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Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K

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Abstract

Lippia origanoides H.B.K. (Verbenaceae) is a plant known in Oriximiná (Brazil) as "Salva-de-Marajó". Its leaves are widely used as of a spice in cooking and in traditional medicine. The chemical composition of the essential oil obtained from its leaves, analyzed by GC and GC/MS, showed a high content of oxygenated monoterpenes (66.0%), carvacrol (38.6%) and thymol (18.5%) being the major constituents. Considering that previous studies on the same plant species showed carvacrol as a trace or absent compound, we propose the existence of a new chemotype for this species. A high carvacrol content in the essential oil determines the plant's suitability for the preparation of oregano condiment. The antimicrobial activity of this essential oil was determined by the drop diffusion method, showing highly significant inhibition zones for all microorganisms tested.

Keywords: *Lippia origanoides*; Essential oil; Oregano; Antimicrobial activity; Thymol; Carvacrol

1. Introduction

Lippia origanoides H.B.K. (Verbenaceae) is a slender, very aromatic shrub or tree, up to 3 m tall (Jansen-Jacobs, 1988; Moldenke, 1965). The species is native to some countries of Central America (Mexico, Guatemala, Cuba) and northern South America, especially in the Amazon Region (Guiana, Venezuela, Brazil and Colombia) (Maisch, 1885; Pascual, Slowing, Carretero, Mata, & Villar, 2001). This plant is popularly known in the North of Brazil by the names of "Salva-de-Marajó" and "Alecrim-d'Angola"; in Pará State it is used for culinary and medicinal purposes (Oliveira, 2004). In Mexico, *L. origanoides* (syn. *Lippia schomburgkiana* Schauer) is called oregano, and the Mexican Pharmacopoeia recognizes it as a substitute for common oregano – *L. graveolens* Kunth (Maisch, 1885). Its characteristic aroma, so similar to that of oregano, may have inspired this name to Humboldt, Bonpland and Kunth (Morais et al., 1972).

An ethnobotanical study carried out in the city of Oriximiná (Pará State, Brazil) during the months of September and October of 2001, pointed out the wide use of this plant as a spice, besides an infusion from its leaves and flowers which was indicated for the treatment of stomachache, baby colic, indigestion, diarrhea, heartburn, nausea, flatus, "ladies belly", vaginal discharges, menstrual complaints and fever as well as a general antiseptic for mouth, throat and wound (Oliveira, 2004).

A review of the literature showed that at least 39 species, in 16 genera, are used throughout the world as condiments or medicines and are called oregano (Fleisher & Sneer, 1982; Lawrence, 1984). The presence of essential oils and

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their composition determine the specific aroma of plants and the flavour of the condiment. Thus, the chemical composition of essential oils is the most important criterion for spice identification and quality. A high carvacrol content in essential oil is the key to the concept of the "oregano" spice and is a requisite for determining a plant's suitability for the preparation of this condiment (Fleisher & Sneer, 1982). The oregano species, which have achieved economic importance are *Origanum vulgare* L. ssp. *viride* (Boiss.) Hayak (Greek Oregano), *Origanum onites* L. (Turkish Oregano), *Thymus capitatus* (L.) Hoffmanns and Link syn. *Coridothymus capitatus* (L.) Rchb. (Spanish Oregano) and *Lippia graveolens* HBK (Mexican Oregano).

The present work aims to study the chemical composition and antimicrobial activity of the essential oil obtained from the leaves of *L. origanoides*, collected at Oriximiná, Pará State, Brazil.

2. Materials and methods

2.1. Plant material

Aerial parts of *L. origanoides* were collected from a cultivated specimen in October of 2002, in the city of Oriximiná, Pará State, Brazil (1°46′01.1″S, 55°51′40.5″W), and identified by Dr. Fátima Regina Gonçalves Salimena, from Universidade Federal de Juiz de Fora. A voucher specimen was deposited at the CESJ, UFJF herbarium, under the registry number CESJ 39532.

