ORIGINAL ARTICLE

A C G publications

Rec. Nat. Prod. 3:4 (2009) 170-177

records of natural products

Chemical Composition and Antibacterial Activity of the Essential Oil of *Lippia multiflora* Moldenke from Nigeria

Moses S. Owolabi[1],*, Akintayo Ogundajo[1], Labunmi Lajide[2], Matthew O. Oladimeji[3], William N. Setzer[4] and Maria C. Palazzo[4]

[1] *Department of Chemistry, Lagos State University, P.M.B. 001, Ojo, Lagos, Nigeria*

[2] *Department of Chemistry, Federal University of Technology, Akure, Ondo State, Nigeria*

[3] *Department of Chemistry, Bowen University, Owo, Osun State, Nigeria*

[4] *Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA*

(Received May 5, 2009; Revised July 9, 2009; Accepted July 21, 2009)

Abstract: The steam distilled volatile oil obtained from dried *Lippia multiflora* Moldenke was examined by gas chromatography-mass spectrometry (GC-MS). The major components were 1,8-cineole (60.5%), sabinene (16.9%), α-terpineol (14.1%) and α-pinene (4.4%). The oil displayed no antibacterial activity against either gram positive *Bacillus cereus* or *Staphylococcus aureus* or gram negative *Escherichia coli*, (MIC = 1250 µg/mL). A cluster analysis was performed for comparison and characterization of *L. multiflora* essential oil from Nigeria with other oils reported in the literature from different locations across central Africa, and reveals much chemical variation in this species with at least 13 different chemotypes.

Keywords: *Lippia multiflora*; Verbenaceae; essential oil; 1,8-cineole; sabinene; α-terpineol; α-pinene; antibacterial activity; cluster analysis.

1. Introduction

The Verbenaceae is a large family of perennial herbaceous plants and is composed of 41 genera with about 200 species. *Lippia multiflora* Moldenke is a shrubby aromatic plant, growing up to 1.2 m with whitish flowers on cone-like heads in a terminal panicle, and nearly 12 mm long. It is widely distributed in West and Central Tropical Africa [1-3]. In Nigeria, it is found along forest savannah transitional and coaster savannah zones. Locally the plant is named 'Efinrin gogoro ', 'Efinrin odan' or 'Efinrin Ajase' according to the specific area. *L. multiflora* has been used in

* Corresponding author: E-Mail: sunnyconcept2007@yahoo.com.

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 10/04/2009 EISSN:1307-6167

traditional and herbal medicine to treat bronchial inflammation, malaria fever, conjunctivitis, gastro-intestinal disturbance, enteritis, coughs and colds [2], and possesses hypertensive, fatigue-relieving, and diuretic properties [4]. Some rural dwellers cook the herbs and use it to relieve stress and enhance sleep [5]. Traditionally, *L. multiflora* has been used as a substitute for tea and as a mouth disinfectant [6]. Scientifically, the oil from the plant has been reported to have insecticidal and pesticidal properties against body lice and has also shown marked antimicrobial activity [7-10].

It has been reported that the essential oil composition of *L. multiflora* from some locations were characterized by high terpenoids content, in particular: 1,8-cineole [11-15], linalool [9,12,13, 16,17], geranial and neral [11,12], ipsdienone and (*Z*)- and (*E*)-ocimenone [18], thymol and thymyl acetate [11,14,19-21], *p*-cymene [11,14,19-21], sabinene [11-13], α-terpineol [12,13], α-phellandrene [12], myrcene and epoxymyrcene [22], myrtenol [17], limonene [9], (*E*)- and (*Z*)-tagetone and ipsenone [19,23], nerolidol [16], geraniol [9], γ-terpinene [11], (*E*)-caryophyllene [19], and β-farnesene [14,18]. The purpose of the present study was to investigate the chemical composition and potential bactericidal activity of *L. multiflora* essential oil from Nigeria, and furthermore to compare and contrast the Nigerianian *L. multiflora* sample with chemotypes previously reported from central Africa.

