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Essential oil composition and antimicrobial interactions of understudied tea tree species

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ABSTRACT

The essential oil composition of three Myrtaceous species (*Leptospermum petersonii*, *Leptospermum scoparium* and *Kunzea ericoides*) belonging to the tea tree group were analysed using gas chromatography coupled to mass spectrometry (GC–MS). The major compounds determined from the mean ± SD of the monthly samples collected for one calendar year in *L. petersonii* are citronellal (11.4 ± 4.3%), citronellol (17.5 ± 7.1%), neral (19.7 ± 1.6%) and geranial (34.7 ± 3.3%). The major compounds in *L. scoparium* are eudesma-4(14)-11-diene (11.6 ± 2.4%), α-selinene (10.4 ± 2.3%) and (*E*)-methyl cinnamate (12.6 ± 3.8%). The major compounds in *K. ericoides* are α-pinene (37.6 ± 6.3%) and *p*-cymene (13.5 ± 4.1%). The essential oils show some promising antimicrobial activity against selected micro-organisms when investigated using the minimum inhibitory concentration assay. Highest sensitivities were noted for the Brevibacteria (lowest MIC value of 0.06 mg/ml), a genus associated with foot odour. When the different essential oils were combined in various ratios and tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, a predominantly additive effect was noted.

1. Introduction

Plants referred to as "tea tree", belong to a group of unrelated plant species, some of which include *Camellia sinensis*, *Kunzea ericoides*, *Leptospermum scoparium*, *Leptospermum petersonii*, and *Melaleuca alternifolia*. Some of these aromatic species have been used historically by the Australian aborigines as well as by the early European settlers for a variety of infectious-related conditions including urinary tract conditions, intestinal complaints, coughs, colds, skin conditions, burns, scalds, mouth washes, gargles and gum disease (Maddocks-Jennings et al., 2005; Carson et al., 2006). The most popular, commercialised and well-studied tea tree species is undoubtedly *M. alternifolia* (Carson et al., 1995, 1996, 2002, 2006; Carson and Riley, 1995; Mann et al., 2000; Hart et al., 2000; Homer et al., 2000; Lis-Balchin et al., 2000; Banes-Marshall et al., 2001; Cox et al., 2001; Christoph et al., 2001; Russel and Southwell, 2003; Hammer et al., 2004). In comparison, research on other tea tree species such as *L. petersonii*, *L. scoparium* and *K. ericoides* have been somewhat neglected. Besides a few reports on the chemical composition and antimicrobial activity (mainly qualitative studies), very little research has been undertaken on these three lesser known species.

L. petersonii Bailey (lemon-scented tea-tree) commonly grows in Australia, but is also commercially grown in Kenya, Democratic Republic of Congo, Guatemala and South Africa. Its odour is described as "extremely pleasant and lemony". It is a tall shrub to small tree of about 5 m in height, with simple leaves, 20–40 mm long. The flowers are white, followed by woody capsules (Nuadha, 2011). Besides the seasonal variation study undertaken by Demuner et al. (2011), no detailed analysis over a 12 month cycle has been reported. The antifungal activity of *L. petersonii* has been reported (Hood et al., 2010; Kim and Park, 2012), however, studies on the antibacterial efficacies have been neglected.

L. scoparium J.R. Forst & G. Forst (manuka) is common in Tasmania and widespread in New Zealand. It is a bushy shrub which has deep green fragrant leaves that bears small white to pink flowers (Coombes, 2002). Manuka trees can reach heights of up to 8 m, especially when found within dense woodland. The tree sheds its bark in long papery strips. *L. scoparium* has been traditionally used to treat many ailments. The leaf decoction is known to offer relief from respiratory and urinary diseases, while the gum exudates are used medicinally for scalds and burns. The bark and seeds are used for infections and inflammation (Brooker et al., 1987). The essential oil composition of this species has been reported (Douglas et al., 2004), however, monthly variation studies have not been undertaken. Antimicrobial efficacies against selected pathogens such as *Candida albicans*, *Staphylococcus* spp., *Escherichia coli* and *Pseudomonas aeruginosa* have been investigated (Williams et al., 1998; Porter and Wilkins, 1999; Lis-Balchin et al., 2000). In a detailed review of this species (Stephens et al., 2005), the antimicrobial efficacies

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were not discussed, possibly due to the lack of anti-infective data known for this species. It was also recommended that further pharmacological studies be given a priority on *L. scoparium* (Stephens et al., 2005).

K. ericoides (A. Rich.) J. Thompson (kanuka), grows to more than 12 m in height. Like *L. scoparium*, the leaves are narrow, thick and less than 1.2 cm long, but *K. ericoides* leaves have rounded tips (Maddocks-Jennings et al., 2005). Although some chemical data has been published (Penfold et al., 1948; Porter and Wilkins, 1999), no detailed seasonal variation studies have been conducted on this species. Although the antimicrobial activity by disc diffusion has been reported by Lis-Balchin et al. (2000), limited attention has been given to the quantitative antimicrobial evaluation of *K. ericoides*.

Notwithstanding the current size of the tea tree oil industry, there is still enormous potential for research and commercial development on lesser known tea tree spp. such as *L. petersonii*, *L. scoparium* and *K. ericoides*. Should commercialization be a point of interest, a detailed analysis of the monthly composition and potential antimicrobial efficacy is warranted. Hence, this comprehensive study on the monthly chemical composition analysis, with an in-depth antimicrobial assay on a range of pathogens associated with the skin and respiratory, urinary and gastrointestinal tract are given. Furthermore, this study also explores the combined antimicrobial activity of these tea tree plants. It is well known that essential oils are often combined in order to increase efficacy (Harris, 2002). It has been shown that essential oils, when used in combination, may produce a heightened pharmacological effect (Van Vuuren and Viljoen, 2011). In this study, *L. petersonii*, *L. scoparium* and *K. ericoides* are combined in varied ratios to determine the interactive effects.

2. Materials and methods

2.1. Collection of plant material and distillation

Plant material consisting of the aerial parts of *L. petersonii*, *L. scoparium* and *K. ericoides* were sampled monthly from February 2007 to January 2008 with the assistance of the resident farmer, Bruce Stumbles, from a cultivated site in Magoebaskloof, north of Polokwane, Limpopo Province, South Africa. Previous studies have demonstrated that the chemical composition of the essential oils of tea tree species varies between early seedlings and more mature plants (Brophy et al., 2000), thus a collective sample, of both young and mature leaves from a selection of trees constituted each of the monthly samples. Voucher numbers were given to all monthly samples and are filed in the medicinal and aromatic plant register kept at the Department of Pharmacy and Pharmacology, University of Witwatersrand. Plant material was stored at 4 °C and distilled within 48 h of harvesting to prevent loss of volatile components. A known quantity (500–1500 g), of weighed fresh plant material was packed into a Clevenger apparatus, followed by hydrodistillation. Volatile oils were collected after 3 h on a cooling

Table 1
GC–MS data (% composition) for *L. petersonii* for the vegetative year of February 2007 to January 2008.

