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Seasonal and geographical variation of *Heteropyxis natalensis* essential oil and the effect thereof on the antimicrobial activity

S.F. Van Vuuren [a], A.M. Viljoen [b,*], T. Őzek [c], B. Demirci [c], K.H.C. Başer [c]

[a] *Department of Pharmacy and Pharmacology, Faculty of Health Sciences, University of the Witwatersrand, 7 York Road, Parktown 2193, Johannesburg, South Africa*
[b] *School of Pharmacy, Tshwane University of Technology, Private Bag X680, Pretoria 0001, South Africa*
[c] *Department of Pharmacognosy, Faculty of Pharmacy, Anadolu University, 26470 Eskişehir, Turkey*

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Abstract

Heteropyxis natalensis (Heteropyxidaceae) is traditionally used to treat respiratory disorders, and as a decongestant and antimicrobial agent. The seasonal variation of the hydrodistilled essential oil was investigated. Three trees in the Johannesburg Botanical Garden (Gauteng) indicated similar chemical profiles with fluctuation in the levels of the two major constituents (1,8-cineole and limonene). Little variation between the antimicrobial activity of seasonally collected samples was documented, with standard deviations of ±0.3 to ±3.3 depending on the pathogen studied. Moderate antimicrobial activity (3.0–16.0 mg/ml) was noted for most pathogens tested with *Cryptococcus neoformans* exhibiting the highest sensitivity (2.0–3.0 mg/ml). The chemogeographical variation of the oil composition from five of the seven distinct localities studied all contains 1,8-cineole and limonene as major constituents. The antimicrobial study of these samples indicated little variability between localities (standard deviation of ±0.5 to ±3.8). As observed in the seasonal variation study, *C. neoformans* displayed the highest sensitivity (0.5–2.0 mg/ml). One oil sample (Lagalametse), was distinctly different both chemically and microbiologically.

Keywords: Antimicrobial activity; Essential oil; *Heteropyxis natalensis*; Limonene; 1,8-Cineole; Geographical variation; Seasonal variation

1. Introduction

The family Heteropyxidaceae has only three representatives in southern Africa; *Heteropyxis canescens* Oliv., *Heteropyxis dehniae* Suess. and *Heteropyxis natalensis* Harv. The most noteworthy is *H. natalensis*, a tree renowned for its therapeutic properties (Watt and Breyer-Brandwijk, 1962; Van Wyk et al., 1997; Van Wyk and Gericke, 2000). *H. natalensis* is used traditionally to treat respiratory disorders, as a decongestant and an antimicrobial agent (Van Wyk et al., 1997). Both the Venda and Zulu communities have reported on the medicinal values of *H. natalensis* (Watt and Breyer-Brandwijk, 1962). The leaves are prepared as a tea and a decoction of the roots is inhaled (Van Wyk and Gericke, 2000).

Seasonal variation studies on *H. natalensis* by Weyerstahl et al. (1992) and Chagonda et al. (2000) reported that the major essential oil constituents varied between plants from the same geographical region. No antimicrobial investigation was undertaken in either of the two studies. Gundidza et al. (1993) studied the phytoconstituents of *H. natalensis* growing in Zimbabwe and reported that the species exhibited antimicrobial properties as determined by the disc diffusion method.

The production of phytochemicals is (often) governed by external factors such as soil quality and climate. The chemical composition of a plant is thus subject to quantitative and qualitative variation. Biological activity which is dependent on the chemical composition is similarly subject to variation. Despite the reported phytochemical variation in *H. natalensis* from Zimbabwe, the species has been identified as a commercial source of essential oil (Chagonda et al., 2000). Even though the antimicrobial activities for *H. natalensis* have been previously reported (Van Vuuren and Viljoen, 2006), this study focused on the seasonal and geographical variation both chemically (quantitative and qualitative composition of essential oil components) and microbiologically (minimum inhibitory concentration determination) of the *H. natalensis* samples from South Africa and Swaziland.

* Corresponding author.
E-mail address: viljoenam@tut.ac.za (A.M. Viljoen).

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2. Materials and methods

2.1. Plant collection

For the seasonal variation study, the aerial parts from three individual trees (A, B and C) in the Johannesburg Botanical Garden (JHB BG) were harvested every month in 2004. Due to the semi-deciduous nature of the tree, no leaves were harvested from June to September and only one tree produced sufficient foliage for harvesting in October. A total number of 22 samples were therefore collected.

For the geographical variation study, plants were collected from Cullinan and Verena (Gauteng); Nelspruit (Mpumalanga); Lagalametse and Waterberg (Northern Province) and Balakane (Swaziland). With the exception of the Swaziland sample (collected in November), all material was harvested in February to avoid possible seasonal variation.

Voucher specimens for the seasonal and geographical variation study were deposited in the Department of Pharmacy and Pharmacology, University of Witwatersrand.

2.2. Isolation of oils

Fresh plant material was hydrodistilled in a Clevenger apparatus and the essential oil collected after 3 h. The oil was stored in amber glass vials at 4 °C.

