	0.75	1.74
Coriandrum sativum (coriander)	*Citrus bergamia* (bergamot)	1.00	1.00	1.00	1.00	0.50	0.50	0.50	1.00	3.00	2.00	1.00	***0.42***
	Piper nigrum (black pepper)	1.00	1.00	0.75	0.75	0.50	0.38	0.50	1.16	3.00	2.00	2.00	0.83
	Salvia sclarea (clary sage)	1.00	1.00	1.00	1.00	0.50	1.00	0.50	0.75	3.00	1.00	2.00	1.33
	Syzygium aromaticum (clove)	1.00	0.50	0.75	1.13	0.50	0.38	0.50	1.16	3.00	0.50	1.00	1.17
	Boswellia carteri (frankincense)	1.00	1.00	0.60	0.60	0.50	0.50	0.50	1.00	3.00	0.75	1.00	0.83
	Zingiber officinale (ginger)	1.00	0.50	0.50	0.75	0.50	0.50	0.50	1.00	3.00	1.00	2.00	1.33
	Santalum album (sandalwood)	1.00	0.31	0.30	0.63	0.50	0.25	0.25	0.75	3.00	0.25	0.50	1.08
	Santalum austrocaledonicum (sandalwood)	1.00	0.01	0.07	3.54	0.50	0.13	0.13	0.61	3.00	0.13	0.50	2.01
Cupressus sempervirens (cypress)	*Salvia sclarea* (clary sage)	0.75	1.00	1.00	1.17	0.50	1.00	1.50	2.25	2.00	1.00	1.50	1.13
	Lavandula angustifolia (lavender)	0.75	1.00	1.00	1.17	0.50	1.00	1.00	1.50	2.00	1.00	1.00	0.75
	Commiphora myrrha (myrrh)	0.75	0.38	0.13	***0.26***	0.50	0.25	0.25	0.75	2.00	0.38	0.50	0.78
	Citrus sinensis (orange)	0.75	1.00	0.50	0.58	0.50	0.75	0.50	0.83	2.00	1.00	2.00	1.50
Cymbopogon citratus (lemongrass)	*Lavandula angustifolia* (lavender)	0.25	1.00	0.50	1.25	0.50	1.00	0.50	0.75	0.50	1.00	1.00	1.50
	Rosmarinus officinalis (rosemary)	0.25	1.00	0.50	1.25	0.50	1.00	0.38	0.56	0.50	1.00	1.00	1.50
Cymbopogon nardus (citronella)	*Citrus bergamia* (bergamot)	0.50	1.00	1.00	1.50	0.25	0.50	0.50	1.50	0.67	2.00	1.67	1.66
	Pelargonium odoratissimum (geranium)	0.50	0.38	0.75	1.74	0.25	0.50	0.50	1.50	0.67	1.00	1.50	1.87
	Pelargonium graveolens (rose geranium)	0.50	0.50	0.50	1.00	0.25	0.50	0.25	0.75	0.67	1.00	1.00	1.25

cont.