RRI	Compound	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Mean
	Essential oil yield (% w/w)	0.4	1.8	0.9	1.3	0.9	0.8	1.1	1.2	1.3	1.0	1.0	1.0	1.1 ± 0.3
1016	α-Pinene	0.3	0.3	0.4	0.3	0.8	0.4	–	0.4	0.4	0.5	0.3	0.2	0.4 ± 0.2
1104	β-Pinene	0.1	0.1	0.1	0.1	0.3	0.2	–	0.2	0.2	0.2	0.1	0.1	0.2 ± 0.1
1117	Sabinene	–	–	–	–	0.2	–	–	–	–	0.3	–	–	ND[a]
1159	Myrcene	1.9	1.8	1.7	1.4	1.6	1.0	0.4	1.2	0.2	1.3	1.3	1.3	0.5
1185	2,3-Dehydro-1,8-cineole	0.2	0.2	0.2	0.1	0.2	0.2	–	0.2	–	0.2	0.2	0.3	0.2 ± 0.1
1193	Limonene	0.1	–	0.1	0.1	–	0.1	–	0.1	–	0.1	–	0.2	0.1 ± 0.04
1242	γ-Terpinene	–	–	–	0.2	–	–	–	–	0.1	0.1	0.2	–	ND
1250	(*E*)-β-Ocimene	0.3	0.3	0.2	–	0.1	0.1	–	–	–	–	–	0.2	0.2 ± 0.1
1339	6-Methyl-5-hepten-2-one	0.4	0.5	0.3	0.3	0.3	0.4	0.1	0.3	0.3	0.5	0.4	0.3	0.3 ± 0.1
1352	(*Z*)-Rose oxide	–	–	–	–	–	0.1	0.1	0.1	0.1	0.2	0.1	–	0.1 ± 0.04
1357	2, 3-Hexen-1-ol	–	–	–	0.1	–	–	–	–	–	–	–	–	ND
1366	(*E*)-Rose oxide	–	–	0.1	–	–	0.1	–	0.1	–	0.1	–	–	ND
1382	(Z)-Hex-3-en-1-ol	–	–	–	–	–	–	–	–	0.1	0.1	–	–	ND
1382	Rose furan	–	–	–	–	–	0.1	–	0.1	–	0.1	–	–	ND
1482	**Citronellal**	**14.8**	**19.1**	**8.6**	**17.2**	**9.2**	**11.6**	**4.3**	**11.8**	**11.3**	**13.1**	**9.9**	**6.1**	**11.4 ± 4.3**
1546	Linalool	2.0	2.6	1.7	1.7	1.7	2.3	1.3	1.9	1.8	2.1	1.6	1.8	1.9 ± 0.3
1577	(*Z*)-Isopulegol	1.5	1.7	0.7	0.9	0.6	1.3	0.4	–	1.2	1.5	0.7	0.7	1.0 ± 0.4
1587	(*E*)-Isopulegol	3.2	3.8	1.9	2.3	1.6	3.1	1.0	–	3.1	3.8	1.7	1.8	2.5 ± 1.0
1591	β-Elemene	0.2	0.2	0.2	0.2	–	0.1	0.1	–	–	–	0.1	–	0.2 ± 0.1
1596	β-Caryophyllene	0.3	0.2	0.2	0.2	–	0.2	0.1	0.2	0.1	0.2	0.2	–	0.2 ± 0.1
1662	Citronellyl acetate	–	0.2	–	–	0.8	0.5	0.8	0.5	–	0.6	1.0	0.8	0.7 ± 0.3
1689	**Neral**	**17.9**	**19.0**	**19.1**	**21.6**	**18.9**	**20.2**	**19.2**	**22.5**	**22.3**	**18.9**	**18.2**	**18.9**	**19.7 ± 1.6**
1715	Germacrene D	0.5	–	0.3	0.3	–	–	–	–	–	0.1	0.3	0.3	0.3 ± 0.1
1740	**Geranial**	**33.8**	**33.3**	**31.5**	**37.0**	**35.7**	**35.2**	**37.0**	**40.7**	**38.9**	**31.4**	**30.4**	**31.7**	**34.7 ± 3.3**
1741	Bicyclogermacrene	1.2	1.0	0.7	1.3	1.0	0.3	0.3	0.3	0.2	0.4	1.1	1.0	0.7 ± 0.4
1758	Geranyl acetate	0.3	0.3	0.2	0.2	0.3	–	0.2	0.1	0.1	0.1	0.2	0.2	0.2 ± 0.1
1765	**Citronellol**	**13.8**	**8.8**	**23.4**	**6.5**	**17.5**	**16.7**	**26.4**	**14.0**	**11.8**	**18.4**	**26.8**	**26.1**	**17.5 ± 7.1**
1800	Nerol	0.5	0.6	0.5	0.5	0.6	0.4	0.6	0.5	0.5	0.4	0.5	0.5	0.5 ± 0.07
1847	Geraniol	3.3	3.4	3.4	3.5	3.6	2.5	3.9	3.0	3.5	2.9	3.2	3.5	3.3 ± 0.4
1848	Palustrol	–	–	–	0.1	–	–	–	–	–	–	–	–	ND
2022	Cubeben-11-ol	0.1	–	–	–	–	–	–	–	–	–	–	–	ND
2090	Globulol	0.1	0.1	–	–	0.2	–	0.2	–	–	–	0.1	–	ND
2098	(*E*)-Methyl cinnamate	–	–	–	0.1	–	–	0.1	–	–	–	–	–	ND
2101	Viridiflorol	0.1	–	–	0.1	–	–	0.1	0.1	0.2	0.1	0.1	0.2	0.1 ± 0.1
2130	Rosifoliol	–	–	–	–	–	–	–	–	0.1	–	–	–	ND
2141	Spathulenol	0.1	0.1	–	0.2	0.3	0.1	0.4	–	–	–	0.1	–	0.2 ± 0.1
2185	T-Cadinol	–	–	–	–	0.1	–	0.2	–	–	–	–	–	ND
2188	Eugenol	0.3	0.2	0.4	0.4	0.6	0.2	0.7	0.2	0.3	0.2	0.5	0.5	0.4 ± 0.17
2199	T-Muurolol	–	–	–	–	0.2	–	–	–	–	–	–	–	ND
	Total area percentage	97.3	97.8	95.9	96.9	96.4	97.4	97.9	98.5	96.8	97.9	99.3	96.7	

Major compounds are given in bold.
[a] Where constituents are present in less than 6 monthly samples, the mean was not taken into account and reported as ND.

column through which ambient temperature tap water was passed. Once cooled and phase separation achieved, the hydrosol in the separating column was discarded and the remaining essential oils were collected, weighed and stored in tightly sealed amber bottles at ~4 °C until further analysis.