2. Materials and Methods

2.1. Plant Material

Dried leaves, stems and flowers of *L. multiflora* were collected in May 2008, from Fasola, Oyo State, Nigeria. Taxonomic identification of the plant was performed by a botanist, Mr. O.K. Oluwa, from the Botany Department, Lagos State University. Air-dried plant material (400 g) of *L. multiflora* was hydrodistilled for 4 h in a modified Clevenger-type apparatus to yield 0.63 g of a light yellow essential oil. After extraction, the volume of essential oil was stored in hermetically sealed glass bottle with screw lid cover under refrigeration at 4^{o}C.

2.2 Gas Chromatography-Mass Spectrometry

The volatile oil sample was subjected to GC-MS analysis on an Agilent system consisting of a model 6890 gas chromatograph, a model 5973 mass selective detector (EIMS, electron energy = 70 eV, scan range = 45-400 amu, and scan rate = 3.99 scans/sec), and an Agilent ChemStation data system. The GC column was an HP-5ms fused silica capillary with a (5% phenyl)-methylpolysiloxane stationary phase, film thickness of 0.25 µm, a length of 30 m, and an internal diameter of 0.25 mm. The carrier gas was helium with a column head pressure of 7.07 psi and flow rate of 1.0 mL/min. Inlet temperature was 200°C and MSD detector temperature was 280°C. The GC oven temperature program was used as follows: 40°C initial temperature, hold for 10 min; increased at 3°/min to 200°C; increased 2°/min to 220°C. The sample was dissolved in dichloromethane to give a 1% w/v solution; 1 µL injections using a splitless injection technique were used. Identification of oil components was achieved based on their retention indices (RI, determined with reference to a $C_9 - C_{21}$ homologous series of normal alkanes), and by comparison of their mass spectral fragmentation patterns with those reported in the literature [24] and stored on the MS library [NIST database (G1036A, revision D.01.00)/ChemStation data system (G1701CA, version C.00.01.08)].

2.3 Antibacterial Screening

The essential oils were screened for antimicrobial activity against the Gram positive bacteria *Bacillus cereus* (ATCC No. 14579) and *Staphylococcus aureus* (ATCC No. 29213); and the Gram negative bacterium, *Escherichia coli* (ATCC No. 25922). Minimum inhibitory concentrations (MIC) were determined using the microbroth dilution technique [25]. Dilutions of the oils were prepared in cation-adjusted Mueller Hilton broth (CAMHB) beginning with 50 µL of 1% w/w solutions of essential oil in DMSO plus 50 µL CAMHB. The oil solutions were serially diluted (1:1) in CAMHB in 96-well plates. Organisms at a concentration of approximately 1.5×10^8 colony forming units (CFU)/mL were added to each well. Plates were incubated at 37°C for 24 h; the final MIC was determined at the lowest concentration without turbidity. Gentamicin was used as a positive antibiotic control.

2.4 Numerical Cluster Analysis

A cluster analysis was performed to determine the chemical relationships between the studied *L. multiflora* oil from Fasola, Nigeria and the oils of this species reported in the literature from other locations in central Africa. Forty-three *L. multiflora* samples (this present work and those from the literature) were treated as operational taxonomic units (OTUs). The percentage composition of the 33 most abundant essential oil components [1,8-cineole, geranial, neral, thymol, *p*-cymene, linalool, sabinene, thymyl acetate, α-phellandrene, α-terpineol, myrcene, 6,7-epoxymyrcene, (*E*)-caryophyllene, (*E*)-tagetone, ipsdienone, limonene, nerolidol, germacrene D, (*E*)-β-farnesene, α-pinene, geraniol, γ-terpinene, myrtenol (*Z*)-tagetone, ipsenone, carvacrol, β-phellandrene, (*Z*)-β-farnesene, (*E*)-β-ocimene, β-pinene, α-thujene, (*E*)-ocimenone, and α-humulene] was used to determine the chemical relationships between the different *L. multiflora* essential oil samples by cluster analysis using the NTSYSpc software, version 2.2 [26]. Correlation was selected as a measure of similarity, and the unweighted pairgroup method with arithmetic average (UPGMA) was used for cluster definition.