▶ **Table 2** *Continued*

Essential oil combinations		Mean MIC (mg/mL) (n = 3) and ΣFIC											
		B. agri (ATCC 51663)				*B. epidermidis* (DSM 20660)				*B. linens* (DSM 20425)			
Essential oil 1	**Essential oil 2**	**MIC1***	**MIC2***	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**
Foeniculum dulce (fennel)	*Pelargonium odoratissimum* (geranium)	1.00	0.38	1.00	1.82	0.25	0.50	0.50	1.50	1.00	1.00	2.00	2.00
	Pelargonium graveolens (rose geranium)	1.00	0.50	1.00	1.50	0.25	0.50	0.50	1.50	1.00	1.00	2.00	2.00
	Santalum album (sandalwood)	1.00	0.31	0.75	1.58	0.25	0.25	0.25	1.00	1.00	0.25	1.00	2.50
	Santalum austrocaledonicum (sandalwood)	1.00	0.01	0.08	3.84	0.25	0.13	0.13	0.73	1.00	0.13	0.75	3.26
	Rosa damascena (rose otto) 1	1.00	0.25	0.50	1.25	0.25	0.25	0.25	1.00	1.00	0.50	1.00	1.50
	Rosa damascena (rose otto) 2	1.00	0.50	0.38	0.56	0.25	0.25	0.25	1.00	1.00	0.50	1.00	1.50
Juniperus virginiana (juniper)	*Styrax benzoin* (benzoin)	2.00	0.50	0.13	***0.16***	1.00	0.50	0.25	***0.38***	1.00	0.75	0.42	***0.49***
	Citrus bergamia (bergamot)	2.00	1.00	0.75	0.56	1.00	0.50	0.50	0.75	1.00	2.00	0.75	0.56
	Salvia sclarea (clary sage)	2.00	1.00	0.75	0.56	1.00	1.00	0.75	0.75	1.00	1.00	2.00	2.00
	Cupressus sempervirens (cypress)	2.00	0.75	0.75	0.69	1.00	0.50	0.38	0.56	1.00	2.00	0.50	***0.38***
	Citrus paradisi (grapefruit)	2.00	1.00	0.75	0.56	1.00	0.38	0.50	0.91	1.00	1.00	0.50	***0.50***
	Lavandula angustifolia (lavender)	2.00	1.00	1.00	0.75	1.00	1.00	0.50	***0.50***	1.00	1.00	2.00	2.00
	Citrus limon (lemon)	2.00	1.00	0.75	0.56	1.00	0.50	0.38	0.56	1.00	1.00	2.00	2.00
	Citrus aurantifolia (lime)	2.00	1.00	1.00	0.75	1.00	0.50	0.25	***0.38***	1.00	1.00	1.50	1.50
	Citrus reticulata (mandarin)	2.00	1.00	0.75	0.56	1.00	1.50	0.63	0.52	1.00	1.00	1.00	1.00
	Citrus sinensis (orange)	2.00	1.00	1.00	0.75	1.00	0.75	0.50	0.58	1.00	1.00	1.00	1.00
	Pinus sylvestris (pine)	2.00	1.00	0.75	0.56	1.00	1.00	0.25	***0.25***	1.00	1.00	2.00	2.00
	Rosmarinus officinalis (rosemary)	2.00	1.00	1.50	1.13	1.00	1.00	0.50	***0.50***	1.00	1.00	1.00	1.00
	Santalum album (sandalwood)	2.00	0.31	0.31	0.58	1.00	0.25	0.25	0.63	1.00	0.25	0.50	1.25
	Santalum austrocaledonicum (sandalwood)	2.00	0.01	0.06	3.14	1.00	0.13	0.13	0.54	1.00	0.13	0.75	3.26
Lavandula angustifolia (lavender)	*Anthemis nobilis* (chamomile)	1.00	1.50	1.00	0.83	1.00	2.00	1.00	0.75	1.00	1.00	1.50	1.50
	Matricaria recutita (German chamomile)	1.00	0.25	0.25	0.63	1.00	0.38	0.50	0.91	1.00	0.50	0.50	0.75
	Citrus limon (lemon)	1.00	1.00	0.75	0.75	1.00	0.50	0.50	0.75	1.00	1.00	1.00	1.00
	Citrus reticulata (mandarin)	1.00	1.00	0.75	0.75	1.00	1.50	0.50	***0.42***	1.00	1.00	1.00	1.00
	Citrus sinensis (orange)	1.00	1.00	1.00	1.00	1.00	0.75	0.50	0.58	1.00	1.00	1.00	1.00
	Pogostemon patchouli (patchouli)	1.00	0.09	0.19	1.14	1.00	0.50	0.63	0.94	1.00	0.75	0.50	0.58
Leptospermum scoparium (manuka)	*Citrus aurantifolia* (lime)	0.38	1.00	1.00	1.82	1.00	0.50	1.00	1.50	0.50	1.00	1.00	1.50
	Allium sativum (garlic)	0.38	0.13	0.13	0.65	1.00	0.75	0.50	0.58	0.50	0.50	1.00	2.00

cont.

