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# Study of the scientific production of the antibacterial activity of the chemical compounds of the essential oil of *Lippia sidoides*

## Estudo da produção científica da atividade antibacteriana dos componentes químicos do óleo essencial de *Lippia sidoides*

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**ABSTRACT**

*Lippia sidoides* Cham. has widespread use in folk medicine because its essential oil (EO) presents great antibacterial properties. In Brazil, this plant has been introduced in governmental programs for herbal medicine, due to its recognized therapeutic activities. This promoted greater interest in the search for new molecules with antimicrobial activity in this EO which have been described by several authors. Thus, this work aimed to present a study of the scientific production of antibacterial activity of the chemical compounds from *L. sidoides* essential oil (LSEO). The inclusion criteria were articles which assessed the chemical components and antibacterial activity through the inhibition diameter, Minimum Inhibitory Concentration (MIC), and Minimal Bactericidal Concentration (MBC), published between 2000 and 2020. Of the 996 studies identified, 55 met the inclusion criteria. In descending order, the most frequently detected chemical components found in LSEO were: thymol, *p*-cymene, and caryophillene. Regarding to the origin, the results showed that the extraction of EO were carried out mainly in the Northeast (55.5%) and Southeast (19%) regions of Brazil; and the part of the plant most used for the production were the leaves. In addition, the essential oil showed strong antibacterial activity against most of the bacteria tested.

**Keywords.** Essential Oil, *Lippia sidoides*, Antimicrobial Agent, Microbial Sensitivity Tests.

**RESUMO**

*Lippia sidoides* Cham. é amplamente utilizada na medicina popular devido às propriedades antibacterianas atribuídas ao seu óleo essencial (OE). No Brasil, esta planta tem sido introduzida em programas governamentais de fitoterapia, por causa de suas atividades terapêuticas. Isso promoveu um maior interesse na busca por novas moléculas com atividade antimicrobiana presentes neste óleo, as quais foram relatadas por diversos autores. Assim, este trabalho objetivou apresentar um estudo da produção científica da atividade antibacteriana dos compostos químicos do OE da *L. sidoide*. Para tanto, foram utilizados artigos que abordaram os componentes químicos e atividade antibacteriana por meio de dados de diâmetro de inibição, Concentração Inibitória Mínima (CIM) e Concentração Bactericida Mínima (CBM), publicados entre 2000 e 2020. De um total de 996 estudos identificados, 55 preencheram os critérios de inclusão. Em ordem decrescente, os componentes químicos mais comumente encontrados no OE da *L. sidoide* foram: timol, *p*-cimeno e cariofileno. Com relação à origem, os resultados mostraram que a extração do OE foi realizada principalmente nas regiões Nordeste (55,5%) e Sudeste (19%) do Brasil; e a parte da planta mais utilizada para a produção foram as folhas. Além disso, o OE apresentou uma forte atividade antibacteriana contra a maioria das bactérias testadas.

**Palavras-chave.** Óleo Essencial, *Lippia sidoides*, Agente Antimicrobiano, Testes de Sensibilidade Microbiana.

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## INTRODUCTION

Essential Oils (EOs) are volatile, natural complex compounds characterized by a strong odor. They are liquid, limpid, rarely coloured and soluble in lipid and organic solvents with a generally lower density than that of water[1]. They can be synthesized as secondary metabolites by all plant organs, i.e., buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark; and are stored in secretory cells, cavities, canals, epidermic cells, or glandular trichomes[1]. These aromatic plants generally grow in warm temperate countries, such as Mediterranean and tropical countries, where they represent an important part of the traditional pharmacopeia[1].

Secondary metabolites are responsible for the synthesis of numerous bioactive substances, protect against insects, pathogens and limit the growth of other plants species[2]. EOs contain a multitude of bioactive substances, including alkaloids, cyanogenic glycosides, glucosinolates, lipids, phenolics, terpenes, polyacetylenes, and polythienyls[2], and are usually obtained by steam or hydrodistillation, which was first developed in the Middle Ages by the Arabs[1]. Known for their antiseptic, i.e., bactericidal, virucidal, fungicidal, medicinal properties and also for their fragrance, they have been used in embalmment, preservation of foods; and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic, and anaesthetic agent in medicine[1]. This have not changed much over the years, but more has been discovered about some of their mechanisms of action, particularly at the antimicrobial level[1].