2.2. Chemical composition (gas chromatography coupled to mass spectrometry)

All essential oils sampled over a one-year period were analysed by gas chromatography coupled to mass spectrometry (GC–MS) using an Agilent 6890N GC system coupled directly to a 5973 MS. A volume of 1 μl was injected using a split ratio (200:1) with an autosampler at 24.79 psi and an inlet temperature of 250 °C. The GC system equipped with a HP-Innowax polyethylene glycol column 60 m × 250 μm i.d. × 0.25 μm film thickness was used. The oven temperature programme was 60 °C for the first 10 min, rising to 220 °C at a rate of 4 °C/min and held for 10 min and then rising to 240 °C at a rate of 1 °C/min. Helium was used as a carrier gas at a constant flow of 1.2 ml/min. Spectra were obtained on electron impact at 70 eV, scanning from 35 to 550 m/z. The percentage composition of the individual components were obtained from electronic integration measurements using flame

Table 2
GC–MS data (% composition) for *L. scoparium* for the vegetative year of February 2007 to January 2008.

RRI	Compound	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Mean
	Essential oil yield (% w/w)	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.04	0.1	0.1	0.1	0.1	0.1 ± 0.1
1016	α-Pinene	0.5	0.4	0.6	0.2	0.8	0.4	0.2	5.6	0.4	1.3	0.7	0.7	1.0 ± 1.5
1104	β-Pinene	0.1	–	0.1	–	–	–	–	0.7	0.2	–	0.1	0.1	0.2 ± 0.2
1159	Myrcene	0.2	0.2	0.2	0.1	0.2	0.1	0.1	0.6	–	0.4	0.4	0.4	0.3 ± 0.2
1192	α-Terpinene	–	0.1	–	–	–	–	–	–	–	–	–	–	ND[a]
1194	Limonene	0.1	0.2	0.1	0.1	0.2	0.2	–	0.5	0.1	–	0.2	0.1	0.2 ± 0.1
1202	1,8-Cineole	1.5	2.1	1.5	1.3	1.3	2.3	1.3	5.7	2.0	2.7	2.4	1.0	2.1 ± 1.2
1242	γ-Terpinene	0.1	0.2	0.1	0.1	0.2	0.2	–	0.2	0.1	–	0.3	0.2	0.2 ± 0.1
1250	(*E*)-β-Ocimene	0.1	0.2	0.1	0.1	0.2	0.1	–	0.3	0.3	–	0.4	0.5	0.2 ± 0.1
1270	p-Cymene	0.1	–	0.2	–	–	–	–	0.8	0.1	–	0.1	0.1	0.2 ± 0.3
1382	(*Z*)-Hex-3-en-1-ol	0.4	–	0.4	0.4	0.3	0.2	–	0.9	0.2	0.5	0.2	0.1	0.4 ± 0.2
1456	α-Cubebene	2.0	1.7	0.8	0.6	0.5	0.6	0.4	0.4	1.7	1.0	2.4	2.0	1.2 ± 0.7
1467	Iso-ledene	–	0.2	–	0.1	0.1	0.2	0.2	–	–	–	–	–	ND
1479	Ylangene	–	–	–	–	0.2	0.2	–	–	–	–	–	–	ND
1493	α-Copaene	0.9	1.1	0.5	0.7	0.6	0.6	0.7	0.7	0.8	0.3	0.8	0.8	0.7 ± 0.2
1533	α-Gurjunene	2.9	0.7	0.4	0.5	0.3	0.4	–	0.3	0.4	0.3	0.7	0.5	0.7 ± 0.7
1546	Linalool	0.1	3.3	3.5	3.2	3.5	4.1	2.3	4.9	3.8	4.1	3.8	2.5	3.3 ± 1.2
1584	Bergamotene	0.6	0.7	0.4	0.4	0.3	0.5	0.3	0.3	0.5	–	0.2	0.3	0.4 ± 0.1
1591	β-Elemene	1.9	2.0	0.7	0.8	0.5	0.6	0.3	0.6	2.9	2.1	1.5	2.4	1.4 ± 0.9
1595	β-Gurjunene	0.6	0.3	0.4	0.7	0.3	0.3	0.6	0.5	0.4	–	0.7	0.4	0.5 ± 0.1
1596	β-Caryophyllene	4.7	4.6	3.9	3.2	2.9	2.7	2.3	2.5	5.1	4.7	6.0	4.6	3.0 ± 1.2
1610	Guaia-1(10),11-diene	3.7	2.9	2.5	3.4	2.6	3.5	2.9	4.7	2.8	2.3	2.2	2.2	3.0 ± 0.7
1636	Cadina-3,5-diene	2.4	2.5	0.4	0.7	0.5	0.6	0.5	0.3	3.1	2.2	4.8	4.9	1.9 ± 1.7
