African Journal of Biotechnology Vol. 9(41), pp. 7009-7013, 11 October, 2010
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.344
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Variation in the chemical composition of the essential oils of different organs of domesticated *Lippia multiflora* Moldenke

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Accepted 20 August, 2010

The essential oils from the different organs of wild and domesticated *Lippia multiflora* Moldenke, analysed by GPC-FID and GPC-MS, were rich in monoterpenes. These made up 95.0, 94.0, 82.5 and 61.0% of oils from leaves, flowers, stems and roots, respectively, with a predominance of aromatic monoterpenes: *p*-cymene, thymol, carvacrol and their acetates together made up 44.0 - 74.0% of the oils, along with β-caryophyllene and its oxide (3.0 - 8.4%). Oils from roots differed from those of flowers, leaves and stems by a higher proportion of β–caryophyllene and its oxide (16.0%), and the absence of *p*-cymene γ–terpinene represented respectively, 6.3 - 18.0 and 0.7 - 11.4% of the oils from the other organs. (*Z*)–β–Ocimene, identified in the oils from flowers (nearly 10%) was absent from oils of leaves, stems and roots. Oils of stems and roots contained very small amounts of hexadecanoïc acid, β–eudesmol, isocaryophyllene and phytol, none of which had previously been reported in oils from the Congo.

Key words: *Lippia multiflora* Moldenke, organs, essential oil, chemical composition.

INTRODUCTION

The genus *Lippia* (Verbenaceae) comprises some 200 reported species (Pascual et al., 2001) growing throughout the countries of Central and South America and tropical Africa (Bouquet, 1967; Bouquet, 1969; Adjanohoum et al., 1988). The characteristic, pleasantly aromatic leaves are consumed in infusions, whence the common names "Gambian tea bush" or "savanna tea". *Lippia multiflora* is used in African traditional medicine to treat illnesses such as malaria and high blood pressure (Noamesi et al., 1985; Koffi Yao, 1985), and also as a cough remedy, disinfectant, antipyretic and diuretic (Kanko, 1995). Earlier studies on essential oils demonstrated pharmacological properties: antimicrobial (Kunle et al., 2003), pediculocidal and scabicidal (Oladimeyi et al., 2000), antioxidant (Agnaniet et al., 2005), antibacterial and antifungal (Abena et al., 2002), analgesic, antipyretic and anti-inflammatory (Abena et al., 2003).

The chemical composition of oils from the wild species of different origins has already been determined and the major constituents identified as followed: camphor (Talalaj et al., 1997), limonene or carvone (46.0%) along

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Abbreviations: GPC-FID, Gas phase chromatography with flame ionisation detection; **GC-MS,** gas phase chromatography coupled with mass spectrometry.

Table 1. Extraction of essential oils from different organs (leaves, flowering tips, stems and roots) of domesticated *L. multiflora* Moldenke.

Parameter		Leaves	Flowers	Stems	Roots
Dry mass of plant material (g)		50.0	52.0	300.0	200.0
Essential oils	Mass (g)	0.8	0.8	0.3	0.2
	Yield (%)	1.6	1.5	0.1	0.1

with linalool and its acetate (Rovesti et al., 1997), linalool (80.0%) (Elakovich and Oguntimein, 1987), thymol (30.3%), thymyl acetate (24.1%), *p*-cymene (14.5%) (Lamaty et al., 1990), (*E*)-tagetone (31.7%), (*Z*)-tagetone (13.8%), ipsenone (12.5%), β-caryophyllene (11.3%) (Lamaty et al., 1990), linalool (46.1%), thymol (15.2%), β-cubenene (11.7%) (Mwangi et al., 1991), (*E*)-tagetone (30.2%), (*Z*)-tagetone (11.3%) (Pelissier et al., 1994), *p*-cymene (18.7%), thymol (53.3%), thymol acetate (9.8%) and carvacrol (5.8%) (Keita et al., 1996), myrtenol (27.1%), linalool (11.9%), 1.8-cineole (11.6%) (Menut et al., 1995a), 6.7-epoxymyrcene (70.3%), myrcene (11.3%), limonene (5.1%), (*E*)-β-farnesene (4.7%) (Menut et al., 1995b), geranial (55.9%), neral (33.4%) (Koumaglo et al., 1996), 1.8-cineole (63.2%) (Koumaglo et al., 1996), thymol (23.1%), thymyl acetate (23.0%), *p*-cymene (16.7%) (Agnaniet et al., 2005), ispedienone (54.6%), (*Z*)-β-ocimenone (11.5%), (*E*)-β-ocimenone (8.8%) (Agnaniet et al., 2005), 1.8-cineole (39.9%), sabinene (11.1%), linalool (10.9%) (Avlessi et al., 2005), limonene (15.4%), linalool (26.7%), geraniol (20.0%) (Oladimeji et al., 2000), *p*-cymene (26.2%), thymyl acetate (11.7%) and thymol (29.9%) (Bassolé et al., 2003), piperitenone, *p*-cymene and linalool (Bissangou, 1993; Bissangou and Ouamba, 1997).

