PERGAMON

Phytochemistry 62 (2003) 209–212

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso

I.H.N. Bassole[a,*], A.S. Ouattara[a], R. Nebie[b], C.A.T. Ouattara[a], Z.I. Kabore[c], S.A. Traore[a]

[a]*Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles, Laboratoire de Microbiologie et de Biotechnologie, Université de Ouagadougou, 03 BP 7131, Ouagadougou 03, Burkina Faso*

[b]*Institut de Recherche en Sciences Appliquées et Techniques, Departement de Substances Naturelles 03, BP 7027, Ouagadougou 03, Burkina Faso*

[c]*Institut de Recherche en Sciences de la Santé, Centre National de la Recherche Scientifique et de la Technologie, 06 BP 9238, Ouagadougou 06, Burkina Faso*

Received in revised form 17 July 2002; accepted 19 September 2002

Abstract

The chemical composition of the essential oils of *Lippia chevalieri* and *Lippia multiflora* obtained from the air-dried leaves by hydrodistillation were analysed using GC and GC–MS. *L. chevalieri* and *L. multiflora* belonged to thymol/*p*-cymene/2-phenyl ethyl propionate and thymol/*p*-cymene/thymyl acetate chemotypes, respectively. The essential oils were also tested against 09 strains using a broth microdilution method. The Gram-negative bacteria were the most sensitive. The essential oil of *L. multiflora* was the most active.

Keywords: *Lippia chevalieri*; *Lippia multiflora*; Verbenaceae; Essential oils; Antibacterial

1. Introduction

Lippia chevalieri and *Lippia multiflora* are West Africa endemic species belonging *Verbenaceae* family of plants. Both species grow widely, possess small white flowers, up to 1 and 2.5 m, respectively. African traditional medicine uses their leaves as tea and in the treatment of malaria, hypertension (Pascual et al., 2001), boils, diarrhoea (Nacoulma/Ouédraogo, 1996) and as a mouth disinfectant (Kerharo and Adam, 1974).

Several chemotypes of the essential oil of *L. multiflora* have been reported according to the major and distribution of its principal compounds: linalool (81.3%) (Elakrovick and Oguntimein, 1987), thymol (30.3%), thymyl acetate (24.1%), *p*-cymene (14.5%) (Lamaty et al., 1990), tagetone (35.5%), ipsenone (12.5%), β-caryophyllene (11.3%) (Lamaty et al., 1990), linalool (46.1%), thymol (15.2%), β-cubenene (11.7%), *p*-cymene (10.4%) (Mwangi et al., 1991), (E)-tagetone (30.2%), (Z)-tagetone (11.3%) (Pélissier et al., 1994) myrtenol (27.1%), linalool (11.9%), 1,8-cineole (11.6%) (Menut et al., 1995a) 6,7-epoxymyrcene (70.3%) (Menut et al., 1995b) geranial (55,9%), neral (33,49%) (Koumaglo et al., 1996), 1,8-cineole (63.2%) (Koumaglo et al., 1996) 1,8-cineole (33,4%), neral (11,5%), geranial (18.3%) (Kanko et al., 1999). Only β-caryophyllene (33.3%), 1.8-cineole (20.2%), germacrene (12.5%) has been reported as chemotypes of *L. chevalieri* (Menut et al., 1993).

Many reports have shown that *L. multiflora* possesses biological properties: hypertensive effects (Noamesi, 1977), muscle relaxant properties (Noamesi et al., 1985a), vascular actions (Noamesi et al., 1985b), hypotensive effects (Chanh et al., 1988) antimicrobial activity (Pélissier et al., 1994), anti-malaria activity (Valentin et al., 1995) insecticidal activity (Koumaglo et al., 1996), antifungal activity (Baba-Moussa et al., 1997), pediculocidal and scabicidal properties (Oladimeji et al., 2000). To our knowledge no data have been published on the biological properties of *L. chevalieri* and there is a lack of information on the antimicrobial activity of the two species from Burkina Faso. Thus we

* Corresponding author. Fax: +226-33-73-73.
E-mail address: ismael.bassole@univ-ouaga.bf (I.H.N. Bassole).

