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Composition and antimicrobial activities of volatile components of *Lippia javanica*

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Abstract

The volatile oil of *Lippia javanica* was prepared by hydrodistillation of leaves, flowers and stems, and characterized by GC–MS. The major component was 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one. The oil was tested for antimicrobial activity on cultures of *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*, and found to inhibit *E. coli* and *S. aureus* at 1% dilution. The oil was also active against *Plasmodium falciparum* in micromolar concentrations.

Keywords: *Lippia javanica*; Verbenaceae; Volatile components; Malaria; Essential oil; Terpenoids; Piperitenone; 3-Methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one

1. Introduction

Malaria, one of the world's deadliest and yet avoidable diseases, is pandemic in over 100 countries in South America, Southeast Asia and Africa (Nchinda, 1998). Although drugs and insecticides have made malaria rare in developed nations, each year the parasite still sickens over 300 million people in the tropical developing countries. Sub-Saharan Africa's poorest citizens suffer the brunt of the illness, accounting for 90% of all cases and deaths world-wide. The malaria pandemic in Africa is aggravated by an increasing prevalence of multi-drug-resistant strains of this parasite, which reduces the efficacy of the current antimalarial drugs for effective treatment of this disease (Kriner, 2000). Therefore, all plant extracts processed in our laboratory are routinely tested for antimalarial activity.

In the ongoing research on and identification of active substances from African traditional medicines, numerous plants have been analysed and tested for their efficacy against malaria and many other ailments (Bassole et al., 2003; Chagonda et al., 1993; Govere et al., 2000; O'Neill et al., 1985). In the course of our studies of Southern African medicinal plants (Dehmlow et al., 2000, and references cited therein), a plant used widely in Africa drew our attention. *Lippia javanica* (Verbenaceae) is commonly used in South Africa against various chest ailments, influenza, measles, rashes, stomach problems and headaches, depending on the traditional healer, and is therefore known as 'fever tea' or 'musudzungwane' in Tshivenda. Its essential oil has also been found to have good insect repellent activity (Govere et al., 2000). In Botswana it is used as a caffeine free tea. In Zimbabwe and Malawi it is used mainly as a nerve tonic. In Zimbabwe *L. javanica* was found to inhibit *Escherichia coli, Staphylococcus aureus*, *Salmonella gallnarum*, *Klebsiella pneumoniae, Candida albicans* and *Pseudomonas aeruginosa* at concentrations of 1 mg/ml

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(Chagonda et al., 1993). *Lippia multiflora* from Ivory Coast has been found to be active against malaria (Valentin et al., 1995). The present work describes the composition of the essential oil of *L. javanica* and its in vitro activity against some common organisms.

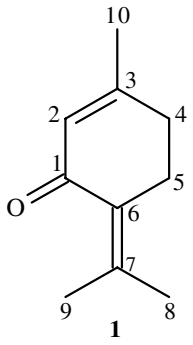
2. Results and discussion

Hydrodistillation of the dried plant material, followed by extraction with diethyl ether and careful evaporation at low temperature produced a light orange oil with a yield of 0.4% (by weight). The identity, retention time, and the percentage composition of the oil of *L. javanica* are presented in Table 1. The percentage composition is presented as relative peak area. Seven components were identified and quantified, accounting for over 94% of the composition of the oil. The constituents are consistent with those of a member of the Verbenaceae. None of the remaining unidentified compounds accounted for more than 0.7% of the total peak area. In contrast with *L. multiflora* (O'Neill et al., 1985), the essential oil was characterized by a high content in one major monoterpene, identified separately as 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (piperitenone, 74.4%).

Silica gel column chromatography of the oil using a hexane:diethyl ether gradient as mobile phase yielded a colourless oil which was analysed by MS, ^{1}H and ^{13}C NMR spectroscopy. A molecular ion at *m/z* 150 (low resolution MS) indicated the presence of a substance with probable molecular formula $C_{10}H_{14}O$. The ^{1}H NMR spectrum showed three methyl signals at 1.74, 1.81 and 1.98 ppm, two methylene signals at 2.17 and 2.54 ppm, exhibiting long-range coupling to one of the methine signals, and a methine proton at 5.76 ppm. The ^{13}C NMR spectrum showed the presence of three methyl quartets at 22.2, 22.6 and 23.5, two methylene triplets (confirmed by DEPT135) at 27.7 and 31.6, three quaternary singlets at 116.1, 142.3 and 159.4 and a carbonyl signal at 191.3 ppm. Finally, comparison with reference spectra (Erman, 1967) (Tables 2 and 3) confirmed the structure as 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (**1**).

Table 1
Volatile components of *L. javanica*

t_R (min:s)	Identification	Percentage of total oil
14:58	Limonene	1.6
21:05	Linalool	1.8
28:37	*o*-Isopropenylanisole	9.7
29:27	α-Terpineol	0.8
31:27	*Z*-Ocimenone	3.9
36:37	Compound **1**	74.4
40:36	β-Caryophyllene	1.0



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The essential oil of *L. javanica* had low activitity in vitro against the Gram-positive *E. coli* and *S. aureus* at a concentration of 10 mg/ml (radius of zone of inhibition of 16–18 mm as shown in Table 4).

As *L. javanica* is known for its indigenous use, *inter alia* as a malaria cure, the plant's hydrodistillate (essential oil) was tested against the D10 chloroquine-sensitive strain of *Plasmodium falciparum*, and gave the following IC_{50} values:

- Chloroquine, 20 ng/ml;
- *L. javanica* oil, 8 μg/ml.