The present work is the first study of the variation in the chemical composition of the essential oil from different organs (leaves, flowers, stems and roots) of domesticated *L. multiflora* Moldenke. These oils from the Congo were compared with those of the same genus types and species in the same region of Africa, and with those of the same genus species in other parts of the world. To the researchers' knowledge, no specific chemical study has been made of the stems and roots of *L. multiflora*.

MATERIALS AND METHODS

Plant material

The different organs of *L. multiflora* were harvested in January 2009, in the study's experimental plot set up in the gardens of the Science Faculty of Marien Ngouabi University. Voucher specimens were identified at the laboratory of Botany of the Centre des Ressources Végétales (CERVE) by comparison with the specimens previously deposited at the National Herbarium of Brazzaville (Congo) (IEC: Koechlin n° 474, January 1950; n° 1229, 21/07/1950; n° 2047, 16/01/1953; Descoings n° 5605, 14/05/1960; De Néré n° 1443, 14/07/1963; n° 1475, 15/07/1963 and Bouquet n° 1411, 24/05/1965).

Extraction

The essential oils of the plant organs (leaves, flower tips, stems and roots) of *L. multiflora* Moldenke were extracted by steam distillation using à Kaiser Lang (Figuérédo, 2007). The plant material, with no prior steeping, was exposed to an ascending stream of water vapour generated from boiling water in a round-bottomed flask placed underneath. The apparatus was fitted with a reflux system for the aqueous phase. The vapour containing the volatile components was condensed and the essential oil isolated from the aqueous phase by extraction with pentane and decantation. The pentane phase was dried on anhydrous sodium sulphate and concentrated by passive evaporation to yield the essential oil (Lamaty et al., 1990). The weights of vegetal materiel used, the amounts and the extraction yields obtained are reported in Table 1.

Chemical analysis of essential oils

The essential oils were analysed in two steps:

1. Preliminary quantitative analysis of chemical constituents by gas phase chromatography with flame ionisation detection (GPC-FID).
2. Fine qualitative analysis by gas phase chromatography coupled with mass spectrometry (GC-MS) to confirm chemical structures.

The quantitative step was carried out using a Hewlett-Packard HP 5890 chromatograph fitted with a flame ionisation detector equipped with HP ChemStation data acquisition software.

The different constituents were separated using a DB5 capillary column (30 m × 0.25 mm, film thickness 0.25 µm) in the following operating conditions: carrier gas helium at 1 ml/min, injection temperature 280°C, detector temperature 280°C, oven temperature program 50°C (5 min) to 300°C (5 min) at 5°C/min and injection in split mode 1-20.

The GPC-MS analysis was performed using a Hewlett-Packard HP 6890 chromatograph coupled with an HP 5973 mass spectrometer, on a capillary column of the same type as above in the same experimental conditions. The mass spectra of the different constituents were analysed and compared with those in the laboratory database (McLafferty and Stauffer, 1989; Adams, 2001).

RESULTS AND DISCUSSION

The masses of plant material used, the masses of essential oils obtained and their extraction yields are given in Table 1. The steam distillation of the different organs of domesticated *L. muliflora* gave yellow oil. The extraction yields were 0.1% from roots and stems, 1.5% from flowers and 1.6% from leaves.

The results of essential oils' analysis from the different organs are given in Table 2. This table shows that the oil from leaves, flowers, stems and roots presented the same chromatographic profile, essentially dominated by

Table 2. Chemical composition of essential oils from different organs (leaves, flowering tips, stems and roots) of domesticated *L. multiflora* Moldenke.