Table 2 *Continued*

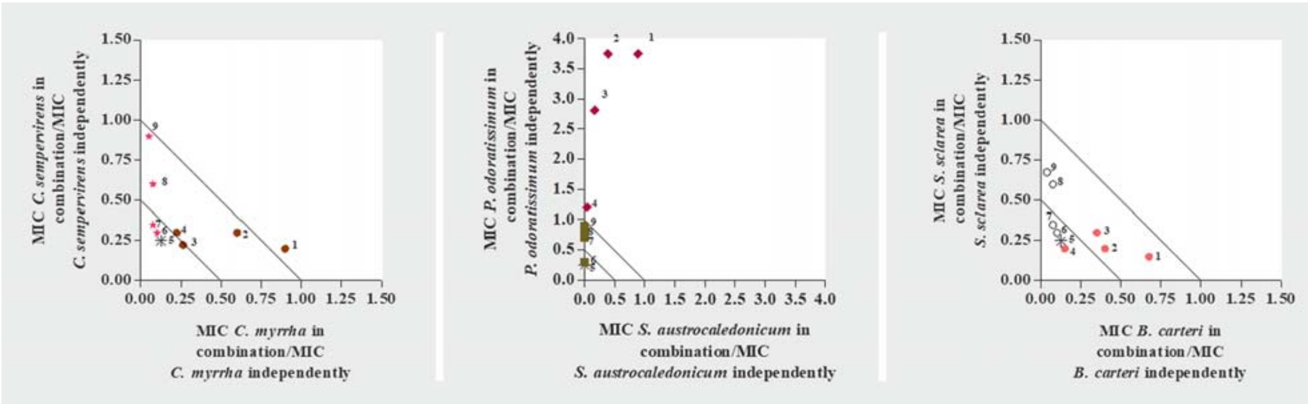
Essential oil combinations		Mean MIC (mg/mL) (n = 3) and ΣFIC											
		B. agri (ATCC 51663)				*B. epidermidis* (DSM 20660)				*B. linens* (DSM 20425)			
Essential oil 1	Essential oil 2	MIC1*	MIC2*	MIC	ΣFIC	MIC1	MIC2	MIC	ΣFIC	MIC1	MIC2	MIC	ΣFIC
Litsea cubeba (may chang)	*Pelargonium odoratissimum* (geranium)	0.13	0.38	0.25	1.29	1.00	0.50	0.50	0.75	0.50	1.00	1.00	1.50
	Lavandula angustifolia (lavender)	0.13	1.00	0.25	1.09	1.00	1.00	1.00	1.00	0.50	1.00	1.00	1.50
	Citrus aurantium var. amara flower (neroli)	0.13	0.50	0.19	0.91	1.00	1.00	0.55	0.55	0.50	1.00	1.50	2.25
	Citrus sinensis (orange)	0.13	1.00	0.22	0.95	1.00	0.75	1.00	1.17	0.50	1.00	1.00	1.50
	Citrus aurantium var. *amara* leaf (petitgrain)	0.13	1.00	0.50	2.17	1.00	1.00	0.75	0.75	0.50	1.00	2.00	3.00
	Cananga odorata (ylang ylang)	0.13	0.50	0.25	1.21	1.00	0.50	0.50	0.75	0.50	1.00	0.30	***0.45***
	Pelargonium graveolens (rose geranium)	0.13	0.50	0.25	1.21	1.00	0.50	0.50	0.75	0.50	1.00	0.50	0.75
	Ocimum basilicum (basil)	0.13	1.25	0.25	1.06	1.00	0.50	1.00	1.50	0.50	1.00	0.65	0.98
	Ocimum tenuiflorum (holy basil aromatics) 1	0.13	0.50	0.25	1.21	1.00	0.50	0.50	0.75	0.50	1.00	0.50	0.75
	Ocimum tenuiflorum (holy basil SE) 2	0.13	1.00	0.25	1.09	1.00	0.50	0.50	0.75	0.50	1.00	0.50	0.75
Pelargonium graveolens (rose geranium)	*Citrus bergamia* (bergamot)	0.50	1.00	1.00	1.50	0.50	0.50	0.42	0.83	1.00	2.00	1.50	1.13
	Citrus paradisi (grapefruit)	0.50	1.00	0.75	1.13	0.50	0.38	0.25	0.58	1.00	1.00	1.00	1.00
	Juniperus virginiana (juniper)	0.50	2.00	1.00	1.25	0.50	1.00	0.42	0.63	1.00	1.00	1.00	1.00
	Lavandula angustifolia (lavender)	0.50	1.00	0.50	0.75	0.50	1.00	0.25	***0.38***	1.00	1.00	0.83	0.83
	Citrus limon (lemon)	0.50	1.00	1.00	1.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00
	Citrus aurantium var. amara flower (neroli)	0.50	0.50	1.00	2.00	0.50	1.00	1.00	1.50	1.00	1.00	1.50	1.50
	Citrus sinensis (orange)	0.50	1.00	0.42	0.63	0.50	0.75	0.42	0.69	1.00	1.00	1.50	1.50
	Rosmarinus officinalis (rosemary)	0.50	1.00	0.50	0.75	0.50	1.00	0.42	0.63	1.00	1.00	1.00	1.00
	Santalum album (sandalwood)	0.50	0.31	0.31	0.81	0.50	0.25	0.13	***0.38***	1.00	0.25	0.25	0.63
	Santalum austrocaledonicum (sandalwood)	0.50	0.01	0.08	3.88	0.50	0.13	0.25	1.21	1.00	0.13	0.75	3.26
	Cymbopogon nardus (citronella)	0.50	0.50	0.50	1.00	0.50	0.25	0.50	1.50	1.00	0.67	0.50	0.62
Pelargonium odoratissimum (geranium)	*Citrus bergamia* (bergamot)	0.38	1.00	1.00	1.82	0.50	0.50	0.42	0.83	1.00	2.00	1.00	0.75
	Citrus limon (lemon)	0.38	1.00	1.00	1.82	0.50	0.50	0.25	***0.50***	1.00	1.00	1.00	1.00
	Citrus paradisi (grapefruit)	0.38	1.00	0.50	0.91	0.50	0.38	0.42	0.96	1.00	1.00	1.00	1.00
	Lavandula angustifolia (lavender)	0.38	1.00	0.50	0.91	0.50	1.00	0.25	***0.38***	1.00	1.00	1.00	1.00
	Juniperus virginiana (juniper)	0.38	2.00	0.50	0.78	0.50	1.00	0.50	0.75	1.00	1.00	1.50	1.50
	Citrus aurantium var. amara flower (neroli)	0.38	0.50	0.50	1.16	0.50	1.00	0.50	0.75	1.00	1.00	1.00	1.00
	Citrus sinensis (orange)	0.38	1.00	0.50	0.91	0.50	0.75	0.42	0.69	1.00	1.00	1.00	1.00
	Rosmarinus officinalis (rosemary)	0.38	1.00	0.50	0.91	0.50	1.00	0.42	0.63	1.00	1.00	1.00	1.00
	Santalum album (sandalwood)	0.38	0.31	0.25	0.73	0.50	0.25	0.19	0.56	1.00	0.25	0.50	1.25
	Santalum austrocaledonicum (sandalwood)	0.38	0.01	< 0.01	***0.46***	0.50	0.13	0.25	1.21	1.00	0.13	0.50	2.17
	Cymbopogon nardus (citronella)	0.38	0.50	0.50	1.16	0.50	0.25	0.25	0.75	1.00	0.67	1.00	1.25

cont.

