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Full Length Research Paper

Composition and antimicrobial activities of the leaf and flower essential oils of *Lippia chevalieri* and *Ocimum canum* from Burkina Faso

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The essential oils of the air dried leaves and flowers of *Lippia chevalieri* Moldenke and *Ocimum canum* Sims from Burkina Faso were analysed by GC-MS. Essential oil of the leaves of *L. chevalieri* is composed mainly of thymol (27.4%), p-cymene (21.1%), and 2-phenyl-ethyl-propionate (12.6%), while the oils from flower is composed of β-elemene (33%), ethyl cinnamate (30.3%) and α-amorphene (12.4%). *O. canum* leaves and flowers oils consisted mainly in 1,8-cineole (60.1%) and cis, trans-piperitol (68.5%), respectively. The antimicrobial activities of the essential oils were evaluated against 9 bacteria by agar diffusion method. The leaves of both plants showed higher activity than their flowers. The leaves of *L. chevalieri* were active against Gram negative and Gram positive bacteria whereas only Gram positive bacteria were sensitive to the essential oil of the *O. canum* leaves. Flower essential oils did not show any significant activity.

Key words: *Lippia chevalieri*, *Ocimum canum*, essential oils, chemical composition, antibacterial activity.

INTRODUCTION

Lippia chevalieri Moldenke and *Ocimum canum* Sims are aromatic species belonging to *Verbenaceae* and *Labiateae* families, respectively. The aerial parts of both species are used in folk medicine for several purposes. Leaves and flowers of *L. chevalieri* are used in the treatment of respiratory diseases, malaria, syphilis and gonorrhoae. Aerial parts of *O. canum* are used to treat conjunctivites and headaches and as spices in a fish sauce (Ngassoum et al., 2004).

Several chemotypes of *O. canum* essential oils have been reported: fenchone type (Lawrence et al., 1980), citral type (Choudhary et al., 1989), eugenol type (Ekundayo et al., 1989), terpineol type (Chalchat et al., 1996), 1,8-cineole type (Zollo et al., 1998), β-caryophyllene /(E)-α-bergamotene/bicyclogermacrene type (Sanda et al., 1998), methyl cinnamate type (Martins et al., 1999), methylchavicol/α-terpineol type (Chalchat et al., 1999), camphor type (Chagonda et al., 2000), linalool-type (Yayi et al., 2001) and limonene type (Ngassoum et al., 2004). Caryophyllene/1,8-cineole / germacene type (Menut et al., 1993) and thymol/p-cymene/thymyl acetate type (Bassole et al., 2003a) of the essential oils of *L. chevalieri* have also been reported.

Previous studies have shown that the essential oils of the leaves of *O. canum* possess antibacterial (Janssen et al, 1989) and insecticidal (Bassole et al., 2003b) properties. Antibacterial properties of the essential oil of the leaves of *L. chevalieri* have been reported (Bassole et al., 2003a). However, to the best of our knowledge, there are no data available on chemical composition of essential oils of the flowers of *L. chevalieri* and *O. canum* from Burkina Faso. Because of the significant difference

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in smell of the aerial parts of both plants, we have focused this study to analyse the volatile oils responsible for the characteristic odour and their antibacterial activities.

MATERIALS AND METHODS

Extraction and chemical analysis of essential oils

L. chevalieri Moldenke and *O. canum* Sims leaves and flowers were collected from a wild population in Ouagadougou (Burkina Faso) during September, 2000. They were taxonomically identified at the Laboratoire de Biologie et d'Ecologie Vegetales (Universite de Ouagadougou), where a voucher specimen of each sample is deposited.