N° of order	I_R	Constituent (*)	Sheet	Flower	Stem	Root
01	978	Oct-1-ene-3-ol	t	t	-	-
02	930	α-Thujene	2.0	2.5	-	-
03	939	α-Pinene	0.3	0.5	-	-
04	976	Sabinene	0.2	0.4	-	-
05	980	β-Pinene	-	0.1	-	-
06	991	Myrcene	1.8	2.7	0.1	-
07	993	Octan-3-ol	-	-	0.5	-
08	1005	α –Phellandrene	0.2	0.4	-	-
09	1011	δ-3-Carene	0.2	0.2	-	-
10	1018	α –Terpinene	1.0	2.3	-	-
11	1026	p-Cymene	18.0	17.2	6.3	-
12	1029	Limonene	0.4	0.5	0.1	-
13	1031	β-Phellandrene	0.2	0.2	-	-
14	1040	(Z)-β-Ocimene	-	9.9	-	-
15	1062	γ-Terpinene	4.7	11.4	0.7	-
16	1068	Sabinene cis-hydrate	0.4	0.3	0.2	-
17	1082	Terpinolene	-	0.1	-	-
18	1093	6.7-Epoxy myrcene	0.2	0.2	0.2	-
19	1096	Linalol	0.4	0.3	0.1	-
20	1101	n-Nonanal	-	-	0.1	-
21	1177	Terpinen-4-ol	0.5	0.5	0.3	0.3
22	1179	Naphtalene	-	-	-	0.4
23	1190	Methyl salycilate	-	-	0.1	-
24	1290	Thymol	35.5	21.4	29.2	23.5
25	1298	Carvacrol	5.0	2.8	4.7	2.5
26	1355	Thymyl acetate	21.4	17.9	37.5	29.6
27	1371	Carvacryl acetate	2.1	1.9	2.6	2.0
28	1386	β-Bourbonene	-	-	0.1	-
29	1409	Isocaryophyllene	-	-	0.5	3.2
31	1418	β-Caryophyllene	3.2	2.7	3.6	5.8
32	1446	(E)-β-Farnesene	0.5	1.0	2.3	5.5
33	1454	α-Humulene	0.6	1.3	0.8	2.2
34	1477	γ-Muurolene	t	-	-	-
35	1481	Germacrene D	0.1	0.1	0.1	-
36	1486	β-Selinène	0.1	0.1	0.2	0.6
37	1491	α-Selinene	-	0.2	0.1	0.8
38	1503	α-Bulnesene	-	-	0.2	-
39	1509	β-Bisabolene	-	-	0.1	-
40	1510	γ-Cadinene	-	-	0.1	0.3
41	1520	δ-Cadinene	-	-	0.1	0.4
42	1561	cis-Iongipinanol	-	-	0.1	-
43	1563	Nerolidol	-	-	0.3	-
44	1581	β-Caryophyllene oxide	0.7	0.3	4.6	10.0
45	1601	Guaiol	-		0.2	0.6
46	1606	1.2-Epoxyhumulene	0.1		0.7	1.2
47	1616	Geranylisovalerate	-	-	0.1	-
48	1641	Caryophylla-4(14).8(15).diene-5-β-ol	-	-	0.3	-
49	1649	β-Eudesmol	-	-	0.7	0.3
50	1656	Citronellyl	-	-	-	0.6
51	1670	Caryophyllene (14-hydroxy-9-epi-(E))	-	-	0.5	-

Table 2. Cont'd

52	1762	Benzyl benzoate	-	-	0.1	-
53	1741	Myrtany l4-methyl-valerate (cis)	-	-	-	0.6
54	1837	Pentadecanone 6. 10.14-trimethyl-	-	-	0.2	-
55	1879	Hexadecanol	-	-	0.1	0.3
56	1949	Phytol	-	-	0.1	0.5
57	1958	NI	-	-	-	2.1
58	1987	Hexadecanoic acid	-	-	0.5	1.8
59	2082	Octadecanol			0.3	0.5
60	2141	NI			0.2	0.9
Total identified compounds (%)			99.8	99.4	99.9	96.5

*: Order of elution DB5.

aromatic monoterpenes, with major constituents as *p*-cymene, thymol, carvacrol and their acetates. The leaves, flowers and stems were respectively rich in *p*-cymene (18.0, 17.2 and 6.3%), thymol (35.5, 21.4 and 29.2%) and its acetate (21.4, 17.9 and 37.5%), while the oils from roots were characterised like the leaves, flowers and stems, by large amounts of thymol (23.5%) and thymyl acetate (29.6%), accompanied by β-caryophyllene (5.8%) and β-caryophyllene oxide in moderate amounts (10.0%). These last two compounds did not individually make up more than 6.0% of the oils from leaves, flowers and stems. The same was the case for carvacrol and its acetate taken individually in all the oils studied.

