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Research Article

Antimicrobial action of essential oil of *Lippia origanoides* H.B.K.

Abstract

The Lippia oils are well known for the treatment of respiratory problems, as well as their oils have compounds that are possibly good antimicrobial agents. In order to confirm the seasonal differences and also to observe the potential to influence the antimicrobial activity, it was evaluated antimicrobial activity of the essential oil of *Lippia origanoides* H K B against the strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans*. Methodology: leaves of *Lippia origanoides* H.B.K. were harvested in the municipality of José de Freitas - PI, followed by their plants by the hydrodistillation process. Afterwards the oil was subjected to the analysis of techniques by Gas Chromatography coupled to GC-MS Mass Spectrometry for the identification of its most important components. The test for its activities was bactericidal and fungicidal at the concentrations of 100, 300, 600 and 900 µg / mL. According to the analysis of the oil in the study, the results are classified as chemotactic C, since it presents 55.53% of thymbrite as the majority of the total area analyzed. In addition, it presents 18.87% of carvacrol and 18.03% of p - cimene, the latter being a precursor of thymol and carvacrol. Events reported to results are due to the major compounds present in the oil. After 24 hours of growth, inhibition can be detected in some of the tested, as well as, it was noticed that the diluted in saline did not present positive results as expected for control. However, the oil at concentrations of 100, 300, 600 and 900 µg / ml inhibited growth against *E. coli.* Inhibition of the *Staphylococcus aureus* strain was observed at concentrations of 300, 600 and 900 µg / mL and against *candida albicans* there was inhibition at the concentrations of 300, 600 and 900 µg / mL. At the concentration of 100 µg / mL against the last strains there was no inhibition. The antimicrobial action is derived from the essential oil itself and the main responsible for it are the main components present, thymol and carvacrol and or association thereof, as well as resulting from hydrophobicity, permeability of the membrane. Conclusion: It was perceived that the essential oil of *Lippia origanoides* H. B. K. presented antimicrobial action as strains of Staphylococcus aureus, Escherichia coli and Candida albicans. At the concentration of 600 µg / mL there was a greater action against bacterial strains and against the fungal strain, 900 µg / mL, with a higher inhibition index. Therefore, a *L. origanoides* presents itself as promising for the development of new phytotherapics, faster and at a lower cost.

Introduction

According to Leal [1] ah long time the use of medicinal plants is recorded in their rural and traditional communities, which according to the Resolution of the Collegiate Board (RCB) da National Health Surveillance Agency (ANVISA) nº 26 de 2015, products derived from the use of the raw material or active principle of natural plants is considered as a herbal product, that is, used for prophylactic and curative treatment [2]. Brazil, being one of the countries richest in biodiversity, fauna and flora, is prone to various empirical and scientific studies of the functional properties of the plant species that it presents [3-5].

Data from the World Health Organization (WHO), together with the Ministry of Health, have shown that over 80% of the population of underdeveloped countries uses traditional medicine through the preparation of teas, bottles and honey, based on observation and experience with medicinal plants, such as *Lippia Origanoides* HBK Research based on natural products, especially those derived from plants, can lead to the discovery of new drugs and the cure of some diseases due to the therapeutic potential they present [6-8]. It allows the discovery of an excellent drug, which according to Leal [1] is one that presents low cost of production, good reproducibility, active pharmacological properties and low toxicity. Despite the advances in the research on the chemical and pharmacological properties of the genus Lippia, there are still species that need studies like *Lippia origanoides* H.B.K. popularly known by Rosemary or Alecrim do Campo (Almeida et al., 2009).

Being the essential oil of L. origanoides Humboldt, Bonpland

Citation: Leal ALAB, Braga AL, de Araújo Barbosa B, Rodrigues AMX, de Carvalho Teixeira FAF, et al. (2019) Antimicrobial action of essential oil of *Lippia origanoides* H.B.K.. J Clin Microbiol Biochem Technol 5(1): 007-012. DOI: http://dx.doi.org/10.17352/jcmbt.000032

e Kunth (H.B.K.) (asthma, bronchitis), gastrointestinal (diarrhea, vomiting), skin and scalp, as well as having analgesic, anti-inflammatory, antifungal, antiparasitic (antimalarial, anti-inflammatory) properties), antibacterial, antiviral and repellent activity (insecticide) [4,9,10].

The search for effective drugs to fight bacterial infections has revolutionized the focus of the research. Taking into account that bacterial resistance is a reality, due to an enormous capacity to transmit genetic material of resistance to the other bacteria, besides the fact to acquire resistance to the effect of drugs used as therapeutic agents happens all the time. However, the use of medicinal plants such as *L. origanoides* H. B. K. against aggressive micro-organisms has been increasing by means of tests that evaluate antimicrobial activity [1].

