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The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils

A.M. Viljoen[a,*], S. Subramoney[a], S.F. van Vuuren[a], K.H.C. Başer[b], B Demirci[b]

[a] *Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, South Africa*
[b] *Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey*

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Abstract

Lippia javanica is widely distributed throughout South Africa where it is used extensively in traditional herbal preparations. An infusion of the leaves is commonly used as a decongestant for colds and coughs. A preliminary study indicated that the essential oil chemistry varies dramatically both within and between natural plant populations. As the antimicrobial activity may be directly related to the specific composition of the oil, the activity may also fluctuate. The aerial parts of *Lippia javanica* were collected from various localities in southern Africa to study the essential oil composition and the antimicrobial activity thereof. The hydrodistilled essential oils were analysed by GC/MS and a cluster analysis was performed on the essential oil dataset. From 16 samples (representing five natural populations), 5 chemotypes were identified; a myrcenone rich-type (36–62%), a carvone rich-type (61–73%), a piperitenone rich-type (32–48%), an ipsenone rich-type (42–61%) and a linalool rich-type (>65%). The myrcenone and linalool chemotypes have been mentioned in the literature but the carvone, ipsenone and piperitenone chemotypes have not previously been reported for *Lippia javanica*. Time kill studies were performed on three microbial respiratory isolates to document the scientific rationale of using *Lippia* to treat respiratory complaints in traditional herbal medicine. *Klebsiella pneumoniae*, *Cryptococcus neoformans* and *Bacillus cereus* showed reduction in microbial populations with the strongest bacteriostatic effect observed for *Klebsiella pneumoniae.*

Keywords: *Lippia javanica*; Essential oil; Antimicrobial; Geographical variation; Chemotypes

1. Introduction

The genus *Lippia* (Houst.), a member of the Verbenaceae family is represented by approximately 200 herbs, shrubs and small trees which are often of an aromatic nature (Terblanché and Kornelius, 1996). The species are distributed throughout South and Central America and Tropical and Southern Africa (Velasco-Negeureula et al., 1993; Van Wyk et al., 1997). *Lippia javanica* (Burm. f.) Spreng. is one of the four indigenous *Lippia* species in South Africa where it occurs as an erect woody shrub approximately 2 m in height. The plant is used extensively in traditional medicine by both lay people and traditional healers to treat minor ailments (Pascual et al., 2001). Many of its uses relate to microbial infections e.g. coughs or colds and also for skin infections or wounds. The leaves and stems are often used and in some cases the roots as well (Van Wyk et al., 1997). Strong leaf infusions are made which are commonly used as inhalants. The leaf infusions are also used topically for scabies and lice (Gelfand et al., 1985; Van Wyk et al., 1997). More commonly, leaf and stem infusions are used as a tea, and this is taken to treat coughs, colds, fever and bronchitis (Watt and Breyer-Brandwijk, 1962; Smith, 1966; Hutchings, 1996). The plant has also been used for bronchial ailments and influenza (Hutchings, 1996). The Vhavenda people use leaf infusions as anthelmintics, for respiratory and febrile ailments and as prophylactic against dysentery, diarrhoea and malaria (Mabogo, 1990). Roots are used as an-

* Corresponding author. Tel.: +27 11 717 2169; fax: +27 11 642 4355.
E-mail address: viljoenam@therapy.wits.ac.za (A.M. Viljoen).

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tidotes for suspected food poisoning and for bronchitis and sore eyes (Hutchings, 1996). Ethnobotanical literature documents its uses for fever and influenza in combination with leaves of *Artemisia afra* (Hutchings, 1996).

Major components in the essential oil were reported to be myrcenone, myrcene and (*E*)- and (*Z*)-tagetenone (Fujita, 1965; Mwangi et al., 1991, 1992; Velasco-Negeureula et al., 1993; Terblanché and Kornelius, 1996). Neidlein and Staehle (1974) reported the components of *Lippia javanica* as caryophyllene, linalool and *p*-cymene while Chagonda et al. (2000) reported variations in major essential compounds for *Lippia javanica* samples taken from the same location in Zimbabwe e.g. linalool, which had a range between 1.7 and 27%. More recently Ngassapa et al. (2003) reported a geranial and neral chemotype from Tanzania. Essential oils are known for their antimicrobial properties and it was the aim of this study to better understand the essential oil composition of this indigenous medical plant and to elucidate the possible role of the volatile constituents in the traditional medicinal uses.