3. Results and Discussion

The essential oil was obtained as yellow oil (0.16% of the dried plant material). Analysis of the volatile constituents of the essential oil by GC-MS facilitated the identification of oil components, which are listed in Table 1. The oil of *L. multiflora* obtained from Nigeria in this work is characterized by its richness in 1,8-cineole (60.5%), sabinene (16.9%), α-terpineol (14.1%) and α-pinene (4.4%). This chemical profile is markedly different from those previously reported [9,11-22]. However, the high concentrations of 1,8-cineole and sabinene in this sample make it similar to those found in oils from Togo [11], Ivory Coast [12], Benin [13], and Ghana [14]. Koumaglo and co-workers [11] had examined a number of different samples of *L. multiflora* and described three different chemotypes: a geranial/neral-type, a thymol-type, and a 1,8-cineole-type. Similarly, an analysis of various samples of *L. multiflora* from the Ivory Coast by Kanko and co-workers [12] revealed a neral/geranial-type, a 1,8-cineole/neral/geranial-type, a 1,8-cineole-type, and a linalool-type. Additionally, a geraniol-type, a tagetone/ipsenol-type, an epoxymyrcene-type, a *p*-cymene/thymol/thymyl acetate-type and a nerolidol-type have also been described (see [12] and references cited therein).

Because of the wide variation in chemical profiles for *L. multiflora* essential oils reported in the literature, a cluster analysis was carried out on the chemical compositions of *L. multiflora* (Figure 1) in order to assess the differences and similarities in these essential oils. The cluster analysis reveals at least 13 distinct chemotypes: a 1,8-cineole-rich cluster, a thymol dominated cluster, a geranial/neral-rich cluster, a cluster rich in β-farnesene, and a tagetone-rich cluster, as well as individual samples rich in epoxymyrcene, linalool, nerolidol, *p*-cymene, myrtenol, farnesol,

germacrene D, and geraniol. In a previous cluster analysis, Juliani and co-workers [14] had found similar chemotypes of *L. multiflora*, with samples from Ghana representing 1,8-cineole (2 samples), thymol (5 samples), and farnesene (3 samples) chemotypes, with individual samples of farnesol-rich and germacrene-D-rich chemotypes. The sample of *L. multiflora* in the current study from Fasola, Nigeria, most closely associates with the 1,8-cineole/sabinene/α-terpineol chemotype from Kofiase-Kubesiase, Ghana [14] (see Figure 1).

Table 1. Chemical composition of *Lippia multiflora* essential oil.

RI	Compound	%	RI	Compound	%
938	α-Pinene	4.4	1051	(*E*)-β-Ocimene	0.3
972	Sabinene	16.9	1060	γ-Terpinene	trace
979	β-Pinene	trace	1092	Terpinolene	trace
988	Myrcene	1.9	1194	α-Terpineol	14.1
1020	*p*-Cymene	trace	1418	(*E*)-Caryophyllene	0.6
1028	Limonene	0.7	1460	(*E*)-β-Farnesene	0.5
1032	1,8-Cineole	60.5	1481	γ-Muurolene	trace

The antibacterial screening of *L. multiflora* essential oil in this study demonstrated no antibacterial activity against *Bacillus cereus*, *Staphylococcus aureus*, or *Escherichia coli*, (MIC = 1250 μg/mL). This is in contrast to the results reported by Bassole and co-workers [21], which may be attributed to the high thymol concentration in their sample [27]. In view of the chemical composition observed in the present study, the lack of antibacterial activity is not surprising. Typically, antimicrobial essential oils contain phenolic constituents such as carvacrol, thymol, or eugenol as the antimicrobial agents [28,29], but this sample of *L. multiflora* essential oil was devoid of these materials. Neither 1,8-cineole, α-terpineol [30], nor sabinene [31] show notable antibacterial activity. In a previous study, *L. multiflora* extract containing carvacrol was found to show antimicrobial activity [32].