1647	*allo*-Aromadendrene	0.7	0.6	0.4	0.7	0.5	0.7	0.7	0.7	0.6	–	0.6	0.6	0.6 ± 0.1
1668	*epi*-Zonorene	1.7	2.3	–	0.9	0.5	0.6	0.5	0.3	1.0	0.9	1.8	1.7	1.1 ± 0.7
1674	α-Humulene	3.7	2.0	0.6	1.6	1.3	0.9	0.9	0.2	1.6	–	0.6	1.7	1.4 ± 0.9
1682	Selina-4,11-diene	–	2.2	–	1.6	1.5	1.7	1.2	1.2	1.8	1.1	0.9	–	1.5 ± 0.4
1700	Methyl nerolate	–	–	–	–	–	–	–	–	–	–	3.4	1.8	ND
1703	Ledene	1.7	2.4	2.2	2.0	2.5	1.8	1.6	2.8	1.5	1.2	1.4	1.4	1.9 ± 0.5
1715	Germacrene D	0.7	1.4	0.5	1.1	0.6	0.8	0.5	0.4	1.1	0.7	0.8	1.2	0.8 ± 0.3
1723	Bicyclosesquiphellandrene	–	–	–	0.5	0.4	0.4	0.3	–	–	–	1.2	–	ND
1735	**Eudesma-4(14),11-diene**	**13.4**	**13.9**	**13.6**	**12.4**	**12.2**	**14.5**	**11.6**	**11.1**	**12.1**	**9.9**	**6.2**	**8.3**	**11.6 ± 2.4**
1735	**α-Selinene**	**13.5**	**13.4**	**12.5**	**10.9**	**10.3**	**12.4**	**9.5**	**8.9**	**10.8**	**9.2**	**5.9**	**7.8**	**10.4 ± 2.3**
1743	γ-Elemene	1.9	1.1	0.4	0.9	0.6	0.6	0.3	0.8	4.3	2.9	3.5	4.7	1.8 ± 1.6
1749	α-Farnescene	1.3	1.9	0.5	0.7	0.4	0.4	0.4	0.2	1.1	0.8	0.7	1.3	0.8 ± 0.5
1758	Geranyl acetate	0.2	0.2	0.3	0.2	0.4	0.4	0.3	0.7	0.5	0.5	0.4	0.8	0.4 ± 0.2
1763	δ-Cadinene	3.6	4.4	2.9	3.6	2.5	2.6	2.7	2.0	2.5	2.2	4.1	3.5	3.0 ± 0.8
1768	γ-Cadinene	0.5	0.6	0.5	0.6	0.5	0.5	0.6	0.5	0.5	0.3	0.5	0.5	0.5 ± 0.1
1773	α-Panasensin	0.5	0.4	0.4	0.3	0.3	0.4	0.3	0.3	0.5	0.4	0.3	0.4	0.4 ± 0.1
1789	Cadina-1,4-diene	1.5	1.9	0.9	1.3	0.9	1.0	1.2	0.6	1.2	1.0	1.9	1.8	1.3 ± 0.4
1844	Calamenene	3.7	2.9	5.7	5.7	5.6	5.2	7.4	6.0	4.9	3.2	5.5	4.0	5.0 ± 1.3
1916	Calacorene	0.2	0.2	0.4	0.6	0.6	0.6	0.7	0.5	0.2	–	0.2	0.1	0.4 ± 0.2
1948	Palustrol	0.1	0.1	0.1	0.2	0.2	0.1	0.2	–	–	0.3	0.2	0.2	0.2 ± 0.1
1960	(*Z*)-Methyl cinnamate	0.2	0.2	0.6	0.6	1.3	1.2	1.3	2.6	0.9	0.7	0.3	0.4	0.9 ± 0.7
2010	Caryophyllene oxide	–	0.4	1.0	1.6	2.7	2.0	3.6	1.5	0.9	0.7	0.6	0.7	1.4 ± 1.0
2021	Methyl eugenol	0.1	0.2	0.2	–	–	–	–	0.2	0.2	–	0.2	0.1	0.2 ± 0.0
2024	*epi*-Globulol	–	–	–	0.5	0.5	0.5	0.6	–	–	–	–	–	ND
2074	Cubenol	0.3	0.3	0.4	0.8	0.6	0.5	1.1	0.6	0.3	0.6	0.4	0.3	0.5 ± 0.2
2022	Cubeben-11-ol	–	–	–	0.4	0.4	0.4	0.5	–	–	–	–	–	ND
2080	1-*epi*-Cubenol	0.4	0.4	–	0.8	0.6	0.5	0.8	0.4	0.5	0.8	0.9	0.8	0.6 ± 0.2
2098	**(*E*)-Methyl cinnamate**	**10.6**	**9.8**	**15.4**	**12.3**	**15.8**	**9.8**	**9.2**	**11.3**	**11.8**	**19.5**	**11.4**	**14.3**	**12.6 ± 3.1**
2101	Viridiflorol	0.3	0.3	0.5	0.6	0.6	0.6	0.8	0.6	0.5	1.1	0.8	0.5	0.6 ± 0.2
2130	Rosifoliol	0.4	0.6	0.7	0.9	0.8	0.8	1.0	0.7	0.5	1.4	0.6	0.6	0.7 ± 0.3
2141	Spathulenol	0.7	0.6	1.2	1.4	1.6	1.5	2.0	1.4	0.7	1.2	1.2	1.1	1.2 ± 0.4
2235	α-Eudesmol	0.8	0.9	1.4	1.1	1.6	1.6	2.3	1.2	0.7	1.4	1.6	0.8	1.3 ± 0.5
2245	β-Eudesmol	0.7	0.8	1.3	1.0	1.7	1.6	2.7	1.3	0.7	1.5	1.6	0.8	1.3 ± 0.6
2268	Eudesm-7-(11)-en-4α-ol	1.1	1.2	2.3	2.1	2.3	2.1	2.6	1.1	1.3	3.5	1.2	1.5	1.9 ± 0.7
	Total area percentage	87.4	91.0	83.7	86.5	87.8	86.5	81.5	90.6	90.1	88.9	88.8	87.5	