2. Materials and methods

2.1. Essential oil extraction and analysis

The aerial parts of *Lippia javanica* were collected in the 2000/2001 growing season from various sites in the wild and voucher specimens of all collections are housed in the Departments of Pharmacy and Pharmacology, University of the Witwatersrand. Localities and corresponding sample abbreviations are given in Table 1. Fresh plant material was hydrodistilled in a Clevenger-type apparatus.

The essential oils were analysed by GC/MS using a Hewlett-Packard G1800A GCD system. An Innowax FSC column (60 m × 0.25 mm diameter, with 0.25 μm film thickness) was used with helium (0.8 ml/min) as the carrier gas. The oven temperature of the GC was maintained at 60 °C for 10 min and the temperature programmed to rise to 220 °C at a rate of 4 °C/min, and then kept constant at 220 °C for 10 min and again programmed to rise to 240 °C at a rate of 1 °C/min. Split flow was adjusted at 50 ml/min. The injector and detector temperatures were 250 °C. Mass spectra were taken at 70 eV. Mass range was from *m/z* 35 to 425. Kovats indices for all compounds were determined. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total ion chromatogram. A library search was carried out using the Wiley GC/MS Library and Başer Library of Essential Oil Constituents. Relative percentage amounts were calculated from TIC by the computer.

2.2. Antimicrobial activity

Time kill studies were used to demonstrate the rate at which the pathogens are killed over a 24 h period. Three respiratory pathogens were selected; *Klebsiella pneumoniae* (NCTC 9633) *Cryptococcus neoformans* (ATCC 90112) and *Bacillus cereus* (ATCC 11778), to determine the efficacy of *Lippia javanica* when used for treating respiratory ailments. The inactivation broth death kinetic method, as described by Christoph et al. (2000), was used. Cultures were grown in Tryptone Soya (Oxoid) broth and centrifuged for 10 min at 5000 rpm. The supernatant was discarded and the pellets resuspended in 10 ml of a 0.9% NaCl solution. Oil concentrations of 0.25, 0.5, 0.75 and 1% were incorporated into 50 ml Tryptone Soya (Oxoid) broth with 0.5% Tween and a final inoculum of approximately 1×10^6 cfu/ml. The different concentrations were incubated at 37 °C in a shaking water bath. At time intervals ranging from 0 min to 24 h, aliquots of 1 ml were transferred to 9 ml inactivation broth consisting of 0.1% peptone (Oxoid), 5% lecithin (Merck) and 5% yeast extract (Oxoid). Five serial dilutions were performed in 0.9% NaCl solution. From the inactivation broth and saline dilutions, 100 μl was plated onto Tryptone Soya (Oxoid) agar for each oil concentration. The plates were incubated at 37 °C for 24–48 h and colony forming units (cfus) counted and death kinetics expressed in $\log_{10}$ reduction time kill plots. Controls were included in the study having the same broth formulation but without the oil. The assay was performed in duplicate.

2.3. Cluster analysis

Cluster analysis was carried out with the NTSYS-PC package version 2.00 (Rohlf, 1997). The quantitative dataset (16 populations and 98 compounds) were analysed using standard clustering algorithms.