2.3. Gas chromatography (GC)

Analysis of the 22 seasonal samples was performed on a Shimadzu 17A gas chromatograph using the following conditions; Column: J and W-DB1 (60 m × 0.25 mm id., 0.25 μm film thickness); Temperatures: injection port 230 °C, column 60 °C for 1 min, 5 °C/min to 180 °C, 180 °C for 2 min, (total = 25 min). Helium was used as a carrier gas. Limonene of 99% purity (Lot 054076) was obtained from Fluka and 1,8-cineole of 98% purity (Lot 1054365) was purchased from Sigma-Aldrich and used as standards.

2.4. Gas chromatography combined with mass spectroscopy (GC–MS)

Three seasonal oil samples (February, plants A, B and C) and the six geographical variation study samples were further analyzed by GC–MS using a Hewlett-Packard 1800A GCD system operating under the following conditions; Column: HP-Innowax (60 m × 0.25 mm id., 0.25 μm film thickness) with helium as carrier gas; Temperatures: injection port 250 °C, column 60 °C for 10 min, 4 °C/min to 220 °C, 220 °C for 10 min, 1 °C/min to 240 °C (total = 80 min). The split ratio was adjusted to 50:1. The MS were taken at 70 eV. The mass range was from *m/z* 35 to 425. Data was recorded after a 5 min lag phase. Component identifications were made by comparing their mass spectra and retention indices using commercial libraries and the Başer Library of Essential Oil Constituents. Relative percentage amounts were calculated from the TIC.

2.5. Cluster analysis

Using quantitative data obtained from the GC–MS analysis, a cluster analysis was performed on the essential oil from three seasonal samples (February, plants A, B and C) and six *H. natalensis* samples from different localities. A dendrogram was generated using NTSYS-pc software (Version 2.0) package (Rohlf, 1998). Correlation of similarity and the unweighted pair-group method with arithmetic average (UPGMA) was used for cluster definition.

Table 1
The mean MIC (mg/ml) for selected *H. natalensis* essential oils for January–December, 2004

Plant sample	Voucher number	*Staphylococcus aureus* ATCC 12600	*Bacillus cereus* ATCC 11778	*Enterococcus faecalis* ATCC 29212	*Escherichia coli* ATCC 11775	*Pseudomonas aeruginosa* ATCC 9027	*Klebsiella pneumoniae* ATCC 13883	*Moraxella catarrhalis* (clinical strain)	*Cryptococcus neoformans* ATCC 90112
January A	SVV897	12	5	8	11	6	16	8	3
January B	SVV898	7	4	8	11	4	8	5	2
February B	SVV923	5	6	8	11	4	8	11	2
February C	SVV924	5	2	8	11	4	6	4	2
March A	SVV954	12	3	8	8	4	8	4	2
March C	SVV956	3	3	8	11	4	8	4	2
April A	SVV972	4	3	8	11	4	8	4	2
April B	SVV973	2	3	8	11	4	8	4	2
April C	SVV974	5	3	8	13	4	8	4	2
May C	SVV995	4	3	13	11	4	8	4	2
October A	SVV1069	6	3	8	11	4	8	4	2
November A	SVV1071	4	3	16	11	4	8	4	2
November C	SVV1073	11	2	8	11	4	8	4	2
December A	SVV1074	11	5	12	11	2	16	6	3
December B	SVV1075	5	1	6	13	12	6	5	2
December C	SVV1076	7	3	6	11	8	8	7	2
Mean		6.4±3.3	3.3±1.2	8.8±2.6	11.1±1.1	4.8±2.3	8.8±2.9	5.1±2.0	2.1±0.3
Control *		0.30×10^{-3}	0.30×10^{-3}	1.25×10^{-3}	0.10×10^{-4}	0.30×10^{-3}	0.60×10^{-3}	0.50×10^{-3}	1.60×10^{-3}

* Ciprofloxacin and amphotericin B served as controls for bacteria and yeast respectively.

2.6. Minimum inhibitory concentration (MIC) determination

Oil samples were investigated for antimicrobial activity using the MIC microtitre plate method (Eloff, 1998). As the traditional use of *H. natalensis* is for respiratory tract infections, eight relevant pathogens were selected as test organisms for the seasonal variation study (Table 1). For the geographical variation study (Table 2), MIC assays were limited to five pathogens, due to low oil yields. All bacterial cultures were subcultured from stock agar plates and grown in Tryptone Soya broth for 18 h. The yeast was further incubated for 24 h. Oil was transferred into the first row of a microtitre plate, at starting stock concentrations of 128 mg/ml. Serial dilutions were performed and the cultures introduced yielding an approximate inoculum size of 1×10^8 colony forming units (CFU)/ml. Optimal incubation conditions followed; 37 °C for 24 h for bacteria and 48 h for the yeast. Ciprofloxacin or amphotericin B at starting stock concentrations of 0.01 mg/ml was used as positive controls. A 0.4 mg/ml *p*-iodonitrotetrazolium violet solution (INT) was prepared and 40 µl transferred to all inoculated wells. The microtitre plates were examined after 6 h to determine a colour change in relation to the concentration of microbial growth. The yeast *Cryptococcus neoformans* was examined after 24 h. All MIC assays were undertaken in triplicate.

3. Results and discussion

3.1. Essential oil composition

3.1.1. Seasonal variation study

All 22 essential oil samples produced similar GC profiles over the entire sampling period with 1,8-cineole and limonene as major oil constituents. Fig. 1 shows the monthly variation of limonene and 1,8-cineole in relation to the sum of the remainder of the constituents as determined by GC.