The air dried leaves and flowers were subjected to hydrodistillation for 3 h using a Clevenger type apparatus recommended by French Pharmacopoeia (1983). The essential oils obtained were dried with anhydrous sodium sulphate and stored at -20°C before using. Gas chromatographic (GC) analyses were performed using a VARIAN 3800 instrument equipped with two fused capillary columns: Supelcowax (30m × 0.25 mm internal diameter; film tickness 0.25 µm) and SPB-1 (30m × 0.25 mm internal diameter; film tickness 0.25 µm). A sample of 1 µl was injected under following conditions: column temperature 40 to 240°C, at 2°C/min and held isothermal for 10 min, injectors and FID detector were at 230°C and 250°C, respectively, injection mode split and carrier gas was Helium at 30 cm s^{-1}. The GC-mass spectrometry analysis was carried out with a Saturn II mass spectrometer. The electron impact was 70 ev and the temperature was 220°C. The mass spectrometry (MS) equipement was coupled with a Varian 3400 GC equipped with a DBWAX capillary column (30 m × 0.25 mm internal diameter; film tickness 0.25 µm). The operating conditions for the oven were the same as above. Injector temperature was programmed for 40 - 240°C at 180°C/min and held isothermal at 240°C for 139 min. The component identification was achieved by comparing of retention indices and mass spectra with those of the standard included in the library (Adams, 1989).

Antibacterial screening

The micro-organisms used were *Bacillus cereus* LMG 13569, *Entrococccus faecalis* CIP 103907, *Escherichia coli* CIP 105182, *Listeria innocua* LMG 13568, *Proteus mirabilis* CIP 104588, *Salmonella enterica* CIP 105150, *Shigella dysenteria* CIP 54.51, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus camorum* LMG 13567. The agar disc diffusion method was employed for the screening of antimicrobial activities of the essential oils (NCCLS, 1997). The test was performed in sterile Petri dishes (90 mm diameter) containing solid and sterile Mueller-Hinton agar medium (Becton, Dickinson, USA). The oils absorbed on sterile paper discs (5 µl per Whatman disc of 6 mm diameter), were placed on the surface of the media previously inoculated with 0.1 ml of microbial suspension (1 µg per Petri dish). One filter paper disc was placed per Petri dish in order to avoid a possible additive activity, exhibited via the vapour phase, of the components from more than one disc. Every dish was sealed with laboratory film to avoid evaporation, then incubated aerobically at 30°C (Gram-negative) or 37°C (Gram-positive) according to strain for 24 h, followed by the measurement of the zone diameter of the inhibition expressed in mm. Gentamicin and Neomycin discs (10 and 30 µg, respectively) were used as antibiotic reference products. All tests were performed in triplicate

RESULTS

Chemical composition of the essential oils

The relative amount (% v/w) of essential oils from *L. chevalieri* and *O. canum* leaves and flowers presented in Table 1 varied from 0.75 to 1.7%. The highest value of yield was obtained for the leaves of both plants. Quantitative and analytical results are shown in Tables 2 and 3. More than 91% of the volatile oils were identified in each sample, and in total 50 components were identified. 14 and 8 constituents were identified by means

Table 1. Essential oils yield (%, v/w) from *Lippia chevalieri* and *Ocimum canum* leaves and flowers.

Plant	Essential oil (%)	
	Leaves	Flowers
Lippia chevalieri	1.5 ± 0.7	0.8 ± 0.2
Ocimum canum	1.7 ± 0.5	0.75 ± 0.1

Table 2. Main components (%) of the essential oils from *Lippia chevalieri* leaves and flowers.

Peak	K. I.	Compound	Amount (%)	
			Leaves	Flowers
1	1019	α-thujene*		1.9
2	1158	myrcene*		1.5
3	1167	α-phellandrene*		1.0
4	1192	α- terpinene*	10.6	
5	1232	β-terpinene*		4.0
6	1256	p-cymene*		21.1
7	1259	cumene***	1.8	
8	1562	longifene**		5.7
9	1564	β-elemene**	33.0	
10	1634	β-himachlene**	1.2	
11	1649	γ-cedrene**		0.9
12	1672	lavandulol**		1.4
13	1674	α-amorphene**	12.4	
14	1824	2-phenyl ethyle propionate*		12.6
15	1936	α-calocorene	5. 2	
16	1936	1-decanol		1.0
17	2051	ethylcinnamate	30.3	
18	2052	benzyl hexanoate		6.1
19	2160	thymol		27.4
20	2186	carvacrol		4.0
21	2186	α-cadinol	3.9	
22	2190	2-phenyl ethyl-tiglate		2.6
Total			**98.4**	**91.2**

Table 3. Main components (%) of the essential oils from *Ocimum canum* leaves and flowers.