3. Results and discussion

3.1. Essential oil composition and variation

The GC/MS data for all 16 essential oil samples are summarised in Table 1 and the cluster analysis on the data set is shown in Fig. 1. The Swaziland (SW) population is represented by four distinct chemotypes; SW1 and SW3 accumulate carvone and limonene. On the basis of this similarity, these samples are united into a cluster, as graphically represented in Fig. 1. SW2 has myrcenone (36.3%), myrcene and α-phellandrene as major compounds. Carvone, which is the major compound in both SW1 and SW3, was not detected in SW2. Limonene (43.4%) and piperitenone (39.9%) are the major compounds in SW4, while SW5 and SW6 yielded high levels of myrcenone, myrcene and β-caryophyllene. The high myrcenone content in SW5 and SW6 was also observed in SW2, however, the high myrcene and α-phellandrene levels in SW2 was not detected in appreciable quantities in any of the other samples.

Two chemotypes are identified in the Nelspruit (N) population. The first type is represented by N1 with major compounds limonene (51.7%) and piperitenone (32.4%), and N3

Table 1
Essential oil composition of 16 samples of *Lippia javanica* representing five natural populations

RRI	Compound	Swaziland						Nelspruit			Warmbaths			Long Tom Pass			Fairland
		SW1	SW2	SW3	SW4	SW5	SW6	N1	N2	N3	W1	W2	W3	LTP1	LTP2	LTP3	F
		0.48%	0.22%	0.38%	0.44%	0.34%	0.33%	0.44%	0.57%	0.37%	0.77%	1.05%	0.48%	0.65%	0.05%	0.45%	0.20%
1017	4-Methyl-2-pentanone	–	–	–	–	–	–	–	–	–	0.14	–	0.11	–	–	–	–
1032	α-Pinene	–	–	–	–	–	–	0.14	–	–	–	–	–	–	0.03	–	0.17
1076	Camphene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.30
1118	β-Pinene	–	–	–	0.11	–	–	0.04	–	–	–	–	–	–	–	–	–
1132	Sabinene	–	–	0.11	–	–	0.28	0.32	–	0.56	0.40	0.50	0.48	–	0.12	–	–
1174	Myrcene	1.11	28.8	0.44	–	5.15	5.62	–	4.49	–	11.33	13.62	13.80	2.20	4.65	4.14	2.60
1176	α-Phellandrene		tr	–	–	–	–	0.94	–	0.77	–	–	–	–	–	–	
1203	2-Methylbutyl isobutyrate	–	–	–	–	–	–	–	–	–	0.09	–	–	–	–	–	–
1203	Limonene	28.21	0.35	13.73	43.35	0.35	0.49	51.67	–	33.52	0.39	–	0.43	0.16	0.11	–	0.45
1213	1,8-Cineole	–	0.55	0.84	0.70	2.14	0.61	1.39	2.20	4.01	0.95	–	0.98	0.31	0.40	–	–
1218	β-Phellandrene	0.15		–	–	–	–	–	–	–	–	–	–	–	0.46		
1225	(*Z*)-3-Hexenal	–	–	–	–	–	–	–	–	–	–	–	–	–	0.13	–	–
1246	(*Z*)-β-Ocimene	0.53	–	–	–	–	–	–	–	–	0.32	1.65	0.17	–	0.32	–	12.97
1266	(*E*)-β-ocimene	0.39	–	–	–	–	–	–	–	–	0.14	1.83	–	–	0.15	–	6.21
1274	Isomyrcenol	–	1.90	–	–	–	0.75	–	–	–	0.75	–	–	0.74	0.82	0.51	–
1280	*p*-Cymene	0.39	0.49	0.10	–	0.81	–	0.04	0.93	0.76	0.97	–	0.97	0.35	0.09	0.64	0.87
1286	2-Methylbutyl-2-methyl butyrate	–	–	–	–	–	–	–	–	–	0.18	–	0.16	0.19	–	–	–