The seasonal fluctuation of 1,8-cineole and limonene showed tree-to-tree variation within a single site as well as variability on a monthly basis (Fig. 1). The highest tree-to-tree variability for limonene is noted in November for plant B, which contains 4% as opposed to plant A with 26%. The total tree-to-tree variation for limonene was between 4 and 29%. Fluctuation of 1,8-cineole levels was highest in January (plant A: 60% and plant B: 79%) and December (plant A: 47% and plant B: 28%). Both these months fall in the mid-summer season. The total tree-to-tree variation for 1,8-cineole was between 13 and 20% (Fig. 1).

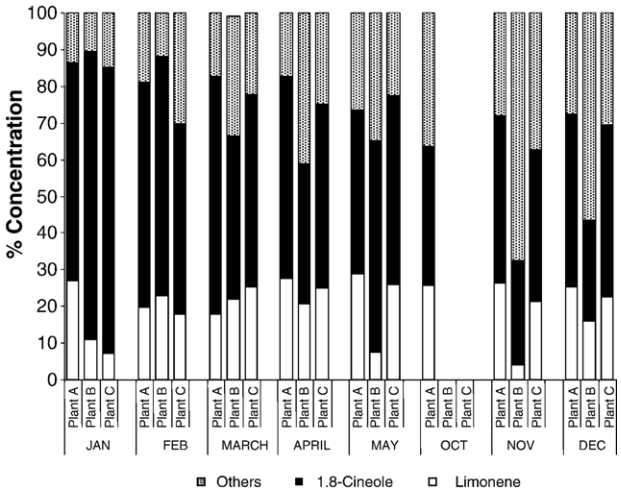

Fig. 1. The monthly variation of limonene and 1,8-cineole recorded for three individual *H. natalensis* plants at the same locality.

Levels of limonene in plant A, showed an 11% seasonal variation throughout the period of analysis. 1,8-Cineole peaked in January, February, March, and April (55–65%), with lower levels obtained for May, October, November and December, (38–47%). The two major compounds (limonene and 1,8-cineole) collectively represent between 44% (plant B, December) and 90% (plant B, January) of the total oil composition.

It is reasonable to assume that there would be similarity between three plants growing in close proximity in a confined area, under cultivated conditions, where irrigation is regular (as is the case in the Johannesburg Botanical Garden). This was found not to be the case, as evidenced by the fluctuation in the oil composition. The sum of the two major constituents shows a fluctuation of up to 46%.

The essential oil yield for the seasonal variation study consistently ranged between 0.1 and 0.2% (wet wt.) for all samples. The same pattern was observed for the geographical variation study (Table 3) with the exception of the Cullinan sample which had a yield of 0.3%. Studies on *H. natalensis* essential oil yields

Table 2
The mean MIC (mg/ml) for *H. natalensis* essential oils selected from different localities

Locality	Voucher number	*Staphylococcus aureus* ATCC 12600	*Enterococcus faecalis* ATCC 29212	*Pseudomonas aeruginosa* ATCC 9027	*Klebsiella pneumoniae* ATCC 13883	*Cryptococcus neoformans* ATCC 90112
Cullinan	AMV771	8.0	16.0	4.0	8.0	0.8
Verena	PMB703	4.0	8.0	3.0	4.0	1.0
JHB BG[a]	SVV923/4	5.0	8.0	4.0	7.0	2.0
Nelspruit	ADC721	6.0	8.0	3.0	6.0	1.0
Lagalametse	ADCAV181	1.6	3.3	2.0	3.3	0.5
Waterberg	PMB738	8.0	8.0	4.5	4.0	1.0
Balakane	ADCAV140	4.0	8.0	4.0	8.0	0.5
Mean		5.2±2.3	8.5±3.8	3.5±0.9	5.8±2.0	1.0±0.5
Control[b]		0.3×10^{-3}	1.25×10^{-3}	0.3×10^{-3}	0.6×10^{-3}	1.6×10^{-3}

[a] Mean of plants B and C harvested in February (included as a comparison with other samples from Gauteng).
[b] Ciprofloxacin and amphotericin B served as controls for bacteria and yeast respectively.

Table 3
Essential oil composition as determined by GC-MS for *H. natalensis* samples