The genus *Lippia* is composed of approximately 200 species of herbs, shrubs, and small trees belonging to the family Verbenaceae that are distributed throughout Africa, South and Central America[3,4]. *L. gracillis* H.B.K., *L. sidoides* Cham., *L. alba* Mill N.E. Brown, *L. mycrophylla* Cham., *L. gravelous*, *L. alnifolia*, *L. aristata*, *L. grata*, *L. triphylla*, *L. thymoides*, *L. citriodora*, *L. adoensise*, and *L. schimperi* are important species of this genus[3].

*L. sidoides* belongs to the division Magnoliophyta (flowering plants), class Magnoliopsida (Dicotyledoneae), and is commonly known as pepper-rosmarin, "alecrim-pimenta", "alecrim-grande", "alecrim-bravo", "alecrim-do-nordeste", and "estrepa cavalo" and was first found in North-Eastern Brazil, where it is extensively used in traditional medicine. It is an aromatic plant typically found in regions with a semiarid climate, such as the Northern part of Minas Gerais state and the Brazilian Northeast, especially in the states of Ceará and Rio Grande do Norte[3,5]. Its EO was first characterized by researchers from the Federal University of Ceará, who collected samples of this plant in Jucuri city (Rio Grande do Norte state) in August 1977[3]. Many studies have been done with the *L. sidoides* Essential Oil (LSEO), which has a high content of isomeric compounds, such as thymol and carvacrol[3]. *L. sidoides* extracts, particularly the EO extracted from its aerial parts, have shown many biological activities such as antioxidant, larvicidal, molluscicidal, antifungal, antibacterial, and insecticidal properties[3].

This plant is used in Brazilian folk medicine[6] and widely used in the social medicine program named "Live Pharmacies", a social phytotherapy program, created by Professor Matos, a pharmacognosist from the Federal University of Ceará State, Brazil[4]. This program is run by the municipal governments of country towns to help poor people with phytotherapy and is performed with local plants that are inexpensive but very effective[4]. As an antiseptic, due to its strong action against many microorganisms[4], the plant extract or its oil is usually applied topically to the skin, mucous membranes, mouth, and throat or used for vaginal washings and its therapeutic effects are attributed to the presence of thymol[4,6,7].

Because of the commercialization of the LSEO, there has been an increased interest in the cultivation of this species[8]. However, factors such as climate and environment, harvest season, and time, post-harvest processing, developmental stage, and plant age that influence the chemical composition and the yield of the EO need to be studied[8].

Variations in the chemical composition of the LSEO occur due to different extraction methods and may also be influenced by abiotic. Therefore, the objective of this study was to locate published studies on the main chemical compounds of the LSEO, with antibacterial activity, in the last 20 years.

## METHODOLOGY

For the present article, an electronic search for articles on antibacterial activity and chemical components of the LSEO were carried out in the databases Web of Science, Science Direct, Scopus, Medline and Lilacs. The search was conducted from April 2000 to June 2020.

The search strategy was to group the descriptors as follows: *Lippia sidoides*, antibacterial, antimicrobial, essential oil, chromatography, chemical composition, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Inhibition Zone Diameter. A total of nine different combinations were used: 1) *Lippia sidoides* AND antibacterial, 2) *Lippia sidoides* AND antimicrobial, 3) *Lippia sidoides* AND essential oil AND chromatography, 4) *Lippia sidoides* AND essential oil AND chemical composition, 5) *Lippia sidoides* AND antibacterial AND essential oil, 6) *Lippia sidoides* AND antimicrobial AND essential oil, 7) *Lippia sidoides* AND essential oil AND Minimum Inhibitory Concentration, 8) *Lippia sidoides* AND essential oil AND Inhibition Zone Diameter, 9) *Lippia sidoides* AND essential oil AND Minimum Bactericidal Concentration.

Inclusion criteria included any study with antibacterial activity and chemical constitution of the LSEO.

The exclusion criteria included studies that: 1) used oil dilutions for the disc diffusion tests to determine antibacterial activity; 2) tested the antimicrobial effects of components in their volatile form; 3) used the extraction and analysis of the chemical composition data, which was published in other studies; 4) analysed only certain components of the EO; 5) did not specify the amount (%) of each component found in the EO; 6) showed the result of only the main component of EO; 7) did not present any of the keywords; 8) did not have the full-text available; 9) did not describe the chemical composition; 10) were published as dissertations, thesis, books or conference papers; and 11) analysed only a chemical fraction of the EO.

To standardize the susceptibility patterns of microorganisms against the LSEO, for antimicrobial studies, MIC range was used as a parameter to determine the intensity of antibacterial activity as mentioned by Freires et al[9].