It is obvious from this as well as from previous studies that the chemical composition of *L. multiflora* demonstrates a great degree of plasticity; the compositions of the essential oils vary greatly and may depend not only on geographical origins [33], but also genetic factors [34-36], culture and environmental conditions [37,38], nutritional status [39], and the effects of mechanical damage [40] or herbivory [41]. Because of the pronounced chemical variability in this plant, care must be exercised in using this plant and its essential oil for potential medicinal applications.

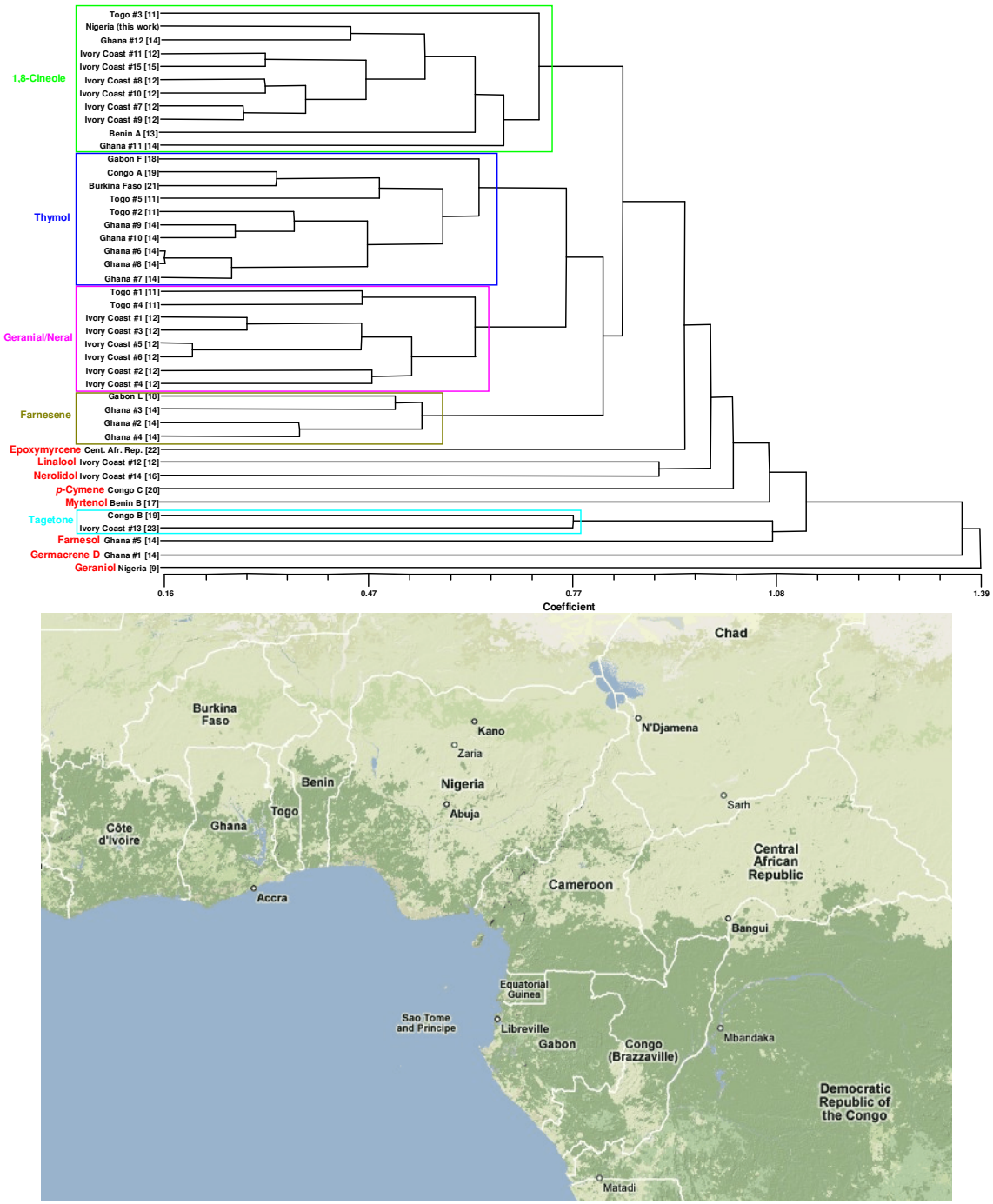

Figure 1. Cluster analysis of *Lippia multiflora* essential oil compositions (map of central Africa from Google Maps, http://maps.google.com/maps).

Acknowledgments

We are very grateful to Dr. A.A. Kasali and Dr. I.A. Ogunwande for their technical assistance.

References

[1] E. Pooley (1998). A Field Guide to Wild Flowers: Kwazulu-Natal and the eastern Region. Natal Flora Publications, Durban.

[2] M.E. Pascual, K. Slowing, E. Carretero, D. Sánchez Mata, and A. Villar (2001). Lippia: traditional uses, chemistry and pharmacology: a review. *J. Ethnopharmacol.* **76**, 201-214.

[3] F.C. Terblanche and G. Kornelius (1996). Essential oil constituents of the genus *Lippia* (Verbenaceae): a literature review. *J. Essent. Oil Res.* **8**, 471-485.

[4] C. Kanco, G. Koukoua, Y.T. N'Guessan, J. Fournier, J.P Pradère and L. Toupet (2004). Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). *C. R. Chemie* **7**, 1029-1032.