The main components found in the essential oil of *L. origanoides* H.B.K. are thymol and carvacrol, oxygenated monoterpenes, which are the real responsible for the antimicrobial and antiseptic action of this plant. The chemical composition of the essential oil depends on biotic factors, ie, genetic and abiotic factors, such as luminosity, moisture, nutrition, development and location. It is necessary to characterize the abiotic and biotic factors of Lippia to determine its main chemical components and to classify it according to the chemotype in A, B or C [11-13].

Lippia origanoides HBK besides allowing the extraction of the essential oil, with its chemical compounds, carvacrol and thymol, and of other chemotypes, have in their composition secondary metabolites, which protect the leaves of the plants of UV-B radiation, these being the flavonoids , acting as natural antioxidants [14].

According to Leal et al. [1], researchers can use natural products in association with other substances or even isolated, aiming to broaden the spectrum of action of commonly used medicines, which are no longer effective. Therefore, the present study presents to the academic and scientific community how important it is to know the pharmacological properties of medicinal plants, for the development of herbal medicines with low cost and effective to the human being. It is of prime importance and of great scientific academic relevance, since it presents itself as revolutionary for the bactericidal and fungicide treatment, with a vast therapeutic and biotechnological potential.

The objective of this study was to evaluate the efficacy of *Lippia Origanoides* H. B. K. essential oil against bactericidal and fungicidal activities at concentrations of 100, 300, 600 and 900 μg / mL. With specific objectives the extraction of the aerial parts *Lippia Origanoides* H.B.K., the characterization of the essential oil, the evaluation of the bactericidal and fungicidal activity of this oil in different concentrations for the strains of *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*.

Methodology

Plant material

It was used as plant material for the study, aerial parts of L. origanoides HBK The collection occurred in the municipality of José de Freitas (latitude 04 ° 45'23 '' south and longitude 42 ° 34'32 '' west), Piauí, Brazil. From the same plant specimen where the collection was made, an exsicata is already deposited in the Herbarium "Graziela Barroso", Department of Biology, Federal University of Piauí, with the number: TEPB 09205.

Extraction and analysis of essential oil

About 500 g of the aerial parts of *L. origanoides* H.B.K. were subjected to the hydrodistillation process for a period of 4 h in a modified Clevenger type apparatus coupled to a 5 L flask, in order to extract the essential oil.

The oil obtained was subjected to the Gas Chromatography coupled to Mass Spectrometry (GC-MS) technique, in equipment belonging to the Petroleum Laboratory of the Federal University of Piauí (LAPETRO - UFPI).

Analyze

The essential oil of *Lippia origanoides* H. B. K., as well as the chemical characterization were carried out at the Petroleum Laboratory of the Federal University of Piauí (LAPETRO - UFPI) following a methodology described by LEAL et al. [1].

Microorganisms

The strains of *E. coli, Candida albicans* and *S. aureus* were transferred from the Central Laboratory of Piauí (LACEN-PI). Where the antibacterial and antifungal tests were performed on the Mauritius University, University of Nassau - Redemption Unit,

Evaluation of possible bactericidal effect

Antifungal and antibacterial activity follows according to methodology described reviewed by [1]. And this descriptive methodology follows in the text.

The study of the antibacterial activity of the essential oil of *Lippia origanoides* H.B.K. was carried out against the standard strain of *Staphylococcus aureus*, ATCC 25922 of *Escherichia coli* ATCC 25923. The bacterial strains were cultured in Müller Hinton agar medium. To standardize the inoculum density for the sensitivity test, the methodology was used according to the CLSI (Clinical and Laboratory Standards Institute). The colonies were suspended in sterile saline (0.85% NaCl) until a turbidity compatible with the Mac Farland scale 0.5 degree (1x106 CFU / mL) was obtained.

Dilution of essential oil: The essential oil was emulsified with 0.05% Tween 80 (Sigma-USA) and dissolved in 0.9% saline, at concentrations of 100, 300, 600 and 900 μg / ml.

Evaluation of antibacterial activity: The evaluation of the antibacterial potential of the essential oil of *Lippia origanoides* H.B.K corresponds to a preliminary test. Müller Hinton agar plates were prepared in advance and removed from refrigeration to reach room temperature before sowing. With a sterile swab, the bacterial inocula were evenly distributed over the surface of the agar until the entire surface of the plaque was complete.