Major compounds are given in bold.
[a] Where constituents are present in less than 6 monthly samples, the mean was not taken into account and reported as ND.

Table 3
GC–MS data (% composition) for *K. ericoides* for the vegetative year of February 2007 to January 2008.

RRI	Compound	Feb	Mar	Apr	May	Jun	Jul	Sep	Oct	Nov	Dec	Jan	Mean
	Essential oil yield (% w/w)	0.4	0.4	0.6	0.3	0.3	0.3	0.4	0.2	0.5	0.5	0.4	0.4 ± 0.1
1016	**α-Pinene**	**36.6**	**41.8**	**26.2**	**36.0**	**42.8**	**30.0**	**32.3**	**35.5**	**40.5**	**46.7**	**42.7**	**37.6 ± 6.3**
1019	α-Thujene	3.0	1.2	1.0	1.1	0.5	1.0	0.7	0.6	1.6	2.0	1.6	1.4 ± 0.7
1104	β-Pinene	0.6	0.5	0.4	0.5	0.5	0.4	0.4	0.5	0.6	0.9	0.7	0.5 ± 0.2
1120	Isoamyl acetate	0.2	–	–	0.2	–	–	–	–	–	–	–	ND[a]
1160	α-Phellandrene	0.2	–	–	0.1	–	–	–	–	–	–	–	ND
1192	α-Terpinene	0.3	0.3	0.1	0.2	0.2	0.2	–	–	0.5	0.4	0.4	0.3 ± 0.1
1193	Limonene	1.8	1.7	1.2	0.2	1.5	1.4	1.3	1.3	1.6	1.7	1.7	1.4 ± 0.4
1202	1,8-Cineole	3.6	3.5	2.2	4	3	3.9	3.8	5.6	2.5	2.9	2.1	3.4 ± 1.0
1218	(*E*)-2-Hexanal	–	–	–	0.3	–	–	–	–	–	–	–	ND
1232	(*Z*)-β-Ocimene	–	–	0.3	0.1	0.1	–	–	–	–	–	–	ND
1242	γ-Terpinene	7.4	7.3	5.5	5.7	7.3	6.3	3.5	3.4	9.7	11.9	9.9	7.1 ± 2.6
1250	(*E*)-β-Ocimene	0.4	0.4	–	–	–	–	–	–	–	–	0.5	ND
1261	Styrene	–	0.5	–	–	–	–	0.5	–	–	–	0.3	ND
1266	Amyl Isovalerate	0.4	0.4	–	0.4	0.3	0.4	–	0.4	–	0.3	0.3	0.4 ± 0.1
1270	***p*-Cymene**	**13.1**	**14.8**	**11.3**	**19.1**	**14.9**	**16.6**	**18.0**	**14.5**	**8.9**	**5.8**	**9.6**	**13.5 ± 4.1**
1281	Terpinolene	1.7	1.6	1.2	1.2	–	1.6	–	0.9	2.7	3	2.3	1.8 ± 0.7
1349	1-Hexanol	–	0.1	–	–	–	–	–	–	–	–	0.9	ND
1357	2, 3-Hexen-1-ol	–	0.2	–	–	–	–	–	–	–	–	–	ND
1382	(*Z*)-Hex-3-en-1-ol	0.2	–	–	0.3	0.2	0.1	–	–	–	–	0.2	ND
1436	*p*-Cymenene	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.3	–	0.1	0.2	0.3 ± 0.1
1441	(*E*)-Linalool oxide (furanoid)	0.3	–	0.2	0.2	0.2	0.3	–	0.2	–	0.2	0.2	0.2 ± 0.1
1441	β-Thujone	–	–	–	0.1	–	–	–	–	–	–	–	ND
1471	(*Z*)-Linalool oxide (furanoid)	0.2	–	–	–	–	0.1	–	0.3	–	0.1	–	ND
1493	α-Copaene	–	–	0.3	–	–	–	–	–	–	–	–	ND
1494	α-Campholenal	–	0.2	–	1.2	0.7	1.1	1.1	1.2	–	0.3	–	0.8 ± 0.4
1495	α-Pinene epoxide	0.4	0.5	0.7	–	–	–	–	–	0.5	–	0.5	ND
1554	Isopinocamphone + Pinocamphone	0.1	–	–	0.2	0.2	–	–	–	–	–	–	ND
1533	α-Gurjunene	0.2	0.1	0.4	–	0.2	–	0.7	–	–	–	0.2	0.3 ± 0.2
1546	Linalool	4.5	4.6	2.1	2.1	2.8	1.4	–	1.3	2.7	3.8	3.8	2.9 ± 1.2
1573	Pinocarvone	0.6	0.6	0.5	1.1	0.8	1.6	1.8	2.4	1.0	0.4	0.4	1.0 ± 0.7
1602	Terpinen-4-ol	1.3	1.1	1.1	1.2	1.1	1	0.8	0.7	1.2	1.4	1.4	1.7 ± 1.7
1604	Hotrienol	0.1	0.1	0.1	0.1	–	0.1	–	–	–	0.1	0.1	0.2 ± 0.0
1604	Aromadendrene	–	1.5	0.1	–	0.1	–	–	–	2.0	–	–	ND
1639	Myrtenal	–	–	0.1	0.2	0.1	0.2	–	–	–	0.3	0.3	0.2 ± 01
1647	*allo*-Aromadendrene	0.3	–	0.6	–	–	0.3	–	–	–	0.9	1.1	ND
1661	(*E*)-Pinocarveol	1.5	0.1	1.2	2.5	1.9	–	4.4	5.6	–	0.1	0.2	1.9 ± 2.0
1674	γ-Terpineol	–	0.3	–	–	–	0.2	–	–	–	0.2	0.4	ND
1674	α-Humulene	–	–	–	–	0.1	–	–	–	–	–	–	ND
1692	Chamigren	0.1	–	–	0.5	0.9	–	–	–	–	–	–	ND
1701	α-Terpineol	1.0	–	–	–		2.0	1.5	–	–	–	–	ND
1701	*p*-Menth-1-en-8-ol	1.0	2.1	1.6	2.0	1.6	0.1	1.1	1.9	1.5	1.5	1.6	1.5 ± 0.6
1703	Ledene	0.7	0.1	1.4	–	–	0.7	–	0.7	0.8	0.2	0.5	0.6 ± 0.4
1719	Verbenone	0.2	0.2	0.3	0.3	0.3	0.5	0.7	0.7	–	0.1	0.2	0.4 ± 0.2
1728	α-Muurolene	–	0.1	0.2	–	0.1	–	–	0.9	–	0.6	0.1	0.3 ± 0.3
1741	Bicyclogermacrene	0.2	0.1	–	–	–	–	–	0.3	–	0.2	0.1	ND
1745	Carvone	–	–	–	0.2	0.4	–	–	–	–	–	–	ND
1743	γ-Elemene	–	–	0.4	–	–	–	–	–	–	–	–	ND
1751	Germacrene B	–	–	0.1	–	–	–	–	–	–	–	–	ND
1758	Geranyl acetate	–	–	0.2	–	–	–	–	–	0.8	–	–	ND
1763	δ-Cadinene	0.2	–	0.6	0.1	0.3	0.2	–	–	–	0.5	–	0.3 ± 0.2
1768	γ-Cadinene	–	–	–	–	–	0.1	–	–	–	0.1	–	ND
1789	Cubebene	0.2	–	0.8	–	0.2	0.3	–	–	0.9	–	0.6	0.5 ± 0.3
1857	Myrtenol	0.1	–	0.1	–	–	0.3	–	–	–	–	–	ND
1839	(*E*)-Carveol	0.5	0.6	–	0.9	0.7	1.3	1.4	1.6	0.8	0.9	0.5	0.9 ± 0.4
1844	Calamenene	1.0	1.1	2.8	0.9	1.6	1.1	2.1	1.2	1.3	0.3	1.0	1.2 ± 0.5
1855	*p*-Cymen-8-ol	0.4	0.5	–	0.7	0.7	1	1.3	1.2	–	–	–	0.8 ± 0.4
1876	(*Z*)-Carveol	0.1	–	0.8	–	–	0.1	–	–	–	–	0.1	ND
1895	Benzyl isovalerate	0.1	–	–	–	–	–	–	–	–	–	–	ND
1916	Calacorene	–	–	0.2	–	–	–	–	–	–	–	–	ND
1945	Cubelol	–	–	1.2	0.4	0.6	–	–	–	–	–	–	ND
1948	Palustrol	0.4	0.4	–	–	–	0.6	1	–	0.9	–	0.4	0.6 ± 0.3
1975	Phenyl ethyl butyrate	0.1	0.1	0.1	0.1	0.2	–	–	–	–	–	0.1	0.1 ± 0.04
2001	Phenyl ethyl propionate	0.2	0.1	0.7	0.2	0.3	–	–	–	–	0.2	0.3	0.3 ± 0.2
2010	Caryophyllene oxide	–	–	–	–	–	–	–	0.7	–	–	–	ND
2050	Ledol	1.0	0.2	4.6	1.7	2.2	2.4	2.9	2.3	0.5	1.3	1.7	1.9 ± 1.2
2022	Cubeben-11-ol	0.2	0.2	0.9	–	–	0.3	–	–	–	0.2	0.2	0.3 ± 0.3
2074	Cubenol	–	–	0.3	–	–	–	–	–	–	–	–	ND
2130	Viridiflorol	3.2	3.7	**14.5**	5.2	7.3	7.5	8.4	7.0	2.2	4.3	4.6	5.3 ± 2.1
2141	Spathulenol	0.4	0.4	0.1	0.9	1.2	1.3	2.3	1.6	7.8	0.6	0.5	1.6 ± 2.2
2199	T-Muurolol	–	–	0.3	–	0.1	–	–	–	–	–	–	ND
2201	α-Cadinol	–	–	0.2	–	–	–	–	–	–	–	–	ND
2211	Thymol	–	–	0.3	–	–	–	–	–	–	–	–	ND
2225	Carvacrol	–	–	0.2	–	–	–	–	–	–	–	–	ND
	Total area percentage	90.6	93.6	90.0	92.7	98.5	88.4	92.5	94.8	93.5	94.5	94.5	