0031-9422/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
PII: S0031-9422(02)00477-6

focused our study on the chemical composition and antibacterial properties of the essential oils of *L. multiflora* and *L. chevalieri*.

2. Results and discussion

The average yields of the essential oils of the air-dried leaves of the representative samples of *L. chevalieri* and *L. multiflora* were 1.5 and 2.25% (v/w), respectively.

Compounds were identified by comparison of their retention indices (RIs) with the ESO data (Aroma Chemical Information Service, 1997) and by comparison of the experimental mass spectra with literature results (Adams, 1989). Retention indices of the compounds were determined relative to the retention times of a serie of n alkanes with linear interpolation. Quantitative and analytical results are shown in Table 1.

Both essential oils consisted mainly of hydrocarbon and phenolic monoterpenes. The *L. chevalieri* exhibited three high percent components: thymol (27.4%), *p*-cymene (21.1%) and 2-phenyl ethyl propionate (12.6%). Others minor compound were benzyl hexanoate (6.1%), longifolene (5.7%), β-terpinene (4.0%), carvacrol (4.0%) and 2-phenyl ethyl tiglate (2.6%).

Table 1
Chemical composition of the essential oils of *Lippia chevalieri* and *Lippia multiflora* air-dried leaves

Pics	KI Supelcowax column	Components	Percentage	
			L. chevalieri	*L. multiflora*
1	1014	α-Pinene		0.7
2	1019	α-Thujene	1.9	
3	1022	2-Methyl-3-buten-2-ol		2.7
4	1158	Myrcene	1.5	2.2
5	1167	α-Phellandrene	1.0	3.0
6	1188	α-Terpinene		1.3
7	1195	Limonene	2.6	1.1
8	1232	β-Terpinene	4.0	
9	1238	γ-Terpinene		4.5
10	1265	*p*-Cymene	21.1	26.2
11	1562	Longifolene	5.7	
12	1571	Hexenyl valerate		4.5
13	1587	*cis*-Isopulegone		0.8
14	1649	γ-Cedrene	0.9	
15	1655	β-Santalene		0.9
16	1672	Lavandulol	1.4	
17	1824	2-Phenyl ethyl propionate	12.6	
18	1840	Thymyl acetate		11.7
19	1859	*Trans*-8-mercapto-3-Methanone		0.5
20	1936	1-decanol	1.0	
21	2052	Benzyl hexanoate	6.1	
22	2160	Thymol	27.4	29.9
23	2186	Carvacrol	4.0	
24	2190	2-Phenyl ethyl tiglate	2.6	
25	2197	(*Z*)-Isoeugenol		4.0

According to our result *L. chevalieri* belonged to thymol/*p*-cymene/2-phenyl ethyl propionate chemotype. This result was different from that reported previously by Menut et al. (1993).

The *L. multiflora* essential oil also contained three major components: thymol (29.9%), *p*-cymene (26.2%), thymyl acetate (11.8%). Its minor components were γ-terpinene (4.5%), hexenyl valerate (4.5%), (Z)-isoeugenol (4.0%), α-phellandrene (3.0%), 2-methyl-3-buten-2-ol (2.7%) and myrcene (2.2%). The *L. multiflora* essential oil chemotype (thymol/*p*-cymene/thymyl acetate) has been reported previously (Lamaty et al., 1990).

The same two major components characterized *L. chevalieri* and *L. multiflora* essential oils: thymol and *p*-cymene. The main difference between both species was found in minor components such as 2-phenyl ethyl propionate, benzyl hexanoate,longifolene, 2-phenyl ethyl tiglate, thymyl acetate, hexenyl valerate, (*Z*)-isoeugenol, methyl-3-buten-2-ol.

The MICs and MBCs of *L. chevalieri* and *L. multiflora* oils for the organism tested are shown in Table 2. Although the MICs and MBCs result varied between test organisms, in the most cases the MIC was equivalent to the MBC, indicating a bactericidal action of the oils.