RRI	Compound	JHB (Feb)		BG	Cullinan	Verena	Nelspruit	Lagalametse	Waterberg	Balakane
		A	B	C						
Essential oil yield (%) wet wt.		0.2	0.1	0.2	0.3	0.2	0.2	0.1	0.1	0.1
1032	α-pinene	1.7	3.2	3.1	3.0	1.9	2.7	tr	3.1	1.9
1035	α-thujene	0.4	1.2	1.1	0.6	0.6	1.0	–	0.7	tr
1076	camphene	–	–	tr	0.1	–	–	–	–	0.1
1118	β-pinene	1.1	2.0	1.9	7.6	3.2	6.2	–	2.5	25.2
1132	sabinene	–	0.1	0.4	0.1	0.1	0.6	–	0.1	tr
1146	2-methylbutyl acetate	–	–	–	–	–	–	0.1	–	–
1174	myrcene	0.5	1.8	2.0	1.6	1.2	3.2	–	1.4	5.1
1183	*p*-mentha-1,7(8)-diene (=*Pseudolimonene*)	5.2	7.1	4.6	6.0	3.7	3.0	–	6.5	tr
1188	α-terpinene	–	–	–	0.1	–	–	–	–	–
1203	limonene	18.1	25.4	16.5	22.8	19.2	15.0	–	23.6	1.8
1213	1,8-cineole	33.6	41.2	23.9	23.5	29.4	21.7	0.2	39.2	–
1246	(*Z*)-β-ocimene	–	0.3	0.1	0.4	tr	0.2	–	–	0.4
1255	γ-terpinene	–	0.2	–	1.1	–	–	–	–	tr
1265	(*E*)-β-ocimene	–	0.8	–	1.5	tr	0.7	–	–	2.5
1274	2-heptyl acetate	–	–	–	0.1	0.1	0.1	0.2	–	0.2
1275	2-methylbutyl butyrate		0.1	tr	0.3	0.3	0.1	0.1	0.4	tr
1280	*p*-cymene	3.5	2.5	3.3	3.7	4.3	4.2	0.1	5.8	0.4
1290	terpinolene	–	0.1	–	0.2	–	–	–	–	0.1
1303	amyl isovalerate	–	–	–	–	–	–	–	0.1	–
1345	4-pentenyl butyrate†	0.4	0.5	1.2	1.2	tr	0.2	0.4	0.3	0.2
1391	(*Z*)-3-hexenol	–	–	–	–	–	–	–	0.4	tr
1395	2-nonanone	–	–	0.3	0.7	0.3	0.6	2.4	–	1.1
1398	3-methyl-2-butenyl butyrate†	0.3	0.3	1.1	0.6	0.4	0.1	0.4	0.2	0.1
1413	rose furan	–	–	0.2	–	–	0.1	–	–	0.1
1429	perillen	–	–	–	–	tr	0.1	–	–	tr
1450	*trans*-linalool oxide (Furanoid)	–	–	–	tr	tr	0.4	8.8	0.1	tr
1458	*cis*-1,2-limonene epoxide	0.3	–	0.1	–	tr	0.1	–	–	–
1466	α-cubebene	–	–	–	–	–	–	–	–	0.1
1468	*trans*-1,2-limonene epoxide	0.2	–	–	–	–	–	–	–	–
1469	3-methyl butyl hexanoate (=Isoamyl hexanoate)	–	–	–	–	–	0.1	–	–	tr
1470	2-nonyl acetate	–	–	–	–	–	–	0.3	–	–
1471	(*Z*)-3-hexenyl butyrate	–	–	0.2	0.1	–	–	–	0.1	–
1473	(*E*)-2-hexenyl butyrate	–	tr	–	–	0.2	–	–	–	–
1474	*trans*-sabinene hydrate	–	–	–	–	–	0.1	–	–	–
1476	(*Z*)-β-ocimene epoxide	–	–	tr	–	–	tr	–	–	tr
1478	*cis*-linalool oxide (Furanoid)	–	–	tr	tr	–	0.3	8.0	0.1	tr
1482	fenchyl acetate	0.1	tr	tr	tr	–	–	–	–	tr
1483	octyl acetate	–	–	–	–	–	tr	–	–	tr
1497	α-copaene	–	–	tr	0.1	0.1	–	–	tr	0.1
1498	(*E*)-β-ocimene epoxide	–	–	0.1	–	0.1	0.1	–	–	0.1
1506	decanal	–	–	–	–	–	–	0.1	–	–
1521	2-nonanol	–	–	–	tr	–	–	0.4	–	–
1541	benzaldehyde	–	tr	tr	–	tr	tr	–	0.1	–
1553	linalool	0.1	1.2	0.5	8.0	0.2	16.7	11.4	2.1	1.0
1559	8,9-limonene epoxide-I	tr	–	–	–	–	–	–	–	–
1565	linalyl acetate	–	tr	–	0.1	–	0.1	0.1	tr	–
1571	*trans-p*-menth-2-en-1-ol	0.1	tr	0.1	tr	0.1	0.1	–	–	–
1586	pinocarvone	–	–	tr	–	–	0.1	–	–	0.1
1589	β-ylangene	–	–	–	–	–	–	–	–	0.1
1590	3-methyl-2-butenyl hexanoate†	–	–	tr	–	–	–	0.1	–	tr
1591	fenchyl alcohol	–	–	–	–	–	tr	–	–	0.2
1597	bornyl acetate	–	–	–	–	–	–	–	–	0.2
1600	β-elemene	–	–	0.1	–	–	0.1	–	–	0.1
1601	β-copaene	–	–	tr	–	0.1	–	–	–	0.2
1602	6-methyl-3,5-heptadien-2-one	–	–	0.2	–	0.1	–	–	–	–
1605	2-undecanone	–	–	–	0.1	–	0.1	0.8	–	–
1611	terpinen-4-ol	1.4	1.6	1.4	0.7	0.8	2.1	–	2.3	0.1
1612	β-caryophyllene	–	0.1	0.5	0.7	0.1	0.1	–	tr	1.9
1616	hotrienol	–	–	–	–	–	–	0.3	–	–
1625	4,4-dimethyl but-2-enolide	–	–	–	–	–	–	0.1	–	–
1628	aromadendrene	–	–	0.1	–	tr	–	–	–	–

Table 3 (*continued*)