A result in the micromolar range is generally accepted (Clarkson et al., 2003) to be significant, and in the light of the traditional use of the plant warrants further investigation of individual (minor) components of the oil.

Table 2
^{1}H NMR data for 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (**1**)

Signal	Chemical shift (ppm)	Reference substance
2-H	5.76 (*m*)	5.76 (*m*)
4-H	2.54 (*t*, *J* = 6.4 Hz)	2.64 (*m*)
5-H	2.17(*m*, *J* = 6.4 Hz)	2.25 (*m*)
8-H	1.81 (*q*)	1.84 (*s*)
9-H	1.98 (*t*)	1.91 (*s*)
10-H	1.74 (*s*)	2.08 (*s*)

Table 3
^{13}C NMR data for 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one (**1**)

Signal	Chemical shift (ppm)	Reference substance
C-1	191.3	191.7
C-2	128.7	128.8
C-3	142.3	142.0
C-4, C-5	27.7, 31.6	28.0, 31.9
C-6	159.4	159.6
C-7	128.9	129.0
C-8, C-9	22.2, 22.6	22.5, 22.9
C-10	23.5	23.7

Table 4
Plate tests of *L. javanica* essential oil

Substance	*B. subtilis*	*S. aureus*	*E. coli*
Imipenem	26	28	28
Cephazolin	19	20	18
Ampicillin	–	–	–
Essential oil	–	18	16

3. Experimental

3.1. Plant material

The plant material was harvested at Thathe Vondo village (Limpopo Province, Republic of South Africa) in March 2001. A voucher specimen was placed in the University of Venda herbarium.

3.2. Essential oil isolation and characterization

The essential oil was prepared by hydrodistillation performed typically with 100 g of fresh plant material in 1.5 l water for 6 h. Analysis by GC–MS was performed on a Finnigan GCQ using the following conditions: RTX-5MS column (5% diphenyl–95% dimethylpolysiloxane, 30 m × 0.25 mm), split injection at 250 °C. Carrier gas was He (head pressure 500 kPa, linear velocity 40 cm/s); transfer line temp. 275 °C, ion source temp. 200 °C, initial column temp. 50 °C held for 3 min, then raised to 150 °C at 2 °C/min, then raised to 290 °C at 5 °C/min and held at 290 °C for 10 min. Peak identification was by means of retention times and spectrum matching, using the NIST and Wiley libraries. NMR spectroscopy: 300 MHz (75 MHz) Bruker AMX-300 spectrometer in $CDCl_3$.

3.3. Antimicrobial assays

The bacteria were cultured in Mueller Hinton Agar (Collins et al. 1995, pp. 178–205), and the oil was tested using imipenem, cephazoline and ampicillin as positive controls. Two dilutions (0.1 ml extract in 100 ml diethyl ether and 0.1 ml extract in 10 ml diethyl ether) were prepared. The organisms were cultured in nutrient broth and the tests carried out on Mueller Hinton agar plates.

The antimalarial assay was provided by Prof Peter Smith of the Medical School, University of Cape Town. A chloroquine-sensitive strain of *P. falciparum* (D10) was continuously cultured and parasite lactate dehydrogenase (pLDH) activity was used to measure parasite viability. Chloroquine diphosphate served as a positive control and was made up in millipore water and diluted in medium to the required concentrations. One mg/ml stock solutions of the plant extracts were made up in methanol (MeOH) and water, and were diluted in complete medium on the day of the experiment. The highest concentration of MeOH that the parasites were exposed to was 0.5%, which had no measurable effect on parasite viability. The antiplasmodial assays were performed in duplicate on a single occasion (Clarkson et al., 2003). The 50% inhibitory concentration (IC_{50}) values were obtained from the dose–response curve, using non-linear dose–response curve fitting analyses with GraphPad Prism v.3.00 software.

3.4. Data for 3-methyl-6-(1-methylethylidene)-cyclohex-2-en-1-one

To elucidate the structure of the major component of the oil, 100 mg of the oil was subjected to column chromatography (SiO_2; hexane–diethyl ether 9:1 to 1:1), followed by preparative TLC (SiO_2; hexane–diethyl ether 8:2). IR (neat): ν 1664 (C=O), 1613 (C=C) cm^{-1} . ^1H NMR (300 MHz, $CDCl_3$): δ 1.74 (3H, *s*, CH_3, 10-H), 1.81 (3H, *q*, CH_3, 8-H), 1.98 (3H, *t*, CH_3, $J = 1.3$ Hz, 9-H), 2.17 (2H, *dt*, $J = 1.2$, 6.4 Hz, CH_2, 5-H), 2.54 (2H, *t*, $J = 6.3$ Hz, CH_2, 4-H), 5.76 (1H, *m*, $J = 1.4$ Hz, 2-H). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 22.2 (*q*, C-8), 22.6 (*q*, C-9), 23.5 (*q*, C-10), 27.7 (*t*, C-4), 31.6 (*t*, C-5), 128.7 (*d*, C-2), 128.9 (*d*, C-7), 142.3 (*s*, C-3), 159.4 (*s*, C-6), 191.3 (*s*, C-1). MS (EI, 70 eV): *m/z* 150 (M^+, 100%), 135 ($M^+ - CH_3$, 59%), 122 ($M^+ - CO$, 19%), 121 (19%), 108 (25%), 107 (83%), 105 (27%), 91 (56%), 79 (51%), 77 (25%).

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