L. chevalieri failed to inhibit *Escherichia coli, Listeria innocua* and *Shigella dysenteria* at the highest concentration (8%). *Bacillus cereus* had the lowest MIC of 0.5%. *L. multiflora* inhibited all microorganisms tested. The lowest MIC was 0.06% for *Bacillus cereus* and *Salmonella enterica*. *Shigella dysenteria* had the highest MIC of 0.5%.

The results of MBC indicated a bactericidal effect of both essential oils. *L. chevalieri* essential oil was bactericidal for *Proteus mirabilis*, *Salmonella enterica* and *Staphylococcus camorum*.

Staphylococcus camorum had the lowest MBC of 0.25% and the highest MBC was 4% for *Staphylocccus aureus*.

The lowest MBC obtained with *L. multiflora* essential oil was 0.06% for *Bacillus cereus, Salmonella enterica* and *Staphylococcus aureus*. *Shigella dysenteria* had the highest MBC of 0.5%.

The MIC and MBC were lower for the Gram-negative than Gram-positive bacteria for both essential oils. The *L. multiflora* was bactericidal for the four Gram-negative bacteria and *Staphylococcus aureus* at the low concentration whereas *L. chevalieri* was bactericidal for two Gram-negatives and *Staphylococcus camorum*. The antibacterial activity of the essential oil of *L. multiflora* was the strongest. Our results on the antimicrobial activity of *L. multiflora* were identical to those reported previously (Pélissier et al., 1994). Both essential oils contained the same two major components (thymol and *p*-cymene). The antimicrobial effect of thymol and the lack of activity of *p*-cymene have been reported (Lattaoui and Tantaoui-Elaraki, 1994). The same authors have shown that carvacrol possessed high antimicrobial

Table 2
Minimum inhibitory concentration and minimum bactericidal concentration data (% v/v) obtained by microdilution method

Bacteria	Essential oils			
	L. chevalieri		*L. multiflora*	
	CMI	CMB	CMI	CMB
Bacillus cereus LMG 13569	0.5	1	0.5	0.5
Enterococcus faecalis CIP 103907	1	4	0.25	1
Escherichia coli CIP 105182	>8	–	0.25	0.25
Listeria innocua LMG 13568	>8	–	0.5	1
Proteus mirabilis CIP 588104	2	2	0.25	0.25
Salmonella enterica CIP 105150	2	2	0.06	0.06
Shigella dysenteria CIP 54051	>8	–	0.5	0.5
Staphylococcus aureus ATCC 25923	1	4	0.06	0.06
Staphylococcus camorum LMG 13567	0.25	0.5	0.25	0.5

activity. Antagonism of high percentage of *p*-cymene and phenolic monoterpenes (thymol and carvacrol), reducing antimicrobial activity of the essential oil, has been reported (Cosentino et al., 1999).

L. multiflora essential oil high antimicrobial activity could be due to its high content antimicrobial components (thymol and its derivatives). An antagonistic effect between *p*-cymene, thymol and carvacrol in the oil of *L. chevalieri* may explain its low antimicrobial activity.

This study shows in vitro high and low antimicrobial activities of the essential oils of *L. multiflora* and *L. chevalieri*, respectively. It supports traditional use of these plants in the treatment of boils, diarrhoea and as a mouth disinfectant.

L. multiflora presented a large antibacterial spectrum. Its oil constitutes a potential source of novel antimicrobial essential oils because of resurgence of interest in aromatherapy (Lis-Balchin, 1997), particularly for the local population which needs cheap medicine. To increase the utilisation of this plant it seems important to investigate different chemotypes, the sites and period of harvest.

3. Experimental

3.1. Plant

L. chevalieri Moldenke and *L. multifora* Moldenke leaves were collected from a wild population in Ouagadougou (Burkina Faso) during September 1999. Plants were identified at the Laboratoire de Biologie et d'Ecologie (Université de Ouagadougou), where a voucher specimen is deposited.