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The plates were then allowed to stand at room temperature for 3 minutes.

The technique used was the one of perforation in agar. In this technique, the removal of the solid culture medium is performed with the aid of cylinders 6-8 mm in diameter for the formation of wells, in which it is possible to apply the substances to be analyzed. Seven wells with 6 mm diameter were made in each plate to analyze the activity against Staphylococcus aureus and Escherichia coli. In each well was placed 50 µL of the suspension of the essential oil of *Lippia origanoides* H.B.K previously diluted in different concentrations. Ampicillin and gentamicin were tested against bacteria Staphylococcus aureus and Escherichia coli as a positive control. As negative control for both pathogens was used 0.05% Tween 80 diluted in 0.9% saline. The plates were made in triplicates and kept in an oven at 35ºC for 24h.

Evaluation of possible fungicidal effect

Fungal strain used and preparation of the inoculum: The study of the antifungal activity of the essential oil of *Lippia origanoides* H. B. K. was carried out against the standard strain 1023 of *Candida albicans*. The fungal strain was grown on Müller Hinton agar medium. To standardize the inoculum density for the sensitivity test, the methodology was used according to the CLSI (Clinical and Laboratory Standards Institute). The colony was suspended in sterile saline (0.85% NaCl) until a turbidity compatible with the Mac Farland scale grade 0.5 (1x106 CFU / mL) was obtained.

Dilution of essential oil: The essential oil was emulsified with 0.05% Tween 80 (Sigma - USA) and dissolved in 0.9% saline, at concentrations of 100, 300, 600 and 900 µM.

Evaluation of antifungal activity: The evaluation of the antifungal potential of the essential oil of *Lippia origanoides* H.B.K corresponds to a preliminary test. Müller Hinton agar plates were prepared in advance and removed from refrigeration to reach room temperature before sowing. With a sterile swab, the fungal inoculum was evenly distributed over the surface of the agar until the entire surface of the plaque was complete. The plates were then allowed to stand at room temperature for 3 minutes.

In this technique, the removal of the solid culture medium was performed with the aid of cylinders 6-8 mm in diameter for the formation of wells, in which it was possible to apply the analyzed substances. Six wells with 6 mm diameter were made in each plate to analyze the activity against *Candida albicans*. In each well was placed 50 µL of the suspension of the essential oil of *Lippia origanoides* H.B.K previously diluted in different concentrations. Miconazole was tested against the fungus used as a positive control. As negative control for the pathogen, 0.05% Tween 80 diluted in 0.9% saline was used. The plates were made in triplicates and kept in an oven at 35ºC for 24h.

Statistical analysis

The results obtained in the antimicrobial tests were evaluated by applying the statistical ANOVA test, followed by Tukey test, when applicable. Statistical analysis and plotting were performed with the aid of Excel® and GraphPad Prisma® 5 programs, considering $p < 0.05$.

Ethical aspects

According to Resolution No. 466 of December 12, 2012, this project did not need to be referred to the Ethics Council, since it was not applied to human beings. The essential oil extracted from Lippia origanoides H. B. K. was used as a source of studies, testing its antimicrobial actions, using strains of Escherichia coli, Staphylococcus aureus and Candida albicans registered in the ATCC and its toxicological activity, thus possessing low risk to the human being.

Results and Discussion

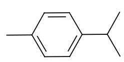
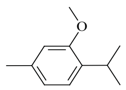
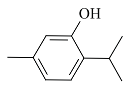
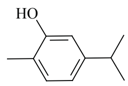
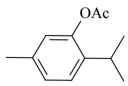
Identification of essential oil

The essential oil of *Lippia origanoides* H.B.K. presents several chemical constituents, according to the literature, which distinguishes one species from another table 1.

The essential oil of *Lippia origanoides* H.B.K presents in its aerial parts oxygenated monoterpene compounds that give them antimicrobial action, in the case of the major compounds thymol and carvacrol. Through gas chromatography and the statistical analysis of ten chemical compounds present in OELO, it can be classified into three chemo types according to the major compound. The chemotype A rich in p-cymene, chemotype B rich in carvacrol and the chemotype C, rich in thymol ([4,15].

Based on this, the essential oil of *Lippia origanoides* H.B.K. used in the present study is classified in the C chemotype, since it presents 55.53% of thymol as major of the total area analyzed. Chemotype C has a similar aroma to the chemo-type B of oregano, and therefore, in the cosmetics industry, as perfume, in addition to thymol, the oil under study has 18.87% of carvacrol and 18.03% of p- cimene, the latter being a precursor of thymol and carvacrol. According to Queiroz et

Table 1: Majority constituents of essential oil de *Lippia origanoides* H.B.K.