Peak	K.I.	Compound	Amount (%)	
			Flowers	Leaves
1	927	α-fenchene		3.8
2	939	Camphene		0.8
3	963	isobutyl valerate		1.5
4	965	β-pinene		5.6
5	984	dehydro-1,8-cineole		2.1
6	1048	acetophenone		0.7
7	1108	campholenal		5.6
8	1167	methyl chavicol		5.4
9	1398	(Z) isopulegone		2.6
10	1423	ethyl cinnamate		3.1
11	1159	α- phellandrene	1	
12	1198	1,8-cineole	0.6	61.2
13	1217	□-phellandrene	2	
14	1225	pentyl isobutyrate	1.8	
15	1231	□-terpinene	1.5	
16	1279	hexylacetate	0.4	
17	1592	trans-bergamotene	4.3	
18	1593	selinadiene	2	
19	1609	carvomenthone	0.6	
20	1667	ledene	1.1	
21	1709	cis + trans-piperitol	68.5	
22	1717	□-bisabolene	0.6	
23	1738	biclogermacrene	0.8	
24	1791	citronellyl-3-methylbutyrate	0.8	
25	1975	oxyde de caryophyllene	9.7	
26	2231	ledol	1.4	
27	2386	vertraldehyde	0.6	
Total			**97.7**	**92.4**

of GC-MS analysis of the essential oils from *L. chevalieri* leaves and flowers, respectively. While 11 and 17 compounds were identified in the essential oil from *O. canum* leaves and flowers.

The three major components of *L. chevalieri* leaf essential oils are thymol (27.4%), p-cymene (21.1%) and 2-phenyl-ethyl-propionate (12.6%), while the essential oils from flower contains p-elemene (33%), ethyl cinnamate (30.3%) and α-amorphene (12.4%). Minor components include hexanoate (6.1%), longifene (5.7%), carvacrol (4%) and β-terpinene (4%) for leaves and α-terpinene (10.6%), α-calocorene (5.2%) and cadinol (3.9%) for the flowers. *O. canum* leaf and flower oils consisted mainly of 1,8-cineole (60.1%) and cis, trans-piperitol (68.5%), respectively. Minor components were β-pinene (5.6%), campholenal (5.6%) and methyl chavicol (5.4%) for leaves whereas flowers contained as minor components caryophyllene oxyde (9.7%) and trans-bergamotene (4.3%).

Antibacterial activity

Table 4 shows the average inhibition zones. Diameter of the inhibition zone of leaves and flowers of *L. chevelieri* varied from 9 to 30 mm and from 6 to 11 mm, respectively. The largest zone of inhibition was obtained for *Enterococcus faecalis* and the lowest for *Shigella dysenteria*. The diameter of the inhibition of leaves and flowers of *O. canum* varied from 6 to 22 mm and from 6 to 10 mm, respectively. The largest zone of inhibition was obtained for *Bacillus cereus* and *Staphylococcus camorum* whereas the lowest was for *Shigella dysenteria*. The inhibition zone of both antibiotics varied from 21 to 30 mm. *Staphylococcus aureus*, *Listeria innocua* and *Bacillus cereus* were the most sensitive and *Escherichia coli* was the least sensitive. The highest activity against bacteria was obtained with the essential oils of leaves while the flowers showed no significant activity. The oil of *L. chevalieri* appeared to be efficient against Gram positive and Gram negative bacteria. The oil of *O. canum* leaves showed significant activity against Gram positive bacteria.