[5] A.W. Etou-Ossibi, J. Nzonzi, J.V. Mombouli, G.E. Nsondé-Ntandou, J.M. Ouamba and A.A. Abena (2005). Screening chimique et effets de l'extrait aqueux du *Lippia multiflora* Moldenke sur le cœur isolé du crapaud. *Phytothérapie* **3**, 193-199.

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

[7] F.R. Irvine (1961). Woody Plants of Ghana. Oxford University Press, UK.

[8] J.W. Mwangi, I. Addae-Mensah, G. Muriuki, R. Munavu, W. Lwande and A. Hassanali (1992). Essential oils of *Lippia* species in Kenya. IV : Maize weevil (*Sitophilus zeamais*) repellancy and larvicidal activity. *Int. J. Pharmacog.* **30**, 9-16.

[9] F.A. Oladimeji, O.O. Orafidiya, T.A.B. Ogunniyi and T.A. Adewunmi (2000). Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. *J. Ethnopharmacol.* **72**, 305-311.

[10] I.H.N. Bassolé, W.M. Guelbeogo, R. Nébié, C. Costantini, N.F. Sagnon, Z.I. Kabore and S.A. Traoré (2003). Ovicidal and larvicidal activity against *Aedes aegypti* and *Anopheles gambiae* complex mosquitoes of essential oils extracted from three spontaneous plants of Burkina Faso. *Parassitologia* **45**, 23-26.

[11] K.H. Koumaglo, K. Akpagana, A.I. Glitho, F.X. Garneau, H. Gagnon, F.I. Jean, M. Moudachirou and I. Addae-Mensah (1996). Geranial and neral, major constituents of *Lippia multiflora* Moldenke leaf oil. *J. Essent. Oil Res.* **8**, 237-240.

[12] C. Kanko, G. Koukoua, Y.T. N'Guessan, M.L. Lota, F. Tomi and J. Casanova (1999). Composition and intraspecific variability of the leaf oil of *Lippia multiflora* Mold. From the Ivory Coast. *J. Essent. Oil Res.* **11**, 153-158.