2.2. Essential oils extraction and analysis

The essential oil from the leaves of *L. origanoides* was obtained by hydrodistillation in a Clevenger-type apparatus for 2 h, yielding 1.0% (v/w). Gas chromatography analyses were performed with a HP 5890 Series II gas chromatograph equipped with a FID detector and an HP-5 (5% phenyl/95% polydimethylsiloxane) fused silica capillary column (25 m × 0.2 mm, film thickness 0.33 μm) using hydrogen as carrier gas (1.0 ml min^{-1}). The injector temperature was 250 °C and the column oven programme was 60–240 °C at 3 °C min^{-1}. The detector (FID) was operated at 280 °C. The GC/MS was performed with an Agilent 5973 MSD coupled to an Agilent 6890 gas chromatograph, using helium as carrier gas, and the same column and oven conditions as above. Transfer line temperature was 240 °C, ion source was at 230 °C, EIMS, 70 eV. Constituents of the oil were identified by comparing the experimental gas chromatographic retention indices RI and MS fragmentation pattern with corresponding reference data (Adams, 1995; Wiley 6th ed.). A standard solution of *n*-alkanes (C_7–C_{26}) was used to obtain the retention indices.

2.3. Antimicrobial assay

The antimicrobial assay was carried out by the drop agar diffusion method described elsewhere (Hili, Evans, & Veness, 1997). The microorganisms tested were the fungi *Candida albicans* Serotype B ATCC 36802, *C. albicans*, *Candida parapsilosis*, *Candida guilliermondii* (Faculdade de Odontologia, Universidade Federal do Rio de Janeiro), *Cryptococcus neoformans* T_1-444 Serotype A (Universidade Federal de São Paulo, UNIFESP-SP), *Trichophyton rubrum* T544, *Fonsecaea pedrosoi* 5VPL (fungal collection from Hospital Clementino Fraga Filho, UFRJ), and the bacteria *Staphylococcus aureus* MRSA (BMB9393) (Hospital Clementino Fraga Filho, UFRJ), *Staphylococcus aureus* ATCC 25923, *Lactobacillus casei* ATTC 4646, and *Streptococcus mutans* ATCC 25175. Microorganisms (2×10^5 cells) were spread over Petri plates containing BHI solid medium (Brain Heart Infusion) and, after 10 min, a 10 μl drop of the essential oil, diluted 1:1 with Tween 80 (0.5% in water), was placed in the center of each plate. Reference antibiotics, amphotericin B, methicillin and vancomycin, were used as positive controls. All plates were incubated at 37 °C for 24 h to 7 days, depending on the microorganism tested, after which the diameter of inhibition zone (cm) was measured. The effect of the Tween 80 (0.5% in water) on microbial growth was also evaluated.

3. Results and discussion

3.1. Chemical analyses of essential oil

Major compounds in the essential oil of *L. origanoides* are listed in Table 1. The essential oil from *L. origanoides* showed a high content of oxygenated monoterpenes (66.0%), and low contents of monoterpene hydrocarbons (20.7%), sesquiterpene hydrocarbons (9.0%), and oxygenated sesquiterpenes (1.1%) (Table 1). The two major compounds among monoterpenes were carvacrol (38.6%) and thymol (18.5%). Also worth noting are the monoterpenes *p*-cymene (10.3%) and γ-terpinene (4.1%), as well as the sesquiterpene (*E*)-caryophyllene (5.9%).

Carvacrol is found as the main component in commercial oregano oils, but some species and chemotypes may contain mainly thymol. The monoterpene hydrocarbons *p*-cymene and γ-terpinene, the biogenetic precursors (via enzymic hydroxylation) of the two phenolic terpenes, thymol and carvacrol, are always found as major monoterpene hydrocarbons (Poulose & Croteau, 1978; Tainter & Grenis, 1993). The same variability in composition was verified in *L. origanoides* essential oil.