1290	Terpinolene	–	–	–	–	0.46	–	–	–	–	–	–	–	–	–	–	–
1319	Dihydrotagetone	–	1.21	–	–	1.92	0.30	–	0.42	–	1.11	1.11	0.62	0.16	0.06	–	0.21
1382	*cis*-Alloocimene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.18
1391	(*Z*)-3-Hexen-1-ol	–	–	–	–	–	0.16	–	–	–	–	–	0.24	–	–	–	–
1398	6,7-Epoxymyrcene	–	1.79	–	–	0.87	1.78	–	1.56	–	–	–	0.73	2.99	1.60	3.53	–
1400	Nonanal	–	–	–	–	–	–	–	–	–	–	–	–	0.12	0.10	–	–
1429	Perillene	–	–	–	–	–	–	–	–	–	0.25	–	0.47	–	0.07	–	–
1444	Ipsenone	–	–	–	–	–	–	–	–	–	60.86	52.58	42.22	–	–	–	0.79
1450	*trans*-Linalool oxide (*furanoid*)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.2
1452	1-Octen-3-ol	0.10	0.65	0.28	0.03	0.53	0.15	–	–	0.35	–	–	–	0.18	0.08	–	0.12
1458	*cis*-1,2-Limonene epoxide	0.43	–	0.09	0.36	–	–	0.19	–	0.10	–	–	–	–	–	–	–
1468	*trans*-1,2-Limonene epoxide	0.14	–	0.01	1.81	–	–	1.11	–	0.30	–	–	–	–	–	–	–
1478	*cis*-Linalool oxide (*furanoid*)	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16
1497	α-Copaene	0.09	–	–	–	–	–	–	–	0.14	–	–	–	0.11	0.02	0.29	0.42
1500	*cis*-Tagetone	–	–	–	–	–	–	–	–	–	1.20	0.95	0.91	–	–	–	0.18
1522	*trans*-Tagetone	–	0.30	–	–	–	–	–	–	–	4.90	4.80	1.07	–	–	–	0.33
1532	Camphor	–	–	–	–	–	0.15	–	–	–	–	–	–	–	–	–	0.18
1535	β-Bourbonene	0.47	0.16	0.41	0.13	–	–	0.10	–	0.08	–	–	–	0.17	0.17	0.30	–
1541	Benzaldehyde	–	–	–	–	–	–	–	–	–	–	–	–	–	0.28	–	–
1553	Linalool	0.51	0.25	0.40	0.19	1.33	0.96	0.41	0.60	0.31	0.42	0.45	0.59	0.49	0.63	2.03	65.19
1568	*trans*-α-Bergamotene	–	–	–	–	–	–	–	–	–	–	–	–	0.12	0.11	0.2	–
1577	α-Cedrene	–	–	–	–	–	–	–	–	0.10	0.18	–	–	0.33	0.35	0.78	–
1586	Myrcenone	–	36.31	0.11	0.38	59.40	55.93	0.30	61.85	0.35	0.47	0.64	12.88	52.30	50.46	48.78	–
1612	β-Caryophyllene	0.81	3.54	1.45	2.16	5.46	5.23	1.85	5.50	1.80	0.47	0.27	0.12	4.69	4.20	4.84	3.58
1626	2-Methyl-6-methylene-3,7-octadien-2-ol	–	–	–	–	–	1.52	–	–	–	0.12	–	0.33	–	–	1.41	–
1639	*trans*-*p*-Mentha-2,8-dien-1-ol	0.27	–	0.19	0.91	–	–	0.85	–	1.10	–	–	–	–	–	–	–
1661	Alloaromadendrene	–	0.12	0.12	0.12	–	–	0.09	–	0.11	–	–	–	1.15	1.21	1.00	0.07
1668	(*Z*)-β-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1.36	–
1678	*cis*-*p*-Mentha-2,8-dien-1-ol	–	–	0.12	0.16	–	–	0.24	–	0.19	–	–	–	–	–	–	–
1678	Ipsdienol	–	0.60	–	–	–	1.26	–	0.98	–	0.82	1.55	1.53	1.16	0.65	1.24	–
1681	(*Z*)-3-Hexenyl tiglate	–	–	–	–	–	0.70	–	–	–	–	–	–	–	–	–	–
1685	Isovaleric acid	–	–	–	–	–	–	–	–	–	0.21	0.13	0.35	–	–	–	–
1687	α-Humulene	0.12	–	0.04	0.13	–	0.34	0.11	–	0.13	–	–	0.11	0.47	1.13	–	0.19
1690	α-Acoradiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.58	–
1693	β-Acoradiene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.24	–
1695	(*E*)-β-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	1.11	–	–