▶ **Table 2** *Continued*

Essential oil combinations		Mean MIC (mg/mL) (n = 3) and ΣFIC											
		B. agri (ATCC 51663)				*B. epidermidis* (DSM 20660)				*B. linens* (DSM 20425)			
Essential oil 1	**Essential oil 2**	**MIC1***	**MIC2***	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**	**MIC1**	**MIC2**	**MIC**	**ΣFIC**
Pinus sylvestris (pine)	*Cedrus atlantica* (cedarwood)	1.00	0.50	0.50	0.75	1.00	1.00	1.00	1.00	1.00	0.25	0.75	1.88
	Eucalyptus globulus (eucalyptus)	1.00	0.50	0.38	0.56	1.00	0.50	1.00	1.50	1.00	1.00	1.00	1.00
	Lavandula angustifolia (lavender)	1.00	1.00	0.78	0.78	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	Melaleuca alternifolia (tea tree)	1.00	1.00	0.75	0.75	1.00	0.50	1.00	1.50	1.00	1.50	1.00	0.83
Pogostemon patchouli (patchouli)	*Pelargonium graveolens* (rose geranium)	0.09	0.50	0.33	2.19	0.50	0.50	0.13	***0.26***	0.75	1.00	0.50	0.58
	Cedrus atlantica (cedarwood)	0.09	0.50	0.25	1.64	0.50	1.00	0.38	0.56	0.75	0.25	0.50	1.33
	Pelargonium odoratissimum (geranium)	0.09	0.38	0.25	1.72	0.50	0.50	0.50	1.00	0.75	1.00	0.50	0.58
	Commiphora myrrha (myrrh)	0.09	0.38	0.25	1.72	0.50	0.25	0.25	0.75	0.75	2.00	0.38	***0.35***
	Santalum album (sandalwood)	0.09	0.31	0.13	0.90	0.50	0.25	0.22	0.66	0.75	0.25	0.38	1.00
	Santalum austrocaledonicum (sandalwood)	0.09	0.01	0.05	2.89	0.50	0.13	0.13	0.61	0.75	0.13	0.25	1.13
Rosa damascena (rose otto) 1	*Citrus reticulata* (mandarin)	0.25	1.00	0.50	1.25	0.25	1.50	0.38	0.88	0.50	1.00	1.00	1.50
Rosa damascena (rose otto) 2	*Citrus reticulata* (mandarin)	0.50	1.00	0.50	0.75	0.25	1.50	0.25	0.58	0.50	1.00	1.00	1.50
Salvia sclarea (clary sage)	*Citrus bergamia* (bergamot)	1.00	1.00	1.00	1.00	1.00	0.50	0.75	1.13	1.00	2.00	1.50	1.13
	Cedrus atlantica (cedarwood)	1.00	0.50	0.45	0.68	1.00	1.00	1.00	1.00	1.00	0.25	0.75	1.88
	Boswellia carteri (frankincense)	1.00	1.00	0.25	***0.25***	1.00	0.50	0.50	0.75	1.00	0.75	1.00	1.17
	Pelargonium odoratissimum (geranium)	1.00	0.38	0.50	0.91	1.00	0.50	0.50	0.75	1.00	1.00	1.00	1.00
	Pelargonium graveolens (rose geranium)	1.00	0.50	0.50	0.75	1.00	0.50	0.50	0.75	1.00	1.00	1.00	1.00
	Citrus paradisi (grapefruit)	1.00	1.00	0.69	0.69	1.00	0.38	0.50	0.91	1.00	1.00	1.00	1.00
	Lavandula angustifolia (lavender)	1.00	1.00	0.75	0.75	1.00	1.00	1.00	1.00	1.00	1.00	1.50	1.50
	Citrus aurantifolia (lime)	1.00	1.00	0.63	0.63	1.00	0.50	0.50	0.75	1.00	1.00	1.00	1.00
	Citrus reticulata (mandarin)	1.00	1.00	0.75	0.75	1.00	1.50	0.88	0.73	1.00	1.00	1.50	1.50
Syzygium aromaticum (clove)	*Citrus bergamia* (bergamot)	0.50	1.00	0.50	0.75	0.38	0.50	0.50	1.16	0.50	2.00	0.50	0.63
	Citrus limon (lemon)	0.50	1.00	0.50	0.75	0.38	0.50	0.50	1.16	0.50	1.00	0.67	1.00
	Lavandula angustifolia (lavender)	0.50	1.00	0.50	0.75	0.38	1.00	0.50	0.91	0.50	1.00	0.50	0.75
Thymus vulgaris (thyme)	*Citrus limon* (lemon)	0.50	1.00	0.50	0.75	0.50	0.50	0.50	1.00	1.00	1.00	0.67	0.67
	Lavandula angustifolia (lavender)	0.50	1.00	0.50	0.75	0.50	1.00	0.50	0.75	1.00	1.00	0.58	0.58
	Citrus bergamia (bergamot)	0.50	1.00	0.50	0.75	0.50	0.50	0.50	1.00	1.00	2.00	0.83	0.63
	Pinus sylvestris (pine)	0.50	1.00	0.50	0.75	0.50	1.00	0.50	0.75	1.00	1.00	0.67	0.67
	Rosmarinus officinalis (rosemary)	0.50	1.00	0.50	0.75	0.50	1.00	0.67	1.00	1.00	1.00	0.83	0.83

*Individual MIC values taken from [16]. § Noteworthy activity (bold). † Synergistic interaction (bold italics). ‡Combinations selected purely from the aromatherapeutic literature (shaded)



► **Fig. 1** Isobologram representation of essential oils in combination against *B. agri* (ATCC 51663). ● *C. sempervirens*, ★ *C. myrrha*, ♦ *P. odoratissimum*, ■ *S. austrocaledonicum*, ● *S. sclarea*, and ○ *B. carteri* in majority volume. *Equal volume of each essential oil. Points 1–9 (► **Table 3**) provide exact concentrations of the essential oils.

► **Table 3** The concentrations of essential oils associated to the volume ratios studied against *B. agri* (ATCC 51663).

