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Chemical composition and antimicrobial activity of the essential oil of *Lippia lasiocalycina* Cham. (Verbenaceae)

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ABSTRACT

The use of plant species of the genus *Lippia* in the treatment of diseases is an old practice, however, some species still needs studies. Thus, the present work aimed to characterize chemically and to evaluate the antimicrobial activity of the essential oil of *Lippia lasiocalycina*. The oil was extracted by hydrodistillation and the analysis of the chemical constituents done using gas chromatography coupled to the mass spectrometer. Minimum inhibitory concentrations were determined by microdilution method against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* strains. The constituents present were identified (90.09%) and piperitenone oxide was defined as the major compound (57.55%) followed by limonene (20.69%). The essential oil of *Lippia lasiocalycina* presented activity against *C. albicans* strain, signaling for a potential application in the treatment of infections caused by this yeast.

1. Introduction

The use of medicinal plants has been constant since the origin of mankind. (Boukhatem et al., 2014). Among the substances of natural origin, essential oils are very important both economically and scientifically, being versatile products with applicability in the most varied sectors (Bernardos et al., 2015).

Essential oils are rich in bioactive substances (Medeiros et al., 2011). Its chemical composition is complex and presents a wide variety of constituents like monoterpenes, sesquiterpenes, and their derivatives such as aldehydes and phenols. This composition varies between plant species and seasons of the year. (Hajlaoui et al., 2010). In plants, the essential oils are directly related to the processes of pollination, dissemination of seeds, and in defense against attacks of herbivores as well as fungi and bacteria (Costa et al., 2015; Li et al., 2013).

The Verbenaceae family has approximately 36 genera of plants and 1000 plant species distributed in pantotropical regions. Brazil is the country with the greatest diversity of taxon with 16 genera and about 290 species. The plants of this family usually present in the form of herbs, shrubs, sub-shrubs and lianas (Costa et al., 2017). Among the genus belonging to this family, we can highlight *Lippia*, constituted by 200 species that exhibits a striking appearance and pleasant odor (Oliveira et al., 2006). The genus *Lippia* is widely used in folk medicine in gastrointestinal disorders and respiratory diseases. The infusion and the essential oil of various parts of plants is used as antifungal, antimicrobial, larvicide, and anesthetic agents (Linde et al., 2010).

In recent years, we have seen the emergence of a problem that permeates the treatment of various diseases, the phenomenon of microbial resistance. This resistance has rapidly proliferated by involving Gram-positive and Gram-negative bacteria, such as *Staphylococcus* and *Escherichia coli* (Silveira et al., 2006) and opportunistic fungal species like *Candida albicans* (Casto and Lima, 2011).

Although the advances in research on the chemical and pharmacological properties of the *Lippia* genus, there are still species that needs clarifying studies such as *Lippia lasiocalycina*. To the best of our knowledge, there have been just one literature sources reporting the preliminary study of the chemical constituents of *L. lasiocalycina* alcohol extract (Funari et al., 2012). No information on the biological activities or chemical composition of the essential oil is available. Therefore, the chemical characterization and determination of the

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antimicrobial activity of the essential oil of *Lippia lasiocalycina* was the objective of the present study.

2. Experimental

2.1. Plant material

The plant material was collected at 9:00 am in the Gilbués city, Piauí State, Brazil, in April 2016. The region presents subhumid tropical climate with high temperatures and low rainfall index being located in the semi-arid region of northeastern Brazil.

Plant material was made available by the Brazilian Agricultural Research Company - EMBRAPA Meio-Norte (-5.036410, -42.797898). Voucher specimen was deposited in the Embrapa Herbarium Genetic Resources and Biotechnology - CEN under the number CEN92437.

2.2. Essential oil extraction

Lippia lasiocalycina essential oil (LLEO) was obtained from fresh leaves of the plant by hydrodistillation using Clevenger type distillation apparatus for approximately 3 h. The essential oils yields ranged from 0.40 to 0.52%. At the end of the process, the resulting oil was collected, dried with sodium sulphate, weighed, and stored under refrigeration. The oil was solubilized in H_2CCl for gas chromatography and mass spectrometry analysis.

2.3. Chromatography conditions

For the characterization of the chemical composition of the volatile compounds of LLEO, a gas chromatograph, coupled to the mass spectrometer (GC–MS) was used. A Shimadzu® Chromatograph, model CGMS-QP2010 SE equipped with AOC-5000 automatic injector and SLB-5 ms column (30 m x 0.25 mm x 0.25 µm) was used. The conditions for the CG-MS analysis were as follows: Helium as carrier gas at a flow rate of 1 mL min^{-1}, a temperature of 250 °C in the injector; a temperature program, starting at 60 °C (3 min), at a rate of 3 °C / min until reach 240 °C (for 10 min); the detector temperature was 250 °C. Previously the essential oil was diluted into dichloromethane (1:10) and so 1 µL was injected. The MS conditions were triple quadrupole type of ion detector operating by electronic impact (70 eV, 45–450 Da).