Table 1 (*Continued*)

RRI	Compound	Swaziland						Nelspruit			Warmbaths			Long Tom Pass			Fairland
		SW1	SW2	SW3	SW4	SW5	SW6	N1	N2	N3	W1	W2	W3	LTP1	LTP2	LTP3	F
		0.48%	0.22%	0.38%	0.44%	0.34%	0.33%	0.44%	0.57%	0.37%	0.77%	1.05%	0.48%	0.65%	0.05%	0.45%	0.20%
1704	γ-Muurolene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.10
1704	*cis*-Tagetenone	–	3.08	–	–	4.93	2.79	–	4.03	–	–	–	–	2.56	3.30	0.28	–
1706	α-Terpineol	–	–	–	0.08	–		0.17	2.35	0.14	0.88	0.63	1.73	0.09	4.58	–	
1719	Borneol	0.10	–	–	–	–	–	–	–	0.11	–	–	0.22	–	–	–	0.37
1725	Verbenone	–	1.31	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1726	Germacrene-D	0.29	4.34	0.83	2.44	0.53	0.86	2.28	1.98	2.19	0.09	0.89	–	1.53	3.20	0.61	1.48
1726	*trans*-Tagetenone	–	3.64	–	–	–	0.60	–	1.06	–	–	–	–	1.41	2.63	0.82	–
1740	α-Muurolene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09
1741	β-Bisabolene	–	–	–	–	–	–	–	–	0.09	–	–	–	0.08	0.15	0.29	–
1747	*trans*-Carvyl acetate	0.87	–	0.58	1.16	–	–	0.90	–	1.20	–	–	–	–	–	–	–
1751	Carvone	61.07	–	72.65	0.97	–	–	0.61	–	0.62	–	–	–	–	–	–	–
1755	Bicyclogermacrene	–	0.15	–	–	–	–	–	–	–	–	–	–	–	–	–	0.09
1755	β-Curcumene	–	–	–	–	–	–	–	–	–	–	–	–	0.08	0.29	0.17	–
1758	(*E*,*E*)-α-Farnesene	–	–	–	–	–	–	–	–	–	–	–	–	–	0.17	–	0.21
1758	*cis*-Piperitol	–	–	–	–	–	–	0.14	–	0.12	–	–	–	–	–	–	–
1771	γ-Bisabolene	–	–	–	–	–	–	–	–	0.14	–	–	–	–	–	–	–
1773	δ-Cadinene	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.16
1786	ar-Curcumene	0.12	–	–	–	–	0.22	–	–	0.25	0.08	–	1.29	0.8	0.57	2.38	–
1808	2-Methyl-2-butenoic acid	–	–	–	–	–	0.16	–	–	–	–	–	–	0.09	–	–	–
1811	*trans*-*p*-Mentha-1(7),8-dien-2-ol	0.10	–	0.09	–	–	–	–	–	–	–	–	–	–	–	–	–
1830	2,6-Dimethyl-3(*E*),5(*E*),7-octatriene-2-ol	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	0.1
1845	*trans*-Carveol	0.33	–	0.26	0.90	–	–	1.22	–	0.65	–	–	–	–	–	–	–
1853	Calamenene	–	–	–	–	–	–	–	–	–	0.08	–	–	–	–	–	–
1856	Carvone oxide	1.01	–	1.98	–	–	–	–	–	–	–	–	–	–	–	–	–
1865	Isopiperitenone	0.86	0.12	1.27	0.96	–	0.08	0.78	–	0.90	–	–	–	0.10	0.14	–	–
1882	*cis*-Carveol	0.10	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
1896	*cis*-*p*-Mentha-1(7),8-dien-2-ol	–	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–	–
1949	Piperitenone	0.14	–	0.17	39.88	–	0.35	32.45	–	47.30	–	–	–	–	–	–	–
2001	Isocaryophyllene oxide	0.13	–	0.09	0.08	0.29	–	–	–	0.21	0.09	–	0.35	0.15	0.08	0.12	0.07
2008	Caryophyllene oxide	0.24	0.11	0.53	0.82	1.20	0.31	0.44	1.10	0.72	0.36	0.67	1.70	0.80	0.46	0.52	0.42
2050	(*E*)-Nerolidol	–	–	–	0.03	–	–	–	–	–	–	–	–	–	–	–	0.06
2071	Humulene epoxide II	–	–	–	–	–	–	–	–	–	–	–	0.25	0.13	–	–	0.03
2131	Hexahydrofarnesyl acetone	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05	–	–
2144	Spathulenol	–	–	0.08	0.07	0.25	–	–	–	–	0.10	–	0.55	0.10	0.08	0.15	–
2186	Eugenol	–	–	0.12	0.06	–	–	–	–	–	–	–	–	0.06	0.19	–	–
2202	Germacrene-D-4-ol	–	–	0.07	0.17	–	–	0.05	–	0.14	–	–	–	–	–	–	–
2316	Caryophylla-2(12),6(13)-dien-5β-ol (=*Caryophylladienol I*)	–	–	0.06	–	–	–	–	–	–	–	–	–	–	–	–	–
2324	Caryophylla-2(12),6(13)-dien-5α-ol (=*Caryophylladienol II*)	–	–	0.16	–	–	–	–	–	–	–	–	–	–	–	–	–
2389	Caryophylla-2(12),6-dien-5α-ol (=*Caryophyllenol I*)	–	–	0.07	–	–	–	–	–	–	–	–	–	–	–	–	–
	Total	99.1	89.8	97.5	98.2	85.6	82.0	98.8	89.1	99.5	88.4	82.3	85.4	76.4	80.4	81.8	99.0