It was noted in particular that γ–terpinene was present in large amounts in the flowers (11.4%) and in lower amounts in the stems (0.7%) and leaves (4.7%), but was absent in the roots. The same was the case for *p*-cymene, which was present in the other organs in amounts ranging from 6.2 to 18.0%. (*Z*)–β–ocimene was identified in oils from flowers in amounts close to 10.0%, but was absent in leaves, stems and roots.

In general, it was found that the proportions of sesquiterpenes increased in the order: leaves, flowers, stems and roots (5.5, 5.7, 16.0 and 31.0%), whereas the proportions of monoterpenes decreased in the same order (95.0, 94.0, 82.5 and 61.0%), reflecting a continuous movement of monoterpenes and sesquiter-penes in the different organs.

The study also notes the presence of stems and roots of compounds in oils hitherto unreported in oils of *L. multiflora* from the Congo: hexadecanoic acid (0.7 - 1.0%), β-eudesmol (0.7 - 0.3%), isocaryophyllene (0.1 - 3.2%) and phytol (0.2 - 0.5%).

The chemical composition of oils of *L. multiflora* from Congo is thus similar to that of the same species from Gabon (Agnaniet et al., 2005) and of *Lippia chevaleri* from Mali (Keita et al., 1996). This confirms that it is an aromatic monoterpene chemotype particularly rich in thymol and its acetate. This chemotype is different from the Benin's type (rich in myrtenol, linalool and 1,8-cineole (Menut et al., 1995a; Avlessi et al., 2005)), Central Africa (rich in 6,7-expoxymyrcene and myrcene (Menut et al., 1995b)), Ghana (rich in camphor) (Talalaj et al., 1997; Rodolfo et al., 2006), limonene or carvone (46.0%), linalool and its acetate (Rovesti et al., 1997), Nigeria (rich in linalool) (Elakovich and Oguntimein, 1987), Togo (rich in sabinene and limonene) (Bissangou, 1993; Bissangou and Ouamba, 1997) and the two other chemotypes found in the Congo (rich in ipsenone, β-caryophyllene, (*E*)-tagetone and (*Z*)-tagetone in the localities of Inoni-plateau and Kiani) (Lamaty et al., 1990) and those of the locality of Mindouli (rich in limonene, linalool and piperitenone) (Bissangou, 1993; Bissangou and Ouamba, 1997).

All these oils of *L. multiflora* differ from other species of the same genus in the same region of Africa and in other parts of the world that have already been studied (Mwangi et al., 1991; Bissangou, 1993; Keita et al., 1996; Bissangou et Ouamba, 1997; Danilo et al., 2006; Argyropoulou et al., 2007; Botelho et al., 2007; Danilo et al., 2007; Mevy et al., 2007; Manterio et al., 2007; Lemos et al., 2008; Tatsadjieu et al., 2009): *Lippia javanica (*syn. *Lippia asperifolia)* from South Africa; *Lippia adoensis* from Ethiopia and Nigeria; *Lippia schimperi* from Ethiopia; *Lippia ukambensis* from Kenya and Tanzania; *Lippia carviodora, Lippia carviodora* var*. minor, Lippia daunesis, Lippia grandifolia, Lippia somalensis* and *Lippia wimsii* from Kenya; *Lippia chevalieri* from Mali and Burkina Faso; *Lippia rugosa* from Cameroon; *Lippia alba* from Argentina and Uruguay; *Lippia grisebachiana, Lippia fissicalyx and Lippia integrifolia* from Argentina*; Lippia alnifolia, Lippia aristata, Lippia sidoides, Lippia grata* and *Lippia organoides* from Brazil and *Lippia chevalieri, Lippia citriodora, Lippia halleri, L. rugosa* and *Lippia savoryi* from unstated origin.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Jean-Marie Moutsamboté

for the botanical identification of *L. multiflora* Moldenke.

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