RRI	Compound	JHB (Feb)		BG	Cullinan	Verena	Nelspruit	Lagalametse	Waterberg	Balakane
		A	B	C						
1630	terpinen-4-yl acetate (=4-Terpinenyl acetate)	–	–	–	–	tr	tr	–	–	–
1638	*cis-p*-menth-2-en-1-ol	–	–	–	tr	–	0.1	–	–	–
1639	*trans-p*-mentha-2,8-dien-1-ol	0.1	tr	0.1	–	0.2	–	–	–	–
1641	*cis*-β-terpineol	–	tr	tr	–	tr	0.1	–	–	–
1648	myrtenal	–	–	–	–	–	0.1	–	–	0.3
1661	alloaromadendrene	–	–	–	–	–	–	–	–	0.3
1664	nonanol	–	–	–	–	–	–	0.2	–	–
1670	*trans*-pinocarveol	0.1		0.1	tr	0.2	0.1	–	–	0.4
1671	acetophenone	–	tr	–	–	–	–	–	0.1	–
1678	*cis-p*-mentha-2,8-dien-1-ol	–	–	0.1	–	0.1	–	–	–	–
1681	4-methyl-4-vinyl butyrolactone	–	–	–	–	–	–	5.3	–	–
1682	δ-terpineol	0.5	0.4	0.2	0.1	0.3	0.2	–	0.4	–
1687	α-humulene	0.1	0.2	0.8	0.3	0.3	0.4	–	0.1	0.5
1688	selina-4,11-diene(=4,11-Eudesmadiene)	–	–	–	–	–	–	–	–	0.2
1700	*p*-mentha-1,8-dien-4-ol(=Limonen-4-ol)	–	tr	tr	–	0.1	–	–	0.1	–
1704	myrtenyl acetate	–	–	–	–	–	tr	–	–	–
1705	γ-muurolene	–	–	–	–	–	–	–	–	0.3
1706	α-terpineol	3.1	3.2	2.3	1.0	3.5	3.0	–	2.8	2.5
1707	δ-selinene	–	–	–	–	–	–	–	–	0.1
1709	α-terpinyl acetate	0.1	tr	–	0.1	0.1	0.1	–	0.1	–
1718	4,6-guaiadiene (=γ-Guaiene)	–	–	–	–	–	–	–	–	tr
1719	borneol	–	–	–	–	–	–	–	–	0.2
1723	*cis*-1,2-epoxy-terpin-4-ol	0.2	tr	0.1	–	0.1	0.1	–	tr	tr
1733	neryl acetate	–	–	–	–	0.1	0.1	–	–	–
1739	α-muurolene	–	0.1	0.2	–	0.3	0.1	–	–	1.0
1740	valencene	–	–	0.1	–	0.1	–	–	–	0.2
1742	β-selinene	–	–	–	0.1	–	0.2	–	–	–
1744	α-selinene	–	tr	0.1	0.1	0.1	0.1	–	–	0.6
1750	*cis*-linalool oxide (Pyranoid)	–	–	–	–	–	–	1.9	–	–
1751	carvone	0.5	0.1	0.2	–	0.4	tr	–	0.2	–
1758	*cis*-piperitol	–	tr	tr	–	tr	tr	–	–	–
1759	(*E*,*E*)-α-farnesene	–	–	–	–	–	–	–	–	tr
1765	geranyl acetate	–	tr	–	0.1	0.2	0.1	0.2	0.1	0.1
1770	*trans*-linalool oxide (Pyranoid)	–	–	–	–	–	tr	2.0	–	–
1773	δ-cadinene	0.1	0.1	0.1	0.1	0.1	0.1	–	0.1	1.2
1776	γ-cadinene	–	0.1	0.2	0.1	0.4	0.1	–	–	0.6
1797	*p*-methyl acetophenone	–	–	–	–	–	tr	–	–	–
1798	methyl salicylate	–	–	–	tr	–	tr	–	–	–
1804	myrtenol	0.1	–	–	–	0.2	0.1	–	–	0.3
1807	α-cadinene	–	–	–	tr	tr	tr	–	–	–
1808	nerol	–	–	–	–	–	0.1	–	–	–
1811	*trans-p*-mentha-1(7),8-dien-2-ol (=*trans*-2-hydroxy pseudolimonene)	–	tr	–	–	–	–	–	–	–
1826	γ-heptalactone	–	–	–	–	–	–	1.1	–	–
1830	2,6-dimethyl-3(*E*),5(*E*),7-octatriene-2-ol	–	tr	–	tr	–	0.1	–	–	–
1845	*trans*-carveol	0.5	0.1	0.3	tr	0.5	0.1	–	0.2	–
1853	*cis*-calamenene	0.1	tr	0.1	–	0.2	0.1	–	0.1	0.6
1857	geraniol	–	–	–	0.1	–	0.3	–	–	–
1864	*p*-cymen-8-ol	0.2	tr	0.1	–	0.2	0.2	–	0.1	0.1
1875	*trans*-2-hydroxy-1,8-cineole	0.1	–	–	–	–	–	–	–	–
1882	*cis*-carveol	0.2		0.1	–	–	tr	–	0.1	–
1883	benzyl butanoate	0.1	0.1	0.3	0.1	0.2	tr	0.2	0.1	–
1896	*cis-p*-mentha-1(7),8-dien-2-ol (=*cis*-2-hydroxy pseudolimonene)	–	tr	–	–	–	–	–	tr	–
1901	geranyl butyrate	–	tr	0.1	0.1	0.2		–	tr	–
1941	α-calacorene	–	tr	tr	tr	–	tr	–	–	0.1
1949	(*Z*)-3-hexenyl nonanoate	–	–	–	–	–	–	16.0	–	–
1969	*cis*-jasmone	–	–	–	0.1	0.1	tr	–	tr	0.1
1989	2,6,10-trimethyl-7,10-epoxy-2,11-dodecadien-6-ol (=*Nerolidol oxide*)	0.4	–	–	–	–	–	–	–	–
2001	isocaryophyllene oxide	–	–	0.2	–	0.5	0.2	–	tr	0.1
2008	caryophyllene oxide	1.3	1.2	2.2	4.2	7.2	1.5	4.6	1.3	4.5
2016	2,6,10-trimethyl-7,10-epoxy-2,11-dodecadien-6-ol isomer (=Nerolidol oxide isomer)	0.6	–	–	–	–	–	–	–	–
2029	perilla alcohol	–	tr	–	–	0.1	0.1	–	tr	0.1