Composto	$K_{(lit.)}$	$IK_{(calc.)}$	Estrutura	Área (%)
p-cimeno	1020	1031		18,03
Timil-metil-eter	1232	1245		4,30
Timol	1289	1318		55,53
Carvacrol	1298	1299		18,87
Acetato detimila	1349	1361		3,72
Others				0,39
			TOTAL IDENTIFIED IDENTIFICATION	100

Source: RODRIGUES, 2015. [9].

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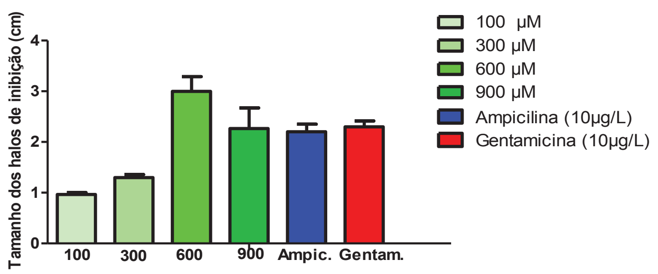
al. [9], these variations on the major compounds are due to genetic, environmental, climatic and age factors, leading to alterations in the production of secondary metabolites by the plant.

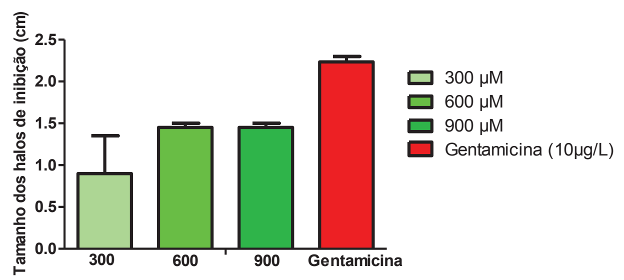
Evaluation of the possible bactericide and fungicide effect

The strains of *Staphylococcus aureus, Escherichia coli* and *Candida albicans* seeded in triplicate were used for the antimicrobial analysis of the essential oil of *Lippia origanoides* HBK at the concentrations of 100, 300, 600 and 900 µg / mL, in addition to those concentrations using positive controls and negative control. After 24 hours, one can detect the formation of inhibition halos (Figures 1-3 in appendix). The data can be observed in graph 1 (*Escherichia coli*), graph 2 (*Staphylococcus aureus*) and graph 3 (*Candida albicans*).

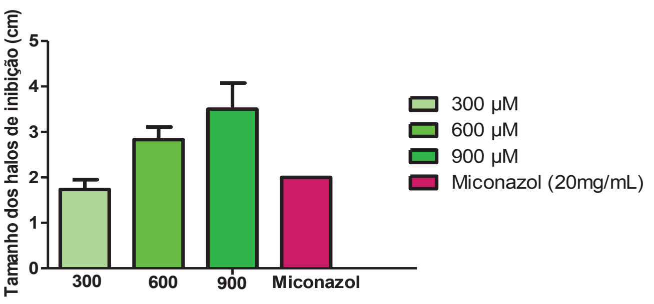
The antimicrobial action of the essential oils has been described since antiquity, with that after 24 hours of growth,



Graph 1: Comparison of lippia essential oil inhibition halos of the *Lippia origanoides* H.B.K against the *Escherichia coli* strain at different concentrations and controls tested.



Graph 2: Comparison of lippia essential oil inhibition halos of the *Lippia origanoides* H.B.K against the *Staphylococcus aureus* strain at different concentrations and controls tested.



Graph 3: Comparison of lippia essential oil inhibition halos of the *Lippia origanoides* H.B.K against the *Candida albicans* strain at different concentrations and controls tested.

one can detect inhibition halos in some of the concentrations tested, as well as it was possible to visualize that the Tween diluted in saline, used as negative control to the three strains, did not influence the antimicrobial activity of the essential oil of *Lippia origanoides* HBK, since it presented sensitivity to the analyzed strains (*Escherichia coli, Staphylococcus aureus* and *Candida albicans*).

According to graph 1, it can be seen that the essential oil of *Lippia origanoides* H.B.K. presented antimicrobial action against the Escherichia coli strain in the four concentrations tested (100, 300, 600 and 900 µg / mL). It is more effective in the concentration of 600 µg / mL, even when compared to the positive controls used, Ampicillin and Gentamicin, antibiotic disks used in laboratory routine for antibiogram.