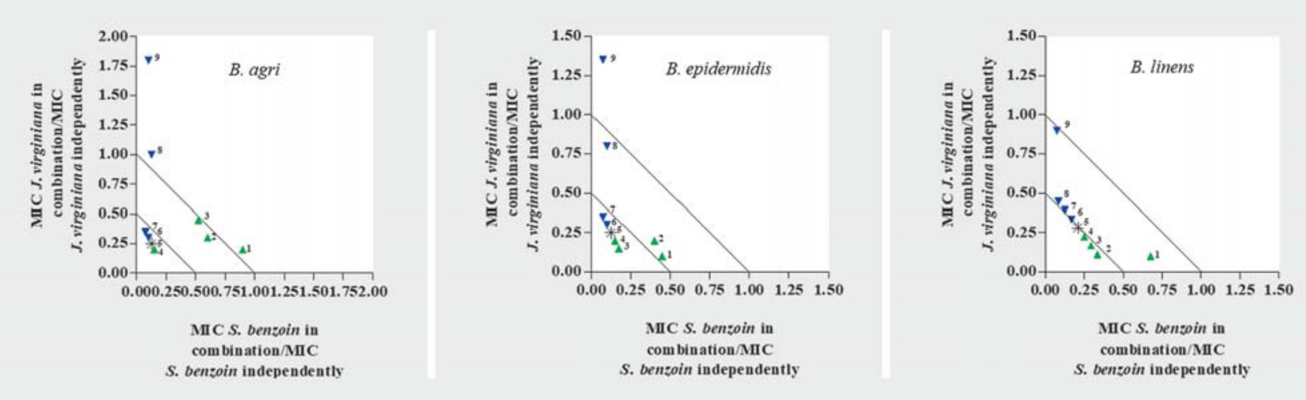
Plot number*	**Volume ratio of essential oil 1: essential oil 2**	**Concentrations of essential oils in combination**		
		C. sempervirens* and *C. myrrha	***P. odoratissimum* and *S. austrocaledonicum***	***S. sclarea* and *B. carteri***
	µL	**mg/mL**		
1	90 : 10	0.50	0.38	0.75
2	80 : 20	0.38	0.19	0.50
3	70 : 30	0.19	0.09	0.50
4	60 : 40	0.19	0.05	0.25
5	50 : 50	0.13	0.03	0.25
6	40 : 60	0.13	0.03	0.25
7	30 : 70	0.13	0.05	0.25
8	20 : 80	0.19	0.06	0.38
9	10 : 90	0.25	0.06	0.38

*Refers to points on the isobologram graphs

A quick search of the top ten perfumes of America (2017) [35] include a number of ingredients of essential oil origin. If one were to identify the most popular essential oils within these fragrances, patchouli and rose spp. are in six of the perfumes. Ylang ylang, mandarin, and bergamot are in three, vetiver and orange are in two, and geranium, lemongrass, lime, neroli, and sandalwood are also present. What is encouraging to note is that the majority of these oils that were used in the combinations investigated in this study displayed noteworthy antimicrobial activity against the malodourous bacteria, and six [*Pelargonium* spp., *P. patchouli, Citrus bergamia* (bergamot), *C. odorata*, *Santalum* spp., and *Citrus aurantifolia* (lime)] were involved in synergistic interactions. The essential oils chosen by the perfume industry were selected for their organoleptic properties. Success of these essential oils is evident by the popularity and the ever-increasing value in the industry. The antimicrobial activity displayed in this study highlights these fragrant essential oils as options for treating malodour.

The essential oil found to predominantly contribute towards synergy was *J. virginiana,* as it was observed in 8 out of the 20 synergistic interactions. This is also an essential oil recommended for body odour [17–19, 21, 22, 25–28]. In addition, two essential oils that are also recommended for odour (*Pelargonium* spp. and *L. angustifolia*) were also observed in several synergistic interactions. A previous study was also able to report on several synergistic essential oil interactions in combination with *L. angustifolia*, although it didn't investigate activity against odour-inducing bacteria [36].

The most frequently recommended combination for bromodosis was *C. sempervirens* (cypress) with *L. angustifolia* [19]. This combination displayed noteworthy antimicrobial activity against each of the *Brevibacterium* spp. (MIC 1.00 mg/mL). Interestingly, the majority of the combinations that demonstrated synergy were those selected based on the noteworthy antimicrobial activity, and not those combinations recommended in the layman's aromatherapeutic literature.



▶ **Fig. 2** Isobologram representation of *J. virginiana* and *S. benzoin* essential oils in combination against the three *Brevibacterium* spp. ▲ *J. virginiana* and ▼ *S. benzoin* in majority volume. *Equal volume of each essential oil. Points 1–9 (▶ **Table 4**) provide exact concentrations of the essential oils.

▶ **Table 4** The concentrations of the essential oil combination of *J. virginiana* and *S. benzoin* against all three *Brevibacterium* spp.