(continued on next page)

Table 3 (*continued*)

RRI	Compound	JHB (Feb)		BG	Cullinan	Verena	Nelspruit	Lagalametse	Waterberg	Balakane
		A	B	C						
2037	salvial-4(14)-en-1-one	–	–	–	–	0.1	–	–	tr	0.2
2045	humulene epoxide-I	0.1	0.1	0.1	0.1	0.1	0.1	–	tr	0.1
2050	(*E*)-nerolidol	12.5	tr	16.3	0.1	0.2	0.8	–	tr	tr
2051	gleenol	–	–	–	–	–	–	–	tr	tr
2071	humulene epoxide-II	1.2	0.6	1.6	1.0	0.9	0.6	–	0.2	0.7
2079	(*E*)-β-ionone	0.1	–	–	–	–	–	–	–	–
2080	cubenol	–	tr	–	–	0.1	tr	–	tr	0.5
2088	1-*epi*-cubenol	0.1	0.1	0.1	0.1	0.2	0.1	–	0.1	1.0
2096	elemol	–	–	–	–	–	0.4	–	–	–
2098	globulol	0.3	0.2	0.6	–	0.8	–	0.3	–	–
2100	heneicosane	–	–	–	0.1	–	–	–	–	–
2104	viridiflorol	–	–	tr	0.1	–	tr	–	0.1	0.8
2106	guaiyl acetate	–	–	–	–	–	–	–	–	0.2
2123	methyl-4-(4′-methyl-3′-pentenyl)-3-cyclohexenyl ketone†	–	–	–	–	–	0.1	–	–	–
2126	3,7-dimethyl-1,7-octadien-3,6-diol	–	–	–	–	–	–	3.4	–	–
2144	rosifoliol	–	–	–	–	0.1	tr	–	–	0.3
2146	spathulenol	0.4	0.6	0.7	0.1	0.9	0.5	1.0	0.1	0.8
2148	(*Z*)-3-hexen-1-yl benzoate	–	–	–	–	0.1	–	–	–	–
2153	neointermedeol	–	tr	–	0.1	–	0.1	–	tr	0.6
2164	6-*epi*-cubenol	tr	tr	0.1	0.1	0.3	0.1	–	tr	0.1
2174	cinnamyl acetate	tr	–	–	–	–	–	–	–	–
2184	*cis*-*p*-menth-3-en-1,2-diol	–	0.1	–	–	0.5	–	–	0.1	–
2185	T-cadinol	0.3	0.1	0.3	0.3	0.8	0.1	–	0.2	1.4
2187	γ-eudesmol	–	–	–	0.2	–	0.9	–	–	5.2
2196	eremoligenol	–	–	–	0.1	–	0.1	–	–	1.7
2206	α-guaiol	–	–	–	–	–	–	–	–	0.5
2209	T-muurolol	0.1	tr	0.1	tr	0.3	tr	–	0.1	0.2
2211	clovenol	–	tr	–	0.1	0.1	–	–	tr	–
2214	torreyol	0.1	tr	0.1	tr	0.2	0.1	–	tr	0.6
2247	*trans*-α-bergamotol	–	tr	0.1	–	–	–	–	–	–
2250	α-eudesmol	–	–	–	0.6	–	1.1		–	7.5
2255	α-cadinol	0.3	0.1	0.3	–	0.8	–		0.2	tr
2257	β-eudesmol	–	–	–	0.8	–	1.4	–	–	3.1
2272	alismol	–	–	–	–	0.4	0.2	–	0.1	0.7
2273	selin-11-en-4α-ol	–	tr	0.2	0.2	0.3	0.1	–	–	0.2
2316	caryophylla-2(12),6(13)-dien-5β-ol (=Caryophylladienol I)	–	–	0.1	–	–	–	–	–	–
2324	caryophylla-2(12),6(13)-dien-5α-ol (=Caryophylladienol II)	–	0.1	–	0.4	0.3	–	–	0.1	0.4
2328	(*E*,*E*)-10,11-epoxyfarnesyl acetate	–	–	0.4	–	–	–	–	–	–
2329	3,7,11-trimethyl-10,11-epoxy-2,6-dodecadien-1-yl acetate † (=10,11-Epoxyfarnesyl acetate)	1.0	–	–	–	–	–	–	–	–
2350	carvone hydrate† (=Aralone)	0.2	–	–	–	–	–	–	–	–
2352	(2*E*,6*E*)-farnesol	–	0.1	0.3	0.3	0.8		–	0.1	–
2375	eudesma-4(15),7-dien-1β-ol	–	tr	0.1	0.1	0.2	0.1	–	0.1	0.5
2385	10-hydroxy calamenene	–	–	0.3	–	0.4	0.1	–	0.1	–
2389	caryophylla-2(12),6-dien-5α-ol (=Caryophyllenol I)	–	0.1	tr	0.4	0.4	–	–	0.1	0.2
2392	caryophylla-2(12),6-dien-5β-ol (=Caryophyllenol II)	0.2	0.1	0.2	0.4	0.5	–	0.2	0.1	0.1
2438	kaur-16-ene	–	–	–	–	0.2	–	–	–	–
2441	3,7-dimethyloct-1-en-3,6,7-triol †	–	–	–	–	–	–	0.5	–	–
2501	phytol acetate†	–	–	–	–	–	–	–	–	1.1
2503	agglomerone †	–	–	–	–	–	–	–	0.4	–
2622	phytol	–	0.1	0.4	–	0.6	0.2	–	–	2.7
	TOTAL	92.3	97.8	93.6	97.6	93.3	95.3	72.0	97.2	89.1