Values in % indicate essential oil yield. Swaziland, Nelspruit, Warmbaths, Long Tom Pass and Fairland represent population.

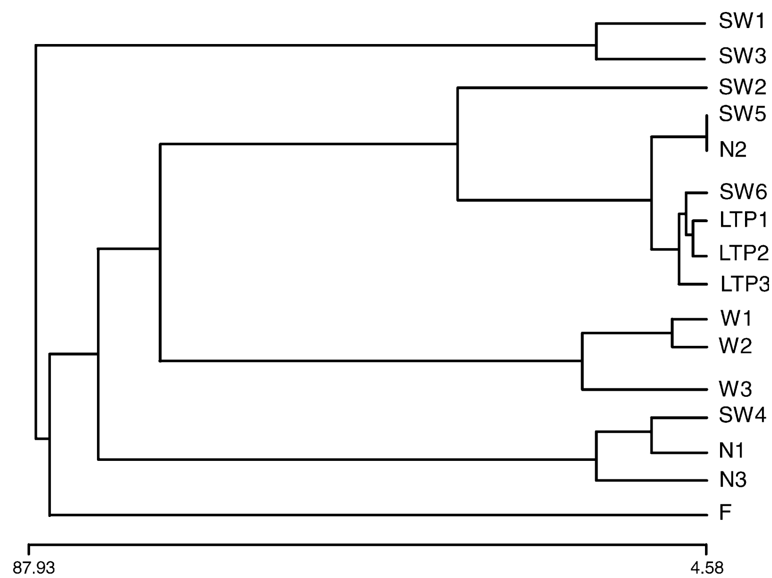

Fig. 1. Dendrogram constructed on sixteen samples of *Lippia javanica* using the quantitative essential oil data matrix in Table 1.

with major compounds limonene (33.5%) and piperitenone (47.3%). This combination resembles SW4 from the Swaziland population. The close resemblance in essential oil composition between these samples is also evident in Fig. 1. N2 belongs to a chemotype also identified in SW5 and SW6, with major compounds myrcenone (61.9%), myrcene (4.5%) and β-caryophyllene (5.5%).