August compositional analysis excluded due to lack of sample. Major compounds are given in bold.
[a] Where constituents are present in less than 6 monthly samples, the mean was not taken into account and reported as ND.

ionization detection (FID, 250 °C). *n*-Alkanes were used as reference points in the calculation of relative retention indices (RRI). Component identifications were made by comparing mass spectra and retention indices. Library searches were carried out using NIST®, Mass Finder® and Flavour® libraries (Van Vuuren et al., 2010).

2.3. Antimicrobial activity (minimum inhibitory concentration assay)

The micro-dilution minimum inhibitory concentration (MIC) assay was used to quantify antimicrobial efficacy according to the NCCLS guidelines (2003). The lowest concentration of the test sample in which no growth occurred was defined as the MIC. Micro-organisms studied included Gram-positive bacteria; *Staphylococcus aureus* ATCC 12600, *Staphylococcus epidermidis* ATCC 2223 (common skin pathogens and commensals), *Mycobacterium smegmatis* ATCC 14468 (non-pathogenic screening strain for tuberculosis infections), *Enterococcus faecalis* ATCC 29212 (which can cause urinary tract and gastro-intestinal tract infections), *Streptococcus pyogenes* ATCC 8668, *Streptococcus agalactiae* ATCC 55618 and *Streptococcus pneumoniae* ATCC 49247 (β-haemolytic bacteria causing a variety of systemic diseases), *Propionibacterium acnes* ATCC 11827 (cause of acne vulgaris) and *Brevibacterium* species (*Brevibacterium brevis* ATCC 8246, *Brevibacterium agri* ATCC 51663 and *Brevibacterium laterosporum* ATCC 64). The Brevibacteria are a group of non-pathogenic micro-organisms which are associated with foot odour. Gram-negative bacteria included *Moraxella catarrhalis* ATCC 23246 (which may cause sinusitis, otitis media and respiratory tract infections), *P. aeruginosa* ATCC 9027 (responsible for nosocomial and respiratory tract infections) and *Klebsiella pneumoniae* ATCC 13883 (causes bacteraemia and pneumonia). The yeast test organisms were *Cryptococcus neoformans* ATCC 90112 (responsible for some nosocomial infections and meningitis) and *C. albicans* ATCC 10231, which infects epithelial tissue (Boyd and Hoerl, 1981; Bannister et al., 2000). All reference cultures were purchased from Davies Diagnostics (South Africa, Pty Ltd).

Using aseptic manipulation 100 μl distilled (Millipore Hemo-Ro*60) sterile water was transferred into each well of a 96 well microtitre plate. The essential oils were diluted to a starting concentration of 128 mg/ml in acetone (Sigma-Aldrich), and 100 μl was transferred into the first row of the microtitre plate. Serial dilutions were performed, starting from 32 mg/ml found in the first well and transferring 100 μl consecutively so that each doubling dilution is reduced by half in each well. Thereafter, 100 μl of the standardized culture suspension was added to each of the wells. Cultures were grown in fresh Tryptone Soya broth (TSB, Sigma-Aldrich) yielding an approximate inoculum size of 1×10^6 colony forming units per millilitre (CFU/ml). Exceptions to the protocol were *P. acnes* which was cultured in Thioglycolate (Sigma-Aldrich) broth and the β-haemolytic bacteria which were cultured in Mueller Hinton broth (Oxoid) with the addition of 2.5% sheep blood. Positive controls, ciprofloxacin (Sigma-Aldrich) at a 0.01 mg/ml stock concentration for bacteria and amphotericin B (Sigma-Aldrich) at a 0.1 mg/ml stock concentration for yeasts were included in each assay to confirm the antimicrobial susceptibility. Negative controls (acetone–water mixture) were included to assess the antimicrobial effect of the solvent. Broth used in each assay was incubated independently to assure sterility. An inoculum of the standardized culture was streaked on an appropriate agar plate for single colonies to assure that the cultures were not contaminated. Each plate was subsequently covered with an adhesive cellophane strip to prevent the escape of any volatile components and incubated at 37 °C for 24 h. *P. acnes* was incubated under 95% CO_2 anaerobic conditions at 37 °C for 7 days without the cellophane sheet to allow for exposure to the CO_2 environment. Forty microlitres of *p*-Iodonitrotetrazolium chloride (0.04% w/v) (Sigma-Aldrich) was added to each well of the plates and the results were read after 6 h. Tests were performed at least in duplicate and on consecutive days.

2.4. Antimicrobial interactions

The combined essential oils of *L. petersonii* with *K. ericoides*, *L. petersonii* with *L. scoparium* and *K. ericoides* with *L. scoparium* were investigated in nine different ratios (i.e. 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8 and 1:9). The adapted microdilution checkerboard method was used (Schelz et al., 2006; Van Vuuren and Viljoen, 2009) to determine interactive antimicrobial efficacies for all ratios against one Gram-positive (*S. aureus* ATCC 12600) and one Gram-negative test organism (*P. aeruginosa* ATCC 9027) and the yeast *C. albicans*, ATCC 10231. Positive controls i.e. ciprofloxacin and amphotericin B were included in each assay to confirm the antimicrobial susceptibility. The interaction between various ratios of the two test oils was plotted on an isobologram. The isobolograms were constructed using GraphPad™ Prism 5 software and represent the results of the MIC assay where the MIC value for each oil in the combination is plotted as a ratio point representative of the effects in combination. Where data points on the isobologram are below or equal to the 0.5 line, they are regarded as synergistic. Points lying between 0.5 and including 1.0 are regarded as additive. Points above 1.0 and including 4.0 are regarded as indifferent and points above 4.0 are regarded as antagonistic (Suliman et al., 2010; Van Vuuren and Viljoen, 2011).

3. Results and discussion

3.1. Compositional analysis of L. petersonii

The annual essential oil yields were more or less constant throughout the year (1.1 ± 0.3%). However, the yield obtained in February was much lower (0.4%), while the March yield was higher (1.8%). Forty compounds representing between 88.4 and 98.5% of the total composition were identified. The major compounds (calculated as a mean of 12 monthly samples) in *L. petersonii* were citronellal (11.4 ± 4.3%), citronellol (17.5 ± 7.08%), neral (19.7 ± 1.6%) and geranial (34.7 ± 3.3%). Composition was mostly constant throughout the year with some variation noted for citronellal and citronellol (Table 1).