RRI Relative retention indices calculated against *n*-alkanes % calculated from TIC data tr Trace (<0.1%).
† Tentative identified from Wiley, MassFinder, Adams Libraries.

(Weyerstahl et al., 1992) from samples collected in Zimbabwe showed higher oil yields (0.9%) to that found in our study.

More interesting, however, was the difference in oil composition. The major compounds found in the Zimbabwean oils in the summer months were (*E*)-β-ocimene (29%) and linalool (26%). In the winter months limonene (21%) and 1,8-cineole (40%) were dominant. In the current study, the GC–MS analysis of the three February samples (A, B and C) from JHB BG indicated that these compounds were present in negligible quantities. (*E*)-β-Ocimene was absent in plant A, with trace amounts detected in plants B and C. Linalool, although present as major constituent in other samples, was a minor constituent

in some samples (e.g. 0.1%, 1.2% and 0.5% for plants A, B and C respectively). It was also determined that limonene and 1,8-cineole, both major constituents, were consistently predominant throughout the winter and summer months. In a seasonal study, also undertaken in Zimbabwe by Chagonda et al. (2000), limonene and 1,8-cineole were found to be the major constituents in winter for both the wild and cultivated trees. In the summer months, linalool (26.5%), limonene (13.3%) and 1,8-cineole (24.7%) were noted as major components.

3.1.2. Geographical variation study

The chemical diversity reported in the Zimbabwean trees from the same geographical region prompted a geographical variation study on the essential oil composition. The essential oil composition of *H. natalensis*, representing samples from seven different localities is given in Table 3 and a dendrogram (Fig. 2) was generated using the 173 different chemical compounds identified by GC–MS. Seven of the nine samples are united in a single cluster based on the similarity in oil composition and dominance of 1,8-cineole and limonene in the essential oil. These major compounds were also present in the Nelspruit and Waterberg samples. The Nelspruit sample showed the highest similarity to the summer (wild type) samples noted by Chagonda et al. (2000). The oil sample from Lagalametse was distinctly different, with (*Z*)-3-hexenyl nonanoate (16.0%) and linalool (11.4%) predominating. Limonene and 1,8-cineole were conspicuously absent. The sample from Balakane was also dissimilar, with β-pinene (25.2%) as the only major constituent. In addition to these major compounds, linalool was present as a major constituent in the Nelspruit sample which differentiated it from the Gauteng and Waterberg samples.

3.2. Antimicrobial activity

3.2.1. Seasonal variation study

As the chemical composition of an essential oil potentially affects the biological activity, the antimicrobial properties of the samples were recorded. Most samples showed similar antimicrobial patterns across the entire season with slight variations for some samples (Table 1). For the Gram-positive organisms *S. aureus*, *B. cereus* and *E. faecalis*, the average MIC values obtained were 6.4, 3.3 and 8.8 mg/ml respectively. The highest variability was noted for *S. aureus* with a standard deviation of ±3.3. Similarly, the Gram-negative test organisms showed little variability. The yeast *C. neoformans* showed the highest sensitivity of all pathogens studied, having an average MIC of 2.1 mg/ml with the least variability (±0.3) noted between samples (Table 1). The antimicrobial activity for the Zimbabwean *H. natalensis* oils, as recorded by Gundidza et al. (1993) indicated good activities against *E. coli*, *K. pneumoniae*, *S. aureus* and *Moraxella* species with lower sensitivities for *P. aeruginosa*. Even though the major constituents 1,8-cineole and limonene, found in the oil studied by Gundidza et al. (1993) correlate well with the composition of the South African oils, results can not be compared directly due to the different antimicrobial methods. Gundidza et al. (1993) made use of disc diffusion techniques whereas in this study, the microtitre plate method was used.