[13] F. Avlessi, G. Alitonou, D.K. Sohounhloue, C. Menut and J.M. Bessiere (2005). Aromatic plants of tropical West Africa. Part XIV. Chemical and biological investigation of *Lippia multiflora* Mold. essential oil from Benin. *J. Essent. Oil Res.* **17**, 405-407.

[14] H.R. Juliani, J.E. Simon, C. Quansah, E. Asare, R. Akromah, D. Acquaye, J. Asante-Dartey, M.L.K. Mensah, T.C. Fleischer and R. Dickson (2008). Chemical diversity of *Lippia multiflora* essential oils from West Africa. *J. Essent. Oil Res.* **20**, 49-54.

[15] C. Kanko, B.E.H. Sawaliho, S. Kone, G. Koukoua and Y.T. N'Guessan (2004). Étude des propriétés physico-chimiques des huiles essentielles de *Lippia multiflora*, *Cymbopogon citratus*, *Cybopogon nardus*, *Cymbopogon giganteus*. *C. R. Chemie* **7**, 1039-1042.

[16] A. Valentin, Y. Pelissier, F. Benoit, C. Marion, D. Kone, M. Mallie, J.M. Bastide and J.M. Bessière (1995). Composition and antimalarial activity in vitro of volatile components of *Lippia multiflora*. *Phytochemistry* **40**, 1439-1442.

[17] C. Menut, G. Lamaty, D.K. Sohounhloue, J. Dangou and J.M. Bessière (1995). Aromatic plants of tropical West Africa. III. Chemical composition of leaf essential oil of *Lippia multiflora* Modenke from Benin. *J. Essent. Oil Res.* **7**, 331-333.

[18] H. Agnaniet, T. Makani, A. Akagah, C. Menut and J.M. Bessière (2005). Volatile constituents and antioxidant activity of essential oils from *Lippia multiflora* Mold. growing in Gabon. *Flavour Fragr. J.* **20**, 34-38.

[19] G. Lamaty, C. Menut, J.M. Bessière, J.A. Ouamba and T. Silou (1990). 2-Methyl-6-methylene-7-octen-4-one, a constituent of *Lippia multiflora* essential oil. *Phytochemistry* **29**, 521-522.

[20] A.A. Abena, M. Diatewa, G. Gakossi, M. Gbeassor, Th. Hondi-Assah and J.M. Ouamba (2003). Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. *Fitoterapia* **74**, 231-236.

[21] I.H.N. Bassole, A.S. Ouattara, R. Nebie, C.A.T. Outtara, Z.I. Kabore and S.A. Traore (2003). Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. *Phytochemistry* **62**, 209-212.

[22] C. Menut, G. Lamaty, J.M. Bessière, J. Koudou and J. Maidou (1995). Aromatic plants of tropical Central Africa. Part XVII. 6,7-Epoxymyrcene, the major unusual constituent of *Lippia multiflora* s.l. Moldenke essential oil from the Central African Republic. *Flavour Fragr. J.* **10**, 75-77.

[23] Y. Pelissier, C. Marion, J. Casadebaig, M. Milhau, D. Kone, G. Loukou, Y. Nanga, J.M. Bessière (1994). A chemical, bacteriological, toxicological and clinical study of the essential oil of *Lippia multiflora* Mold. (Verbenaceae*). J. Essent. Oil Res.* **6**, 623-630.

[24] R.P. Adams (2007). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th Ed. Allured Publishing Corporation. Carol Stream, Illinois.

[25] D.H. Sahm, D.H. and J.A. Washington (1991). Antibacterial susceptibility tests: Dilution methods. In: Manual of Clinical Microbiology, *eds:* A. Balows, W.J. Hausler, K.L. Herrmann, H.D. Isenberg and H.J. Shamody. American Society for Microbiology, Washington DC, USA.

[26] F.J. Rohlf (2005). NTSYSpc, Numerical Taxonomy and Multivariate Analysis System. Applied Biostatistics, Inc., New York.