Plot number*	Volume ratio of essential oil 1: essential oil 2	Concentrations of essential oils in combination		
		J. virginiana and *S. benzoin*		
		B. agri (ATCC 51663)	*B. epidermidis* (DSM 20660)	*B. linens* (DSM 20425)
	µL	mg/mL		
1	90:10	0.50	0.75	1.50
2	80:20	0.38	0.75	1.00
3	70:30	0.38	0.50	1.00
4	60:40	0.13	0.50	1.00
5	50:50	0.13	0.50	1.00
6	40:60	0.13	0.50	1.00
7	30:70	0.13	0.50	1.00
8	20:80	0.31	0.75	1.00
9	10:90	0.50	1.00	1.50

*Refers to points on the isobologram graphs

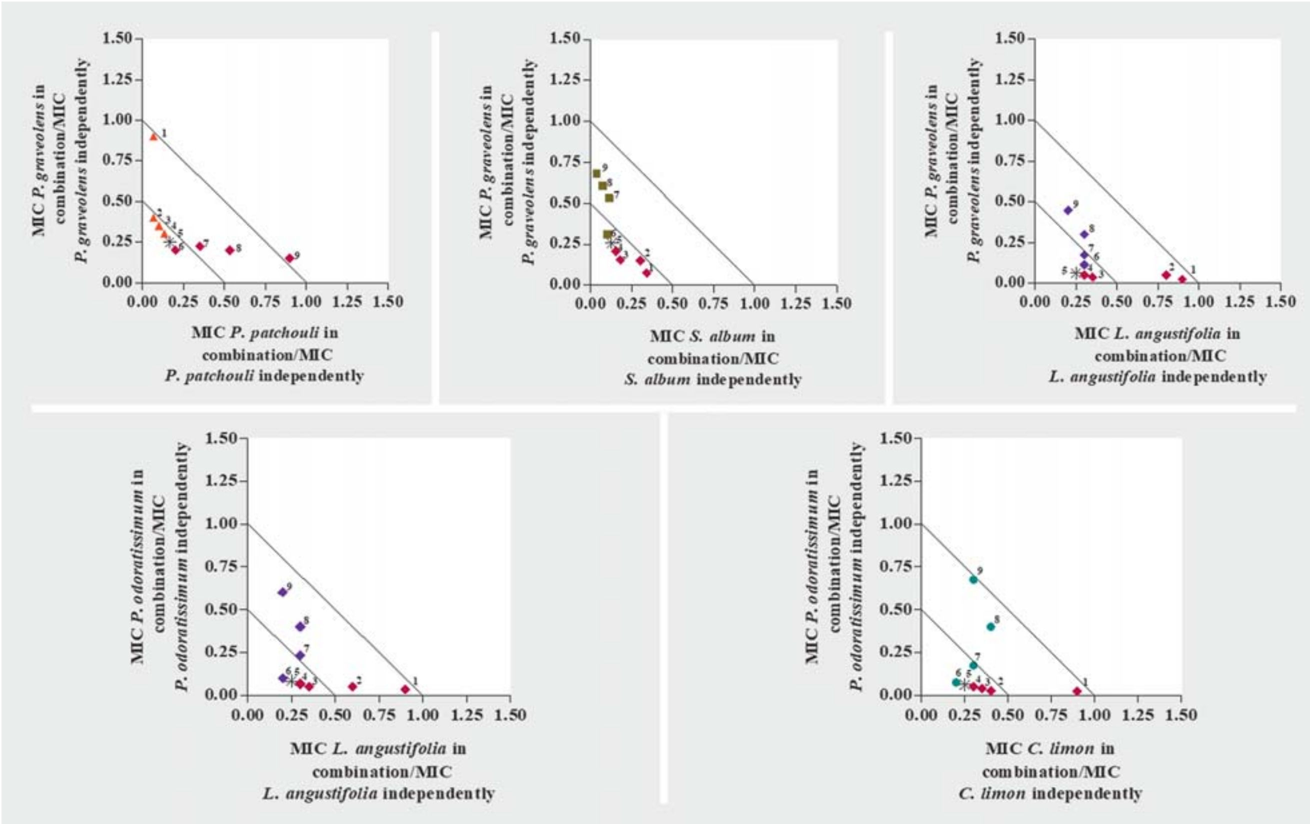
No chemotype or variation in the plant species tested offered superior antimicrobial activity when tested in combination against the different *Brevibacterium* species. This is reassuring as the design of fragrant deodorants may not necessarily be limited to one chemotype. What is important, however, is that the results herein reported be considered when selecting ingredients to formulate blends for treating malodour. Besides the offered organoleptic properties, the selection should be based on combinations that inhibit all three of the *Brevibacterium* species. There is rarely one bacterium present on the skin, thus antimicrobial activity targeting all bacteria implicated in odour is desirable.

This is the first investigation to study the influence of the ratios against *Brevibacterium* spp. The dual action offered by essential oils regarding their array of pleasant fragrance and noteworthy antimicrobial activity highlights some of these combinations as credible options for the fragrant treatment of foot odour. Several combinations (such as *C. atlantica* with *V. zizanioides*, *P. patchouli* with *S. austrocaledonicum* and *J. virginiana* with *S. benzoin*) could be highlighted for not only use against bromodosis, but also as potential combinations for developing formulations. This study provides scientific evidence for the use of selected essential oil combinations for the treatment of bromodosis and provides convincing preliminary data for their use in products promoting personal hygiene.