Identification of the essential oil components was performed by comparing their GC–MS retention indices. The spectra were considered coincident if the similarity index was equal to or greater than 90%. The Kovats index was estimated by comparison between some known compounds in the chromatogram and the compatible Kovats indices of the database records (WILEY, NIST, PHEROBASE).

2.4. Microbial strains

Evaluation of the antimicrobial activity of EOLL was performed against standard strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans* ATCC 10231, as well as, against a multidrug-resistant *S. aureus* strain (SA-1199B). Bacterial strains were maintained on Brain Heart Infusion Agar (BHIA, Himedia, India) slant at 4 °C, and prior to assay the cells were grown overnight at 37 °C in Brain Heart Infusion (BHI, Himedia, India). The yeast strain was maintained on Sabouraud Dextrose Agar (SDA, Himedia, India) slant at 4 °C and prior to assay the cells were grown for 24 h at 37 °C in Sabouraud Dextrose Broth (SDB, Himedia, India).

2.5. Determination of the minimum inhibitory concentration

The determination of the minimum inhibitory concentration (MIC) was performed according to the microdilution method (CLSI, 2003). In this assay, 96-well microplates (12 columns and 8 lines) with flat bottom were used. A stock solution of the test product was previously prepared by dissolving 10.000 µg of the product in 1 mL^{-1} of dimethylsulfoxide (DMSO). This initial solution was then diluted in sterile distilled water to a concentration of 1024 µg mL^{-1}. MIC of the LLEO was determined by microdilution in BHI with microbial suspensions of 10^5 CFU mL^{-1} and with LLEO at concentrations 8, 16, 32, 64, 128, 256, 512 µg mL^{-1}. Microplates were incubated at 37 °C for 24 h. MIC was defined as the lowest concentration of the drug in which no microbial growth is observed. All experiments were done in triplicate. The control group used in this study consisted of culture medium and inoculum without the addition of essential oil. The control of the inoculum and the control of the sterility of the culture medium were made.

Antifungal assays were performed by the microdilution method in SDB double concentrated with a yeast suspension of 10^5 CFU mL^{-1} and LLEO solutions ranging from 8 to 512 µg mL^{-1}. Microplates were incubated at 37 °C for 48 h.

2.6. Determination of the minimum microbicide concentration

Inhibition of the bacterial or fungal growth was confirmed transferring an aliquot of 10 µl from each well of the MIC test microplates to a Petri dish containing BHIA (or SDA) and checking cell viability after incubation at 37 °C for 24 to 48 h. Minimal microbicide concentration (MMC) was defined as the lowest concentration of the drug in which no microbial growth was observed.

3. Results and discussion

In the last years we have seen a change in the habits of life of the population, a change that includes a greater concern with the quality of the food ingested as well as to the safety of the industrialized products. At the same time, the industry has been looking for options to reduce the amount of synthetic chemicals in its products, signaling a global trend towards adherence to more natural means of conservation and food production (Machado et al., 2011). In this scenario, essential oils appear as a promising alternative. One of the main factors that make them interesting for the food industry is the fact that these oils are mostly used routinely in cooking or in folk medicine, making their acceptability much higher when compared with synthetic additives (Pereira et al., 2006).

The volatile compounds of *L. lasiocalycina* essential oil were detected for the first time by GC–MS. Representing 90.09% of the essential oil fourteen constituents were identified of which the major ones, piperitenone oxide (57.55%) and limonene (20.69%), accounted for 78.24% (Table 1).

The chemical composition of species of the genus *Lippia* has been extensively studied. Gonçalves et al. (2015) and Guimarães et al. (2014)

Table 1
Constituents of *Lippia lasiocalycina* essential oil analyzed by GC–MS.

RT[a]	Compound	KI[b]	Area
3.677	Methylbenzene	770	0.31
7.121	α-pinene	939	1.17
8.816	Myrcene	991	0.20
10.195	*p*-Cimene	1026	0.47
10.399	Limonene	1031	20.69
10.986	*Trans*-β-ocimene	1050	1.01
11.538	γ-Terpinene	1062	0.18
19.849	Carvone	1242	0.26
21.998	*m*-Thymol	1290	0.83
22.354	Tridecane	1299	1.03
24.199	Piperitenone	1342	3.04
25.283	Piperitenone oxide	1365	57.55
31.175	Bicyclogermacrene	1494	2.67
31.597	β-Bisabolene	1509	0.68

[a] Retention time.
[b] Kovats index.