[27] A. Nostro, A.R. Blanco, M.A. Cannatelli, V. Enea, G. Flamini, I. Morelli, A.S. Roccaro and V. Alonzo (2004). Susteptibility of methicillin-resistant staphylococci to oregano essential oil, carvacrol and thymol. *FEMS Microbiol. Lett.* **230**, 191-195.

[28] H.J.D. Dorman and S.G. Deans (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J. Appl. Microbiol.* **88**, 308-316.

[29] K. Knobloch, A. Pauli, B. Iberl, H. Weigand and N. Weis (1989). Antibacterial and antifungal properties of essential oil components. *J. Essent. Oil Res.* **1**, 119-128.

[30] J.M. Schmidt, J.A. Noletto, B. Vogler and W.N. Setzer (2006). Abaco bush medicine: Chemical composition of the essential oils of four aromatic medicinal plants from Abaco Island, Bahamas. *J. Herbs Spices Med. Plants* **12**(3), 43-65.

[31] N. Filipowicz, M. Kaminski, J. Kurlenda, M. Asztemborska and J.R. Ochocka (2003). Antibacterial and antifungal activity of juniper berry oil and its selected components. *Phytother. Res.* **17**, 227-231.

[32] O. Kunle, J. Okogun, E. Egamana, E. Emojevwe and M. Shok (2003). Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* extract. *Phytomedicine* **10**, 59-61.

[33] B.M. Lawrence (1988). A further examination of the variation of *Ocimum basilicum* L. In: Flavors and Fragrances: A World Perspective, *eds:* B.M. Lawrence, B.D. Mookherjee and B.J. Willis. Elsevier Science, Amsterdam.

[34] Y. Holm, I. Laakso, R. Hiltunen and B. Galambosi (1997). Variation in the essential oil composition of *Artemisia annua* L. of different origin cultivated in Finland. *Flavour Fragr. J.* **12**, 241-246.

[35] R.F. Vieira, R.J. Grayer, A. Paton and J.E. Simon (2001). Genetic diversity of *Ocimum gratissimum* L. based on volatile oil constituents, flavonoids and RAPD markers. *Biochem. Syst. Ecol.* **29**, 287-304.

[36] J.D. Thompson, J.C. Chalchat, A. Michet, Y.B. Linhart and B. Ehlers (2003). Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J. Chem. Ecol.* **29**, 859-880.

[37] A. Gil, E.B. de la Fuente, A.E. Lenardis, M.L. Pereira, S.A. Suárez, A. Bandoni, C. van Baren, P. Di Leo Lira and C.M. Ghersa (2002). Coriander essential oil composition from two genotypes grown in different environmental conditions. *J. Agric. Food Chem.* **50**, 2870-2877.

[38] M.A. curado, C.B.A. Oliveira, J.G. Jesus, S.C. Santos, J.C. Seraphin and P.D. Ferri (2006). Environmental factors influence on chemical polymorphism of the essential oils of *Lychnophora ericoides*. *Phytochemistry* **67**, 2363-2369.

[39] N. Borlina Maia, O. Alves Bovi, M.O. Mayo Marques, N. do Prado Granja and Q.A. Cargo Carmello (2001). Essential oil production and quality of *Mentha arvensis* L. grown in nutrient solutions. *Acta Hort. (ISHS)* **548**, 181-188.

[40] E. Banchio, J. Zygadlo and G.R. Valladares (2005). Effects of mechanical wounding on essential oil composition and emission of volatiles from *Minthostachys mollis*. *J. Chem. Ecol.* **31**, 719-727.

[41] E. Banchio, G. Valladares, J. Zygadlo, P.C. Bogino, L.V. Rinaudi and W. Giordano (2007). Changes in composition of essential oils and volatile emissions of *Minthostachys mollis*, induced by leaf punctures of *Liriomyza huidobrensis*. *Biochem. Syst. Ecol.* **35**, 68-74.