Materials and Methods

Essential oil procurement and quality confirmation

The essential oils (56 in total) were selected and obtained from international flavour and fragrance industries such as Givaudan (Dübendorf, Switzerland), Robertet (Grasse, France) Burgess and Finch, PranaMonde, Essentia, Scatters Oils (Gauteng, South Africa), Aromatics International, and Subtle Energies (Ayurveda aro-



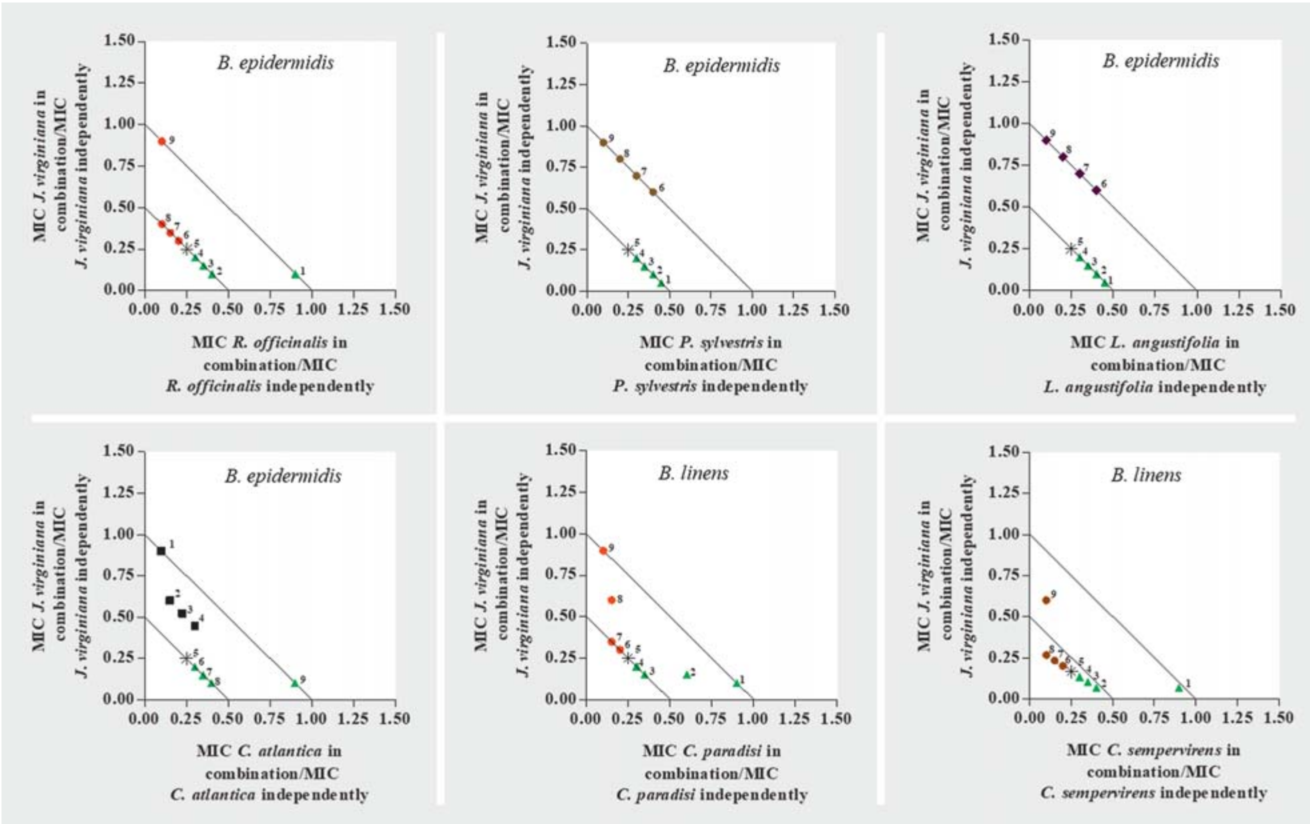
▶ **Fig. 3** Isobologram representations of essential oils in combination containing *Pelargonium* spp. against *B. epidermidis* (DSM 20660). ♦ *Pelargonium* spp., ■ *S. album*, ♦ *L. angustifolia*, ● *C. limon*, and ▲ *P. patchouli* in majority volume. *Equal volume of each essential oil. Points 1–9 (▶ **Table 5**) provide exact concentrations of the essential oils.

▶ **Table 5** The concentrations of essential oil combinations involving the *Pelargonium* spp. against *B. epidermidis* (DSM 20660).

Plot number*	Volume ratio of essential oil 1: essential oil 2	Concentrations of essential oils in combination				
		P. patchouli and *P. graveolens*	*P. graveolens* and *S. album*	*P. graveolens* and *L. angustifolia*	*P. odoratissimum* and *L. angustifolia*	*P. odoratissimum* and *C. limon*
	µL	mg/mL				
1	90 : 10	0.38	0.25	0.50	0.50	0.50
2	80 : 20	0.25	0.25	0.50	0.38	0.25
3	70 : 30	0.19	0.19	0.25	0.25	0.25
4	60 : 40	0.13	0.19	0.25	0.25	0.25
5	50 : 50	0.13	0.19	0.25	0.25	0.25
6	40 : 60	0.13	0.19	0.38	0.25	0.25
7	30 : 70	0.13	0.25	0.50	0.50	0.50
8	20 : 80	0.13	0.25	0.75	0.75	1.00
9	10 : 90	0.25	0.25	1.00	1.00	1.50

*Refers to points on the isobologram graphs

matherapy). Additional chemotypes and samples were included (▶ **Table 1**) in addition to the essential oils reported previously [16] to determine the consistency in results from different fragrance companies and the influence of the differences in major compound concentration. These are indicated with numbers, e.g., *Rosa damascena* (rose otto) **1** and *R. damascena* (rose otto)



▶ **Fig. 4** Isobologram representations of *J. virginiana* and essential oils in combination against the three *B. epidermidis* and *B. linens*. ▲ *J. virginiana*, ● *P. sylvestris*, ● *R. officinali*, ♦ *L. angustifolia*, ■ *C. atlantica*, ● *C. paradise*, and ● *C. sempervirens* in majority volume. *Equal volume of each essential oil. Points 1–9 (▶ **Table 6**) provide exact concentrations of the essential oils.