Fig. 1 shows that the three Warmbaths (W) samples are chemically coherent. For the three plants collected in the Warmbaths (W) area, W1 accumulated ipsenone (60.9%) and myrcene (11.3%) and W2 with major compounds ipsenone (52.6%) and myrcene (13.6%) belong to a type different from those already noted in the other populations. W3, with major compounds ipsenone (42.2%) and myrcene (13.8%) is very similar to W1 and W2 with notable difference in the myrcenone levels. The myrcenone level in W3 is 12.9% compared to W1 (0.5%) and W2 (0.6%). Plants collected on Long Tom Pass (LTP) belong to a chemotype already identified in the Swaziland population (SW5 and SW6), with major compounds myrcenone, myrcene and β-caryophyllene. LTP3 showed the highest content of α-terpineol across all populations. The chemical affinity between plants in the N and SW populations are also expressed in Fig. 1.

The plants in the Fairland population belongs to a chemotype not identified in any of the other samples, hence it occupies an outlying position in Fig. 1. Linalool (65.2%), (*Z*)-β-ocimene (13%) and β-caryophyllene (3.6%) accumulate as the major compounds in the essential oil.

Certain studies have mentioned that *Lippia javanica* displays chemical variation but most of these studies have mentioned myrcenone as a major component (Fujita, 1965; Mwangi et al., 1991; Velasco-Negeureula et al., 1993). Myrcenone as a major compound occurred in seven of the sixteen samples (43.8% occurrence). Chagonda et al. (2000) also recorded high amounts of limonene in *Lippia javanica* samples collected from three locations in Zimbabwe. The carvone and ipsenone chemotypes, however, was not mentioned. The literature makes mention of high amounts of linalool in *Lippia javanica* found in varying quantities in plants collected at approximately the same time of year. (Neidlein and Staehle, 1974; Chagonda et al., 2000).

3.2. Antimicrobial activity

Lippia javanica has been used traditionally to treat various respiratory ailments, which may be either bacterial or

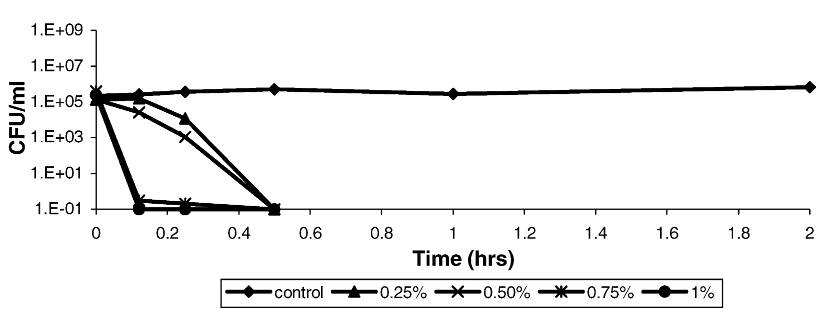

Fig. 2. Time kill plot on *Lippia javanica* essential oil showing death kinetics of *Klebsiella pneumoniae* (NCTC 9633) represented over 2 h.

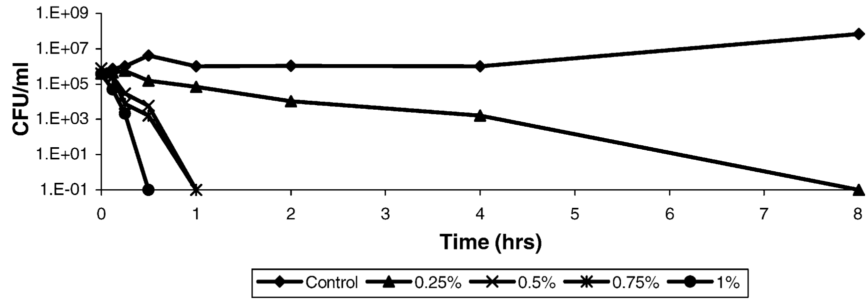

Fig. 3. Time kill plot on *Lippia javanica* essential oil showing death kinetics of *Cryptococcus neoformans* (ATCC 90112) represented over 8 h.