DISCUSSION

The extraction yield reported in Table 1 shows that the leaves of *L. chevalieri* and *O. canum* contained more oil than their flowers. Similar results have been obtained with *Helianthus annuus* L. (Ceccarini et al., 2004). The oil of the leaves of *L. chevalieri* presented high amount monoterpenes hydrocarbons whereas the oil from the flowers consisted mainly of sesquiterpenes. Both leaves and flowers of *O. canum* contained mainly hydrocarbon monterpenes. The chemical composition of the essential of the leaves and the flowers of *L. chevalieri* and *O. canum* are different. The results of the chemical analysis of the leaves of *L. chevalieri* are quite similar to those reported previously (Bassole et al., 2003a) but are different from those obtained by Menut et al. (1993). The chemical composition of the leaves and the flowers of *O. canum* reported here also differ from those observed by Ngassoum et al. (2004).

The essential oils showed variable activities against

Table 4. Antimicrobial activity* (mm) of the essential oils of *Lippia chevalieri* and *Ocimum canum* using agar disc diffusion method.

Bacteria	Gentamicin	Neomycin	*L. chevalieri* oil		*O. canum* oil	
			Leaves	Flowers	Leaves	Flowers
Bacillus cereus	26	30	16	8	22	7
Enterococcus faecalis	28	23	30	10	12	10
Escherichia coli	20	17	14	6	10	6
Listeria innocua	30	24	14	7	17	10
Proteus mirabilis	23	16	21	6	8	6
Salmonella enterica	22	23	28	11	10	9
Shigella dysenteriae	25	20	9	6	6	6
Saphylococcus aureus	30	28	15	6	21	6
Saphylococcus camorum	21	22	19	6	22	6

*Diameter of zone of inhibition (mm).

tested bacteria. The highest activities were obtained with the essential oils of the leaves of both plants. The essential oil of the *L. chevalieri* was more potent than that of *O. canum*. The variation of the antimicrobial activity could be correlated to chemical composition variability (Burt, 2004; Lahlou, 2004). Indeed, four chemotypes of the essential oils have been identified. It is also known that some components of the essential oils exerted high antimicrobial activities. Cosentino et al. (1999) and Lattaoui and Tantaoui-Elaraki (1994) showed that carvacrol and thymol possess high activity against bacteria whereas 1,8-cineole, piperitol, terpinene (Lis-Balchin et al., 1998; Carson et al., 1995; Chalchat et al., 1995; Lattaoui and Tantaoui-Elaraki, 1994) exerted weak antimicrobial activity. Sesquiterpenes also possess weak antimicrobial activity. The interaction between essential oils component play an important role in the determination of the antimicrobial activity. Synergetic effect of carvacrol and thymol has been reported (Didry et al., 1993; Cosentino et al., 1999). P-cymen could also enhance the antibacterial effect of carvacrol (Ultee et al., 2000).

Additional effect of these three components could explain the relatively high antimicrobial activity of *L. chevalieri* leaves. In addition, it has already shown that the antimicrobial activity of volatile compounds results from the combined effect of direct vapour absorption on microorganisms and indirect effect through the medium that absorbed the vapour (Moleyar and Narasimtram, 1986). The vapour absorption on microorganims is determined by their membrane permeability. Gram-negative microorganisms are less susceptible to essential oils than gram-positive ones because they possess outer membrane surrounding the cell membrane (Ratledge and Wilkinson, 1988), which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Vaara, 1992). Absorption into aqueous media is determined by solubility, volatility and stability of volatile compounds. Thus carvacrol and thymol, which are very stable, moderately soluble in water and of low volatility, were accumulated into the agar layer in greater amounts than others which are unstable and of moderate volatility (Inouye et al., 2001).

L. chevalieri and *O. canum* leaves and flowers are used differently by local healers. This study has shown the variability of the chemical composition and antimicrobial activities of the aerial parts of two plants. The leaves (rather than flowers) should be used against bacteria.

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