4. Essential oil extraction and analysis

The air-dried leaves were distilled for 3 h using a Clevenger type apparatus. The essential oils obtained were dried with anhydrous sodium sulphate and stored at −30 °C before using.

GC analysis were performed using a VARIAN 3800 instrument equipped with two fused capillary columns: Supelcowax (30 m × 0.25 mm × 0.25 μm) and SPB-1 (30 m × 0.25 mm × 0.25 μm). The oven temperature was programmed from 40 to 240 °C at 2 °C/mn and held isothermal for 10 min. Injectors and FID detector were 230 and 250 °C, respectively; carrier gas was Helium at 30 cm s−1. Volume of injection was 1 μl.

The GC–MS analysis was carried out with a Saturn II mass spectrometer (EI 70 ev), coupled with a Varian 3400 GC equipped with a DBWAX capillary column (30 m × 0.25 mm × 0.25 μm). The operating conditions were the same as described above. Constituents were identified by their retention indices (RIs) and mass spectra.

4.1. Bacterial strains

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, *Stahpylococcus aureus* ATCC 25923 and *Stahpylococcus camorum* LMG 13567.

4.2. Microbiological methods

A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) (Carson et al., 1995). A serial doubling dilution of each essential oil was prepared in a microtiter tray over the range 0.03–8%. The broth was supplemented with tween 80 (Merck, Germany) at a concentration of 0.5% in order to enhance essential oils solubility.

Overnight broth cultures of each strain were prepared in Nutrient Broth (Diagnostic Pasteur, France) and the final concentration in each well was adjusted to 5 × 105 CFU/ml following inoculation. The concentration of each inoculum was confirmed by viable count on Plate Count Agar (Merck, Germany).

Positive and negative growth controls were included in every test. The tray was incubated aerobically at 30 °C (Gram-negative) or 37 °C (Gram-positive) according to strain and MICs were determined. The MIC is defined as the lowest concentration of the essential oil at which the microorganism tested does not demonstrate visible growth. The bacteria growth was indicated by the turbidity.

To determine MBCs, 10 μl broth was taken from each well and inoculated in Mueller–Hinton Agar (Becton Dickinson, USA) for 24 h at 30 or 37 °C. The MBC is defined as the lowest concentration of the essential oil at

which 99.99% or more of the initial inoculum was killed. The number of surviving organism was determined by viable count.

All tests were performed in Mueller–Hinton Broth (Becton Dickinson, USA) and triplicate.

References

Adams, R.P., 1989. Identification of Essential Oils by Ion Trap Mass Spectrometry. Academic Press, New York.

Aroma Chemical Information Service (ACIS), 1997. ESO 97 Database of Essential Oils.

Baba-Moussa, F., Koumaglo, K., Ayedoun, A., Akpagana, K., Moudachirou, M., Bouchet, P., 1997. Antifungal activity of essential oils extracted in the African states of Togo and Benin. Cryptogamie, Mycologie 165–168.

Carson, C.F., Hammer, K.A., Riley, T.V., 1995. Broth micro-dilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). Microbios 82, 181–185.

Chanh, P.H., Koffi, Y., Chanh, A.P.H., 1988. Comparative hypotensive effects of compounds extracted from Lippia multiflora leaves. Planta Medica 54, 294–296.

Cosentino, S., Tuberoso, C.I.G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., Palmas, F., 1999. In-vitro antimicrobial activity and chemical composition of *Sardinia thymus* essential oils. Letter in Applied Microbiology 29, 130–135.

Elakovich, S.T., Oguntimein, B.O., 1987. The essentials oils of *Lippia adoensis* leaves and flowers. Journal of Natural Products 50, 503–506.

Kanko, C., Koukoua, G., N'Guessan, Y.T., Lota, M.L., Tomi, F., Casanova, J., 1999. Composition and intraspecific variability of the leaf oil of *Lippia multiflora* Mold. from the Ivory Coast. Journal of Essential Oil Research 11, 153–158.