Previous studies on the essential oil from other samples of *L. origanoides*, obtained from cultivated plants originating from Belém (Pará State, Brazil) (Morais et al., 1972) and from a sample growing wild in Minas Gerais State, Brazil (Gallino, 1987), showed thymol as a major compound (20.6–38.4%), while carvacrol was present only as a trace constituent or was absent (0–0.4%). In contrast to the above findings, in the present study carvacrol was identified as a major compound in the essential oil of *L. origanoides*. This suggests that there are different chemotypes for this species, in a similar way to what happens with

Table 1
Qualitative and quantitative chemical composition of *Lippia origanoides* essential oils

Compounds	$RI_{calc.}$	$RI_{lit.}$	%	Identification method
α-Thujene	931	931	0.9	1,2
α-Pinene	937	939	0.9	1,2
Camphene	953	953	0.1	1,2
Sabinene	976	976	0.1	1,2
1-Octen-3-ol	981	978	0.4	1,2
Myrcene	992	991	1.8	1,2
α-Phellandrene	1006	1005	0.2	1,2
δ-3-Carene	1012	1011	0.1	1,2
α-Terpinene	1019	1018	1.1	1,2
ρ-Cymene	1028	1026	10.3	1,2
Limonene	1032	1031	0.5	1,2
1,8-Cineole	1035	1033	0.5	1,2
(*Z*)-β-Ocimene	1040	1040	0.1	1,2
(*E*)-β-Ocimene	1051	1050	0.2	1,2
γ-Terpinene	1062	1062	4.1	1,2
(*Z*)-linalool oxide	1076	1074	0.1	1,2
Terpinolene	1090	1088	0.3	1,2
Linalool	1101	1098	2.5	1,2
Ipsdienol	1150	1147	0.2	1,2
Borneol	1168	1165	0.2	1,2
Umbellunone	1177	1171	0.3	1,2
Terpinen-4-ol	1180	1177	0.9	1,2
p-Cymen-8-ol	1187	1183	0.1	1,2
α-Terpineol	1192	1189	0.2	1,2
Thymyl methyl ether	1237	1235	2.2	1,2
Carvacryl methyl ether	1247	1244	0.1	1,2
Phenyl ethyl acetate	1258	1256	0.1	1,2
Thymol	1289	1290	18.5	1,2
Carvacrol	1303	1298	38.6	1,2
Thymyl acetate	1358	1355	0.4	1,2
Carvacryl acetate	1375	1371	1.2	1,2
Geranyl acetate	1386	1383	0.1	1,2
β-Elemene	1392	1391	0.2	1,2
(*E*)-caryophyllene	1420	1418	5.9	1,2
β-gurjunene	1430	1432	0.2	1,2
(*E*)-α-bergamotene	1437	1436	0.1	1,2
(*Z*)-β-farnesene	1445	1443	0.3	1,2
α-Humulene	1454	1454	0.1	1,2
(*E*)-β-Farnesene	1459	1458	0.1	1,2
allo-Aromadendrene	1461	1461	0.6	1,2
Germacrene D	1481	1480	0.2	1,2
β-Selinene	1486	1485	0.5	1,2
β-Bisabolene	1509	1509	0.1	1,2
γ-Cadinene	1514	1513	0.1	1,2
δ-Cadinene	1524	1524	0.1	1,2
γ-Bisabolene	1533	1533	0.6	1,2
Germacrene B	1557	1556	0.1	1,2
Caryophyllene oxide	1583	1581	0.2	1,2
2-Phenyl ethyl tiglate	1588	1585	0.8	1,2
1-*Epi*-cubenol	1633	1627	0.4	1,2
Epi-α-cadinol	1643	1640	0.1	1,2
α-Muurolol	1648	1645	0.2	1,2
α-Cadinol	1656	1653	1.3	1,2
Monoterpene hydrocarbons			20.7	
Oxygen containing monoterpenes			66.0	
Sesquiterpene hydrocarbons			9.0	
Oxygen containing sesquiterpenes			1.1	
Other			1.3	
Total identified			98.1	

Identification methods: 1, retention indices; 2, Wiley library.

Origanum vulgare (Fleisher & Sneer, 1982), *Lippia alba* (Matos, Machado, Craveiro, & Alencar, 1996; Zoghbi, Andrade, Santos, Silva, & Maia, 1998), *Mentha* species (Kokkini, 1992) and thyme (*Thymus zygis*) (Lawrence, 1992). However, it is also known that cultivation conditions can affect secondary metabolite production (Edris, Shalaby, Fadel, & Abdel-Wahab, 2003), and this should be borne in mind.