3.2.2. Geographical variation study

The MIC data of the oil distilled from different geographical regions is presented in Table 1. The Gauteng samples (Cullinan, Verena and JHB BG) all exhibited consistent, moderate antibacterial activity (MIC values between 3.0 and 16.0 mg/ml). Only *C. neoformans* displayed a higher sensitivity (0.8 to 2.0 mg/ml). Nelspruit, Waterberg and Balakane samples also displayed similar antimicrobial profiles. With the exception of the Balakane sample, all these samples had 1,8-cineole and limonene as major constituents. β-Pinene, which was present as a major constituent only in the Balakane sample, has previously shown either poor (Hinou et al., 1989; Kang et al., 1992) or moderate (Dorman and Deans, 2000; Van Zyl et al., 2006) antimicrobial activity against Gram-negative organisms. The low MIC values (0.5 mg/ml) noted for this sample against *C. neoformans* could possibly be attributed to alterations in mitochondrial and plasma membrane function of the yeast cells. Previous studies by Uribe et al. (1985) have shown that β-pinene has an adverse effect on yeast membrane functions. The composition of the essential oil of the Lagalametse sample is very different from the rest of the samples, where (*Z*)-3-hexenyl

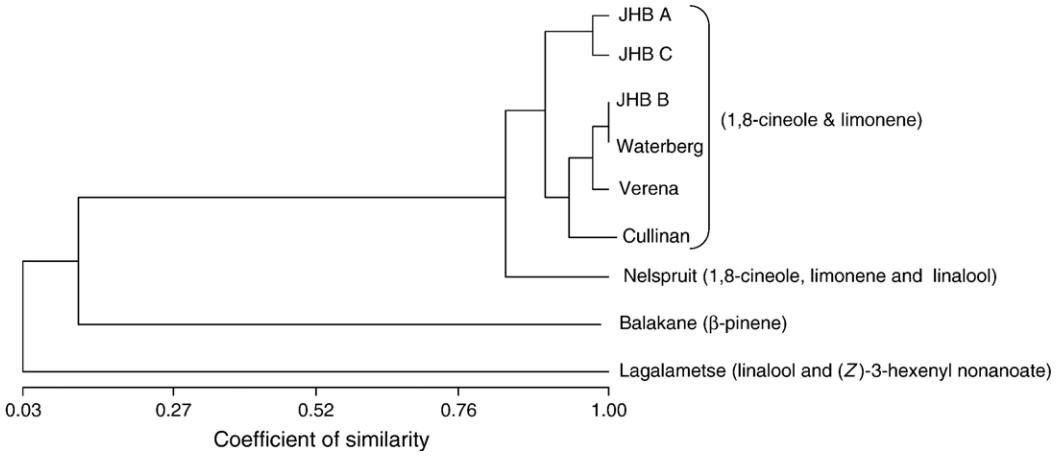

Fig. 2. Dendrogram constructed on the essential oil data matrix (Table 3) with major compounds indicated in brackets.

nonanoate and linalool were identified as major constituents. Linalool was present as a major compound in both the Nelspruit sample (16.7%) and Lagalametse sample (11.4%). The antimicrobial activity of the oil obtained from the two localities differed with the greatest variation seen for *S. aureus*. The Lagalametse sample had an MIC value of 1.6 mg/ml and the Nelspruit sample had an MIC value of 6.0 mg/ml. Hence, the antimicrobial activity found in the Lagalametse sample can not be attributed to linalool. (*Z*)-3-Hexenyl nonanoate exclusively found in the Lagalametse sample, could possibly account for the increased antimicrobial activity noted. A previous study (Dorman and Deans, 2000) has shown that linalool has far greater broad-spectrum antimicrobial activity than limonene. The authors presented disc diffusion data reporting that inhibition was greater for linalool against 22 of the 25 test organisms studied. Carson and Riley (1995) demonstrated higher efficacies for linalool when comparatively assessed with 1,8-cineole. Higher efficacies were found for linalool in both disc diffusion and MIC methodology. Pattnaik et al. (1997) also demonstrated increased efficacies for linalool for 21 of the 30 pathogens studied.

4. Conclusions

All essential oil studies showed moderate antimicrobial activity (MIC values ranged between 3.0 to 16.0 mg/ml) against most of the pathogens investigated. *Cryptococcus neoformans*, a respiratory pathogen, exhibited higher sensitivities towards the essential oil. As *Heteropyxis* (and especially its volatile fraction) is traditionally used to treat respiratory disorders, efficacies exhibited against this pathogen, may give credibility to the ethnobotanical use. The seasonal variation of *H. natalensis* samples selected from three trees in the Johannesburg Botanical Garden indicate similar chemical profiles with respect to seasonal fluctuation in the levels of major constituents. In the antimicrobial study, results indicated little variation between samples. The geographical variation study of *H. natalensis* revealed similar major profiles for Gauteng, Nelspruit and Waterberg samples, which had 1,8-cineole (21.7–41.2%) and limonene (15.0–25.4%) as major constituents. The Lagalametse sample showed distinct variation both chemically and in terms of antimicrobial activity. Linalool (11.4%) and (*Z*)-3-hexenyl nonanoate (16.0%) was identified as the major constituents and antimicrobial efficacy, possibly due to the presence of (*Z*)-3-hexenyl nonanoate, was significantly enhanced.

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