▶ **Table 6** The concentrations of essential oil combinations involving *J. virginiana* against *B. epidermidis* and *B. linens*.

Plot number*	Volume ratio of essential oil 1: essential oil 2	Concentrations of essential oils in combination					
		***B. epidermidis* (DSM 20660)**				***B. linens* (DSM 20425)**	
		J. virginiana* and *R. officinalis	***J. virginiana* and *P. sylvestris***	***J. virginiana* and *L. angustifolia***	***J. virginiana* and *C. atlantica***	***J. virginiana* and *C. paradisi***	***J. virginiana* and *C. sempervirens***
	µL	**mg/mL**					
1	90 : 10	1.00	0.25	0.50	0.50	1.00	1.00
2	80 : 20	0.50	0.25	0.50	0.25	0.75	0.50
3	70 : 30	0.50	0.25	0.50	0.25	0.50	0.50
4	60 : 40	0.50	0.25	0.50	0.25	0.50	0.50
5	50 : 50	0.50	0.25	0.50	0.25	0.50	0.50
6	40 : 60	0.50	0.50	1.00	0.38	0.50	0.50
7	30 : 70	0.50	0.50	1.00	0.38	0.50	0.50
8	20 : 80	0.50	0.50	1.00	0.38	0.75	0.50
9	10 : 90	1.00	0.50	1.00	0.50	1.00	1.00

*Refers to points on the isobologram graphs

2. The chemical compositions of the test essential oils have all previously been characterised [16, 37].

Combination selection

The selection of 119 essential oil combinations was made firstly based on the frequency of citation in the aromatherapeutic literature in treating treating body odour [17–28]. Also included were essential oils where noteworthy activity was previously reported against *Brevibacterium* spp. [16] and essential oils that were found by the researchers to exhibit a pleasant fragrance, as these may add to the organoleptic selection for future formulation possibilities.

Preparation of cultures

The microorganisms used in this study were from ATCC and Deutsche Sammlung von Mikrooganismen (DSM) strains. *B. agri* and *B. epidermidis* were grown in Tryptone Soya broth (TSB) (Oxoid) for 18 to 24 h at 37 °C and *B. linens* was grown in TSB and incubated at 30 °C for 4 days. All three *Brevibacterium* spp. were streaked onto Tryptone Soya agar (TSA) (Oxoid) plates and incubated accordingly to confirm purity. A waiver for the use of these microorganisms was granted by the University of the Witwatersrand Human Research Ethics Committee (Reference W-CJ-131026-3).

Minimum inhibitory concentration

The selected essential oil combinations were tested in 1 : 1 ratios using the broth microdilution assay [37], where the total volume of 100 µL was comprised of 50 µL of each essential oil used (Essential oil 1: Essential oil 2). Ciprofloxacin (purity ≥ 98.0 %, Sigma-Aldrich), at a concentration of 0.01 mg/mL, was included as a positive control to ensure microbial susceptibility, and 32.00 mg/mL water in acetone was used as a negative control to determine the antimicrobial effects of the solvents. A volume of 100 µL of an approximate inoculum concentration of 1×10^6 colony forming units per mL (CFU/mL) of the tested microorganisms was added to each well.

After the respective incubation periods, microtiter wells received 40 µL of 0.04 % w/v *p*-iodonitrotetrazolium violet solution (INT) (Sigma-Aldrich), and the MIC was evaluated as the lowest concentration displaying no colour change. MIC values ≤ 1.00 mg/mL were considered noteworthy [15, 16]. The individual and combined values were recorded and the ΣFIC was calculated.

The ΣFIC was calculated according to the following equations [38]:

$$\text{FIC (i)} = \frac{\text{MIC of (a}*\text{) combined with (b}*\text{)}}{\text{MIC of (a) independently}}$$

$$\text{FIC (ii)} = \frac{\text{MIC of (b) combined with (a)}}{\text{MIC of (b) independently}}$$

*Where (a) is the MIC of the first essential oil in the combination and (b) is the MIC of the second essential oil.

The FIC index was calculated to the sum ΣFIC = FIC (i) + FIC (ii). The ΣFIC for each essential oil combination was interpreted as follows: ≤ 0.5 indicates synergy, > 0.5–1.0 is additive, > 1.0 – ≤ 4.0 indicates indifference, and > 4.0 indicates antagonism [38].

Varied ratio combinations

Combinations that resulted in synergistic interactions were further evaluated at various ratio combinations according to the described MIC assay; however, the oils were placed in different ratios of 9 : 1, 8 : 2, 7 : 3, 6 : 4, 5 : 5, 4 : 6, 3 : 7, 2 : 8, and 1 : 9. The subsequent MICs of the different ratios were then captured and recorded on an isobologram using GraphPad Prism (Version 5) software and the ratio points were expressed graphically. This allowed for a graphical representation of the overall interactive influence of each essential oil in combination [38]. Synergy was displayed where the data points fell beneath or on the 0.5 : 0.5 line. Ratio points in the area above the 0.5 : 0.5 line and below and inclusive of the 1 : 1 line represent additive interactions. For data points above the 1 : 1 line and below and inclusive of the 4 : 4 line, non-interactive effects were observed. Points above the 4 : 4 line would indicate antagonism [38].

Supporting information

Essential oil voucher codes and analysis data are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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