In the course of preparation of this manuscript, a paper (Santos et al., 2004) appeared reporting the identification of carvacrol as the principal constituent of the essential oil of three different samples of *L. origanoides* (33.5–42.9%) growing wild in the State of Piauí (Brazil). This finding strongly suggests that the high carvacrol content in the sample analyzed by us is not due to environmental conditions, since both climate and soil are very different in Piauí and Pará states, supporting the existence of two different chemotypes for this species – thymol and carvacrol.

Despite the existence of several chemotypes for different oregano species, which account for a great variability in the contents of carvacrol and thymol, the Europeans are considered to be the best ones, and they have, in general, a high content of carvacrol (Fleisher & Sneer, 1982; Lawrence, 1984; Tucker & Maciarello, 1994).

Therefore, *L. origanoides* collected at Oriximiná, which showed good essential oil yield (1.0%) and a high carvacrol content (38.6%) can be considered as promising for future utilization as a food ingredient.

3.2. Antimicrobial activity

The essential oil of *L. origanoides* inhibits all microorganisms assayed (bacteria and fungi) (Table 2). The inhibitory effect of several terpenoids on microbial oxygen uptake and oxidative phosphorylation has also been demonstrated. In particular, phenolic and non-phenolic alcohols exhibited the strongest inhibitory effects, followed by aldehydes and ketones. The monoterpene hydrocarbons were less active and it has been suggested that this behaviour depends on the free hydroxyl group from the alcohols (Griffin, Wyllie, Markham, & Leach, 1999).

The antimicrobial activities of carvacrol and thymol have also been demonstrated (Bagamboula, Uyttendaele, & Debevere, 2004; Didry, Dubreuil, & Pinkas, 1994; Griffin et al., 1999; Salgueiro, Cavaleiro, Gonçalves, & Cunha, 2003). Both substances appear to make the cell membrane permeable, and are able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Burt, 2004).

Several studies have demonstrated the above activity for many species of the genus *Lippia* rich in thymol and carvacrol. This is the case of *L. sidoides* (Lemos et al., 1990), *L. gracilis* (Lemos et al., 1992), *L. graveolens* (Salgueiro et al., 2003), *L. chevalieri* (Bassole et al., 2003); *L. multiflora* (Bassole et al., 2003), and *L. origanoides* (Santos et al., 2004). In

Table 2
Growth inhibition zones of microorganisms in millimetres

Microorganisms	Inhibition halo diameter (mm)			
	Lippia origanoides	Amphotericin B	Vancomycin	Methicillin
Candida albicans Serotype B ATCC 36802	25	20	–	–
Candida albicans	27	16	–	–
Candida guilliermondii	40	24	–	–
Candida parapsilosis	35	18	–	–
Cryptococcus neoformans T_1-444 Serotype A	24	20	–	–
Trichophytum rubrum T544	30	20	–	–
Fonsecaea pedrosoi 5VPL	40	18	–	–
Staphylococcus aureus ATCC 25923	25	–	21	20
Staphylococcus aureus MRSA (BMB9393)	25	–	18	08
Lactobacillus casei ATTC 4646	20	–	10	15
Streptococcus mutans ATCC 25175	26	–	10	10

the latter case, however, the authors assayed only a limited number of microorganisms and no reference antibiotic was used.

The very good antimicrobial activity of *L. origanoides* can thus be related, at least in part, to the major compounds in the essential oil, carvacrol and thymol.

4. Conclusion

The high content of carvacrol in the essential oil of *L. origanoides* collected at Oriximiná (Pará state, Brazil), suggests the occurrence of a new chemotype for this species. In addition, due to its essential oil yield and composition, *L. origanoides* can be considered as promising for future utilization as a spice and condiment. Also, the extensive use of this plant, by the local people of Oriximiná, as carminative, as a general antiseptic (mouth, skin, vagina) and to treat respiratory infections, can be related to the antimicrobial activity.

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