Therefore, the antimicrobial action is derived from the essential oil itself and according to some authors, the main responsible for it are the major components present in this, thymol and carvacrol. Others claim that this action is due to the hydrophobicity, ie, membrane permeability, which allows a leak of essential molecules, causing bacteria and / or fungi death [9,16].

In Figure 2, an inhibition of the strain of *Staphylococcus aureus* is observed when at the concentrations of 300, 600 and 900 µg / mL, however, when compared to the positive control Gentamicin, it was observed that these are inferior to it. And that when compared to Ampicillin are superior, since there was no growth in that. It can also be seen that there was no inhibition at the concentration of 100µM. However, tests have already been carried out with *Lippia origanoides against S. aureus*, proving that it has an antimicrobial action due to its rich source of secondary metabolites useful in the treatment of diseases caused by this bacterium [17].

According to Almeida [11], the essential oil of *Lippia origanoides* has an inhibitory effect, according to tests carried out at a concentration of 120 µL / mL, that is, a higher concentration than those tested, thus, OELO presents antimicrobial action against strains of *Staphylococcus aureus* and *Escherichia coli*, in concentrations well below those already described in the literature. Another study conducted by Queiroz (2014) handled concentrations between 15 and 240 µL / mL. Confirming the hypothesis that the essential oil of *Lippia origanoides* H. B. K. can be used as antimicrobial.

Miconazole was used only in bacterial cultures, where two positive controls were used in fungal sowing, but this was handled in the version sold to the population with a concentration of 20 mg / mL. Thus, when we observed graph 3, we can see that there was inhibition of *C. albicans* at concentrations of 300, 600 and 900 µg / mL, being effective in the three concentrations mentioned. When compared to the positive control, the concentrations of 600 and 900 µg / mL were superior to him, and in 300 µg / mL obtained a similar inhibition to the control. However, at the concentration of 100 µg / mL, the essential oil of *Lippia origanoides* H.B.K, did not influence fungal growth.

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The essential oil against the fungus *Candida albicans* presented good antifungal activity. A study carried out by Ribeiro [18] shows a fungal activity of the essential oil of *Lippia origanoides* found in the Carajás National Forest against the phytopathogen Fusarium sp., where a concentration of 0.25 to 5.0 μL of the oil was used. Having its minimum inhibitory activity at the concentration of 0.25 μL. However, when compared to the present study, there was a difference in the time of analysis of the plaques, where it was 24 hours and in the study performed by Ribeiro [18] was 240 hours.

A large variation of the chemical compounds of the same species or genus can be observed, if we observe a study done by [19], the major compounds found in the species *Lippia lasiocalycina* are completely different since cavacrol and thymol are quite found in essential oils of the genus, taking into account the same methodology of microdilution used to test antibacterial with small modifications the results of the present work showed to be active against fungal and bacterial strains, this reveals that the presence of compounds with facility of oxidation may be favoring the rupture of membra [1].

A study carried out by Medeiros [20], corroborates with the present research the antifungal results of the essential oil of *Lippia origanoides* HBK, where in its study the oil was evaluated at the concentrations of 100, 25, 25 and 12.5% (volume / volume), with its antifungal activity confirmed against rotting fungi of wood, Gloeophyllum trabeum at all concentrations tested showed oil sensitivity. Against Trametes versicolor the inhibition was somewhat lower, however, still quite significant mainly considering that there was no significant difference between the first three concentrations.

Conclusion

It is possible to observe that the essential oil of *Lippia origanoides* HBK is of the chemotipo C, since it presents / displays the major component, thymol, with 55,53% of the total analyzed area, the oil presented antibacterial and antifungal action against the strains of *Staphylococcus aureus* , *Escherichia coli* and *Candida albicans*, respectively. In the analyzed concentrations of 100, 300, 600 and 900 μg / mL, in contrast to the *E. coli* strain, the oil presented 100% inhibition, whereas the strains of *S. aureus* and *C. albicans* presented a concentration of 100 μ sensitive to them. However, in 600 μg / mL, it was more effective, even when compared to the positive controls gentamicin and ampicillin, in the case of *S. aureus*, however, the concentration of 900 μg / mL, in contrast to the fungus, was the most inhibitory.

It was also observed that Miconazole, when it was already used in the population, was less effective than the concentrations of 600 and 900 μg / mL of the oil when compared to the antifungal control used. It is possible to conclude that the essential oil of *Lippia origanoides* H.B.K. analyzed can be used for the production of new antimicrobial herbs, more efficient and at a lower cost. And that when compared to literature, the present work presents satisfactory and promising results. Further study is still needed as to the possible toxic effect, as well as its clinical applicability *in vivo*.

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