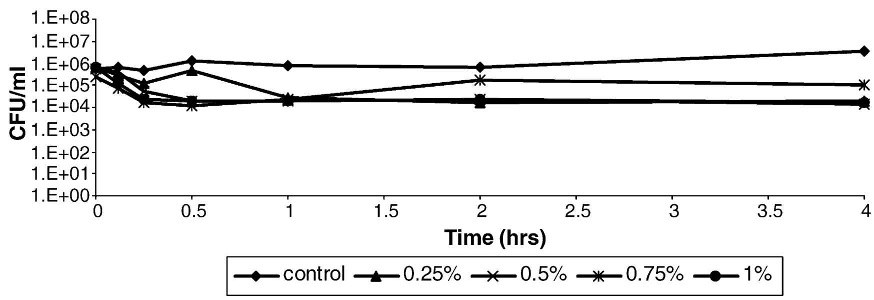

Fig. 4. Time kill plot on *Lippia javanica* essential oil showing death kinetics of *Bacillus cereus* (ATCC 11778) represented over 4 h.

fungal. Time kill assays were performed on the three respiratory pathogens from three different microorganism groups; *Klebsiella pneumoniae* (gram-negative), *Cryptococcus neoformans* (yeast) and *Bacillus cereus* (gram-positive). The essential oil from the Fairland population was used for the time kill studies, chosen on the basis of proximity and its positive antimicrobial activity determined by the disc diffusion assay (Subramoney, 2003). Due to the large volume of essential oil required for the time kill method, individual plants were not sampled and analysed. Instead, a collective sample was harvested from many plants in the Fairland population and pooled. Comparison of the time kill plots for the three organisms studied showed that the killing rate was the greatest for *Klebsiella pneumoniae* (Fig. 2), then *Cryptococcus neoformans* (Fig. 3) and very little reduction of microbial populations for *Bacillus cereus* (Fig. 4). The efficacy of *Lippia javanica* oil for *Klebsiella pneumoniae* showed a killing rate within 30 min at all concentrations tested. To a lesser extent, *Cryptococcus neoformans* showed a killing rate for concentrations 0.5, 0.75 and 1% within 1 h. The lowest concentration of 0.25% took 8 h before a bactericidal effect was noted. *Bacillus cereus* showed some reduction in colonies. However, no bactericidal activity was noted for the full 24 h. This confirms the use (albeit in vitro) of *Lippia javanica* to treat gram-negative and yeast-borne respiratory ailments. The Fairland (F) population had a high linalool yield (65.2%). Antimicrobial activity has been reported for linalool (Knobloch et al., 1989; Hinou et al., 1989; Kim et al., 1995; Pattnaik et al., 1997; Ngassapa et al., 2003) and could possibly be attributed to the positive antimicrobial activity recorded in the time kill study.

4. Conclusions

Lippia javanica displays quantitative and qualitative variations both within and between natural plant populations. This variation seems to be random and is not correlated to the geographical distribution of the plant. From the natural plant populations assessed, five distinct chemotypes have been noted. Chemotypic variability is an important factor in selecting favourable chemotypes for commercial development, especially in terms of chemical fingerprinting often required in quality control.

Lippia javanica is mainly used in African traditional medicine to treat respiratory disorders such as coughs, colds and bronchitis. The essential oil displays moderate antimicrobial activity against respiratory pathogens, which justifies its wide use in African traditional medicine to treat symptoms associated with colds and flu. The highest activity was observed against pathogens such as *Klebsiella pneumoniae* and *Cryptococcus neoformans* which are commonly associated with opportunistic infections in immune-compromised patients.

Further studies on *Lippia javanica* should be cognizant of the occurrence of chemotypes in natural plant populations and the impact that this would have on the results of such studies. It should be noted that this study has acutely focused on the volatile constituents as this aromatic plant is often used in inhalation therapy. As *Lippia javanica* is also administered in the form of tinctures and teas, it is most probable that the non-volatile compounds could be acting in a synergistic/additive manor to produce enhanced medicinal properties.

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