The chemistry of *L. petersonii* oil was first reported under the name *Leptospermum citratum* by Challinor et al. in 1918 (Brophy et al., 2000) and described to have a "pleasant lemon scented odour" due to its principle components citronellal and citral (Penfold et al., 1948; Brophy et al., 2000). Recently, a seasonal variation study was undertaken in Brazil, where compositional results were collectively reported for dry and rainy seasons. Even though harvested on different continents, the major compounds reported in the earlier study and the current investigation are similar. The only incongruence noted was that citronellol, present in this study was not found in the *L. petersonii* species from Brazil (Demuner et al., 2011).

3.2. Compositional analysis of L. scoparium

The monthly essential oil yields were consistent (0.1 ± 0.1) throughout the year (Table 2). Fifty-six compounds representing between 81.5 and 91.0% of the total composition present in *L. scoparium* were identified. The major compounds (calculated as a mean of 12 monthly samples) in *L. scoparium* are eudesma-4(14), 11-diene (11.6 ± 2.4%), α-selinene (10.4 ± 2.3%), and (*E*)-methyl cinnamate (12.6 ± 3.8%) (Table 2).

Previous studies by Costa et al. (2010), have reported major compounds as α-copaene (36.0%) and (*E*)-caryophyllene (13.1%) from *L. scoparium*. α-Selinene was only present in minor (0.9%) quantities. Eudesma-4(14), 11-diene and (*E*)-methyl cinnamate, found as major compounds in this study, were not reported in the previous study. This compositional variation is not surprising as Douglas et al. (2004) reported on the high degree of infraspecific essential oil compositional variation from several geographical regions in New Zealand. Of the eleven different chemotypes recognised, none closely resembled that found in this study.

3.3. Compositional analysis of *K. ericoides*

The average oil yield was (0.4 ± 0.1%) with the lowest yields in October (0.2%) and highest yields in April (0.6%). Seventy-four compounds, representing between 88.4 and 94.5% of the total oil in *K. ericoides* were identified. The mean of the major compounds found in all the monthly samples analysed was α-pinene (37.6 ± 6.3%) and *p*-cymene (13.5 ± 4.1%) (Table 3). The chemical profile was qualitatively and quantitatively consistent of the sampling period. Porter and Wilkins (1999), also reported α-pinene as a major compound from *K. ericoides* essential oil. In a later study, by Wyatt et al. (2005), globulol at 18.4% was observed as a major constituent of *K. ericoides*.

3.4. Antimicrobial activity of tea tree essential oils

The antimicrobial efficacy, expressed as an MIC in mg/ml, against 16 test organisms are summarised in Table 4. Previously, Van Vuuren (2008) recommended when analysing the antimicrobial activity of essential oils that activities with MIC values ≤2.00 mg/ml should be considered as noteworthy. Thus, notable activity for *L. petersonii* was observed for 11 of the 16 pathogens studied, particularly against *B. agri* (MIC 0.06 mg/ml). The other Brevibacteria species (*B. brevis* and *B. laterosporum*) also showed some of the most prominent sensitivities (1.00 and 0.25 mg/ml, respectively). A similar, but slightly less effective trend was noted for the antimicrobial activities of *L. scoparium* and *K. ericoides*.

Previous antimicrobial investigations on *L. petersonii* include a number of disc diffusion studies, the most recent of which has been reported by Demuner et al. (2011). Several limitations of disc diffusion studies and the recommendation to use a quantitative (MIC) method of antimicrobial analysis (Kalemba and Kunicka, 2003) make these earlier studies somewhat redundant. A few studies have focused on specific organisms i.e. the investigation on phytopathogenic fungi (Lee et al., 2008; Hood et al., 2010; Kim and Park, 2012). A recent study focusing on dermatophytes (*Microsporum canis*, *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *Epidermophyton floccosum* and *Microsporum gypseum*) found *L. petersonii* oil to be more than 90% effective against all strains tested (Park et al., 2007). Interestingly, the *Brevibacterium* genus, having the highest efficacy in this study is also associated with dermatophytic conditions, clearly suggesting that this oil may be of commercial importance in the treatment of skin conditions. Less attention has been devoted to the antimicrobial efficacies of *L. scoparium*. Some earlier studies, either disc diffusion (Williams et al., 1998; Lis-Balchin et al., 2000) or more quantitative MIC studies (Porter and Wilkins, 1999) have reported on activity against *C. albicans*, *S. aureus*, *E. coli* and *P. aeruginosa*, but little is known on the antimicrobial efficacies against other pathogens as detailed in this study. In a review of *L. scoparium* by Stephens et al. (2005), the antimicrobial efficacy is under reported, emphasising the need for a more detailed antimicrobial screening. The activities noted for the Brevibacteria (0.06–1.00 mg/ml) and *S. agalactiae* (0.50 mg/ml) clearly show some interesting noteworthy activities (Table 4), and hence warrants further investigation for potential applications in infection control.

Even less is known on the antimicrobial efficacies of *K. ericoides*. Some studies have been undertaken on the extracts (Wyatt et al., 2005) and on some food spoilage organisms (Lis-Balchin et al., 2000). Only one other quantitative study (Porter and Wilkins, 1999), reporting on activity on the commonly studied test organisms *S. aureus*, *E. coli*, *P. aeruginosa* and *C. albicans* has been undertaken. Other pathogens have been neglected and the promising activities observed for the Brevibacteria warrant noting.

3.5. Antimicrobial interactions

The combinations of *L. petersonii* with *L. scoparium*, *L. petersonii* with *K. ericoides* and *L. scoparium* with *K. ericoides* (▼, ●, ■ respectively) are presented in Fig. 1. For the combination where *L. petersonii* was combined with *L. scoparium*, non-interactive effects were noted for all ratios tested against both the Gram-positive test organism *S. aureus* and the Gram-negative test organism *P. aeruginosa*. More favourable (additive) interactions were noted for all ratios when testing against *C. albicans*. When *L. petersonii* was combined with *K. ericoides*, all ratios tested were found to have an additive effect for the three test organisms. The combinations where *L. scoparium* was added to *K. ericoides* in various ratios showed consistent additive interactions against *P. aeruginosa* and *C. albicans*. However, for *S. aureus*, varied interactions were noted ranging from non-interactive (four ratios) to additive (four ratios). No pattern could be observed where a higher concentration of one oil resulted in a predominantly favourable interaction. An exception to this trend was presented by the combination of *K. ericoides* with *L. petersonii* where one ratio (different concentrations depending on the pathogen) demonstrated a synergistic effect. Note, that none of the data points on the isobologram in this study approaches the antagonistic reference line of 4:00 (not displayed as a matter of simplicity), and thus prove promising for favourable combination formulations. Other than the earlier tea tree combination studies by Christoph et al. (2001), Cassella et al. (2002), and more recently de Rapper et al. (2013) no combination studies have been undertaken on the specific interaction between the tea tree species reported here. This is surprising,

Table 4
Antimicrobial activity (mean MIC expressed in mg/ml) of *L. petersonii*, *L. scoparium* and *K. ericoides* essential oils.