Kerhaho, J., Adam, J.G., 1974. La Pharmacopée Senégalaise Traditionnelle: Plantes Medicinales et Toxiques. Vigot Frères, Paris.

Koumaglo, K.H., Akpagana, K., Glitho, A.I., Garneau, F.X., Gagnon, H., Jean, F-I., Moudachirou, M., Addae-Mensah, I., 1996. Geranial and neral major constituents of *Lippia multiflora* moldenke leaf oil. Journal of Essential Oil Research 8, 237–240.

Lamaty, G., Menut, C., Bessiere, J.M., Ouamba, J.M., Silou, T., 1990. 2-methyl-6-methylène- 7-octen-4-one, a constituent of *Lippia multiflora* essential oil. Phytochemistry 29, 521–522.

Lattaoui, N., Tantaoui-Elaraki, A., 1994. Individual and combined antibacterial activity of the main components of three thyme essential oils. Revista Italiana Eppos 13, 13–19.

Lis-Balchin, X., 1997. Essential oils and "aromatherapy" their modern role in healing. Journal of the Royal Society for the Health 117, 324–329.

Menut, C., Lamaty, G., Samaté, D., Nacro, M., Bessière, J.-M., 1993. Contribution à l'étude des *Lippia* africaines: Constituants volatils de trois espèces du Burkina Faso. Rivista Italiana Eppos 11, 23–29.

Menut, C., Lamaty, G., Sohounhloue, D.K., Dangou, J., Bessière, J.-M., 1995a. Aromatic plants of Tropical West Africa. III. Chemical composition of leaf essential oil of *Lippia multiflora* Moldenke from Benin. Journal of Essential Oil Research 7, 331–333.

Menut, C., Lamaty, G., Bessière, J.-M., Koudou, J., Maidou, J., 1995b. Aromatic plants of tropical Central Africa. XVII: 6,7-epoxymyrcene, the major unusual constituent of *Lippia multiflora* s.l. Moldenke essential oil from the Central African Republic. Flavour and Fragrance Journal 10, 75–77.

Mwangi, J.W., Addae-Mensah, I., Lwande, W., 1991. Essentials oils of two *Lippia* species. Flavour Fragrance Journal 6, 221–224.

Nacoulma/Ouédraogo, O.G., 1996. Plantes Médicinales et Pratiques Médicales Traditionnelles au Burkina Faso: Cas du Plateau Central. Thèse d'état, Université de Ouagadougou.

Noamesi, B.K., 1977. Power tea (*Lippia multiflora*) a potent hypertensive therapy. West Africa Journal of Pharmacology Drug Resource 4, 33–36.

Noamesi, B.K., Adebayo, G.I., Bamgbose, S.O., 1985a. Muscle relaxant properties of aqueous extract of *Lippia multiflora*. Planta Medica 3, 253–255.

Noamesi, B.K., Adebayo, G.I., Bamgbose, S.O., 1985b. The vascular actions of aqueous extract of *Lippia multiflora*. Planta Medica 3, 256–258.

Oladimeji, F.A., Orafidiya, O.O., Ogunnivi, T.A., Adawunmi, T.A., 2000. Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. Journal of Ethnopharmacology 72, 305–311.

Pascual, M.E., Slowing, K., Carretero, E., Sánchez Mata, D., Villar, A., 2001. *Lippia*: traditional uses, chemistry and pharmacology: a review. Journal of Ethnopharmacology 76, 201–214.

Pelissier, Y., Marion, C., Casadebaig, J., Milhau, M., Kone, D., Lokou, G., Nanga, Y., Bessière, J-M., 1994. A chemical, bacteriological, toxicological and clinical study of the essential oil of *Lippia multiflora* Mold. (Verbenaceae). Journal of Essential Oil Research 6, 623–630.

Valentin, A., Pélissier, Y., Benoît, F., Marion, C., Koné, D., Mallie, M., Bastide, J.M., Bessière, J.M., 1995. Composition and antimalaria activity in vitro of volatile components of Lippia multiflora. Phytochemistry 40, 1439–1442.