Test Organism	*L. petersonii*	*L. scoparium*	*K. ericoides*	Control[a]
Staphylococcus aureus ATCC 12600	4.00	4.00	8.00	$6.250\ e^{-4}$
Staphylococcus epidermidis ATCC 2223	**2.00**	4.00	8.00	$1.563\ e^{-4}$
Mycobacterium smegmatis ATCC 14468	**1.50**	**2.00**	**2.00**	$3.906\ e^{-5}$
Enterococcus faecalis ATCC 29212	8.00	4.00	12.00	$6.250\ e^{-4}$
Streptococcus pyogenes ATCC 8668	**0.50**	**1.00**	**2.00**	$1.563\ e^{-3}$
Streptococcus agalactiae ATCC 55618	**2.00**	**0.50**	**2.00**	$1.563\ e^{-3}$
Streptococcus pneumoniae ATCC 49247	**2.00**	8.00	8.00	$7.813\ e^{-4}$
Brevibacterium brevis ATCC 8246	**1.00**	**1.00**	**1.00**	$1.563\ e^{-4}$
Brevibacterium agri ATCC 51663	**0.06**	**0.06**	**1.00**	$1.563\ e^{-4}$
Brevibacterium laterosporum ATCC 64	**0.25**	**0.25**	**1.00**	$1.563\ e^{-4}$
Propionibacterium acnes ATCC 11827	**1.00**	**1.00**	4.00	$6.25\ e^{-4}$
Klebsiella pneumoniae ATCC 13883	8.00	8.00	8.00	$7.813\ e^{-5}$
Pseudomonas aeruginosa ATCC 9027	4.00	4.00	4.00	$1.563\ e^{-4}$
Moraxella catarrhalis ATCC 23246	4.00	**2.00**	8.00	$3.125\ e^{-4}$
Cryptococcus neoformans ATCC 90112	**1.00**	**1.00**	**1.00**	$3.125\ e^{-3}$
Candida albicans ATCC 10231	**2.00**	8.00	4.00	$3.125\ e^{-3}$

Noteworthy activity is given in bold.
[a] Ciprofloxacin was used as the control for bacteria and amphotericin B for the yeasts.

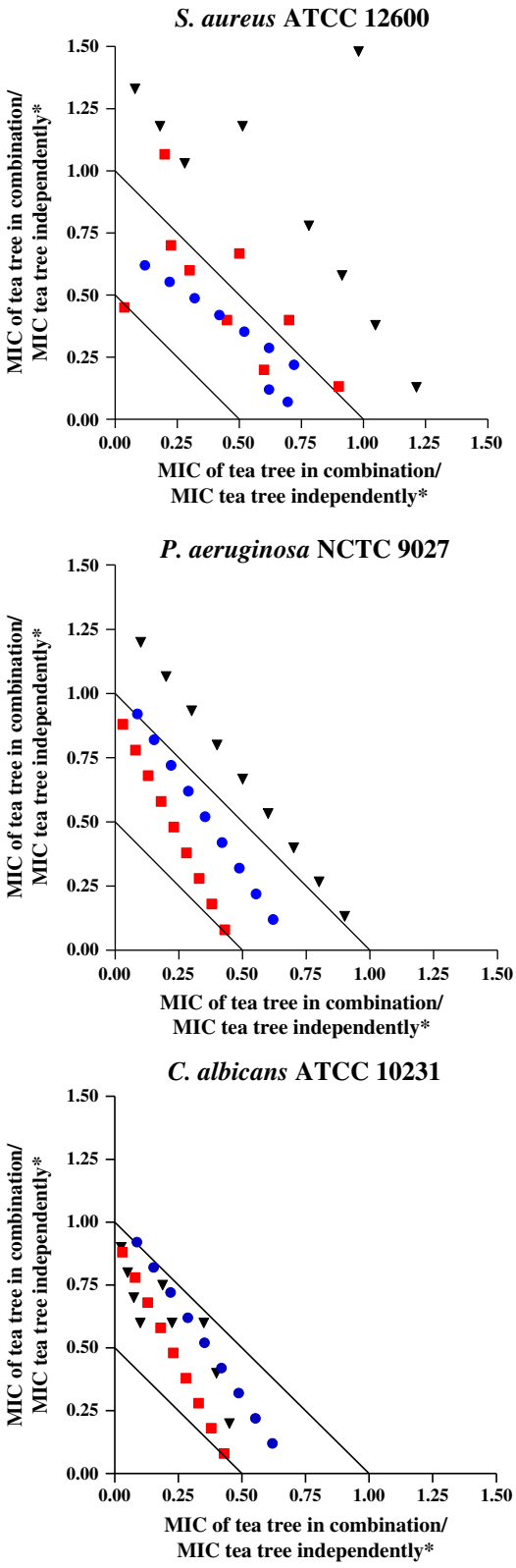

Fig. 1. Isobolograms representing interactions of tea tree oils where * = ▼ is the combination of *L. petersonii* with *L. scoparium*; ● is the combination of *L. petersonii* with *K. ericoides* and ■ is the combination of *L. scoparium* with *K. ericoides*.

as oils are more frequently used in combination than independently. The basic practice of aromatherapy, which should not be ignored when investigating possible efficacy, involves the blending of a selection of oils for either an enhanced effect or to achieve a more pleasant aromatic outcome. The most commercially known tea tree species (*M. alternifolia*) has a distinctive, almost unpleasant odour. The potential to use other more fragrant tea tree oils in combination, such as *L. petersonii*, as presented in this study, demonstrates a more favourable approach.

4. Conclusion

This study demonstrated negligible monthly variation in the essential oil composition for *L. petersonii*, *L. scoparium* and *K. ericoides*. *L. petersonii* displays noteworthy antibacterial activity, and it remains a mystery why this species has been neglected in the scientific literature. The most noteworthy antimicrobial activity was recorded for *L. petersonii* essential oil assayed against the *Brevibacterium* genus. While these pathogens are not pathogenic, they are closely linked to micro-organisms that are responsible for bothersome foot odour. *K. ericoides* does not display broad-spectrum inhibitory activity, yet when combined with *L. petersonii* can act in an additive manner (Fig. 1). The commercial potential of combining *K. ericoides* and *L. petersonii* oil holds enormous promise as the oil yields are good and the pleasant smell of *L. petersonii* not only masks foot odour but also shows potential on the micro-organisms related to this unpleasant condition.

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