## RESULTS AND DISCUSSION

A total of 996 articles were retrieved from the five consulted databases for a preliminary review, through search strategy with the appropriate terms.

The majority of articles containing the combination of descriptors were found in Science Direct (52%), following by Web of Science/Scopus (18%), Medline (9%), and Lilacs (4.7%).

After the removal of articles according to the exclusion criteria, 55 articles underwent a full-text review for the antibacterial activity and chemical composition of the LSEO.

The distribution of the remaining 55 articles for each database alone or in combination is shown in **Figure**.

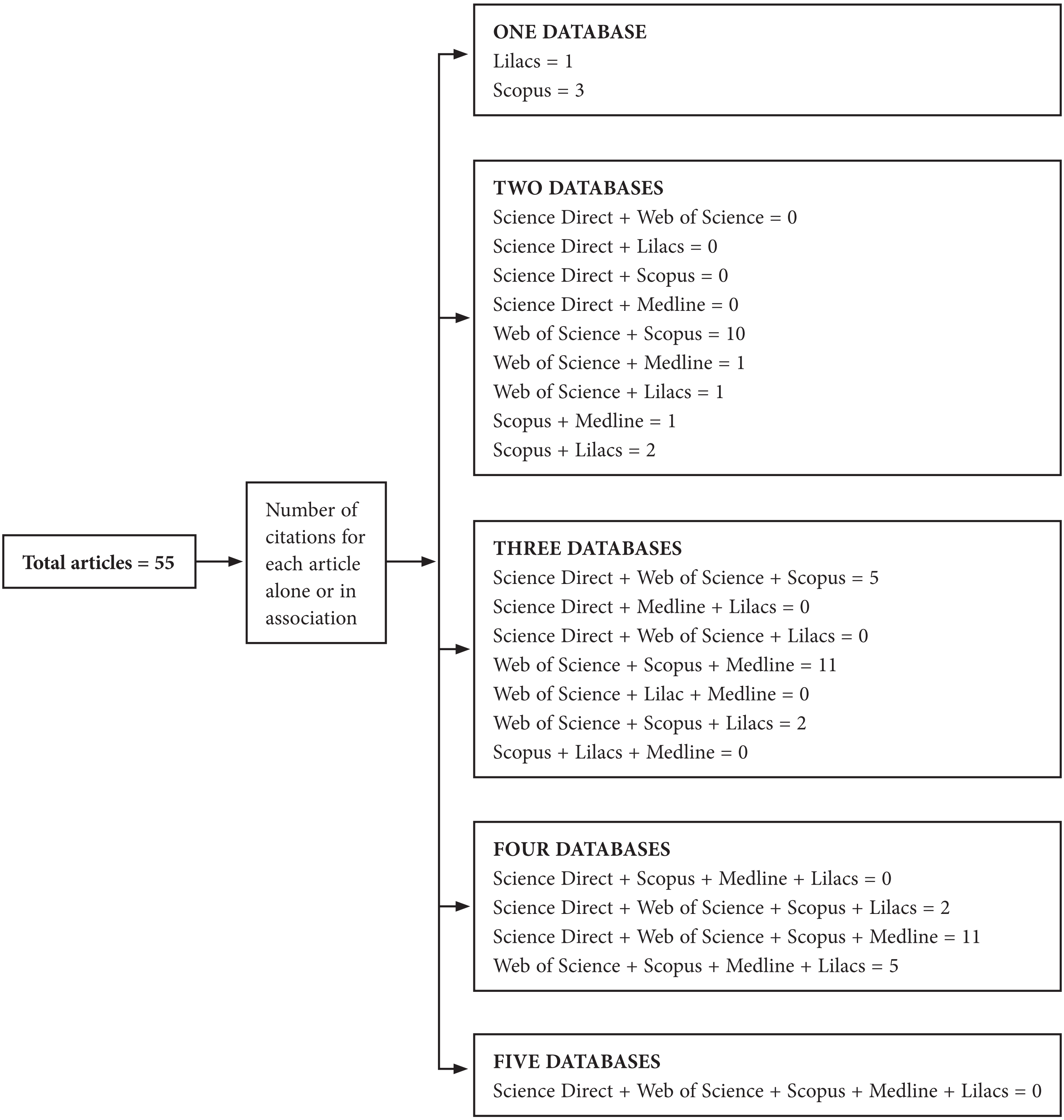


**Figure.** Distribution of articles for chemical composition and antibacterial activity of *L. sidoides* essential oil for each database alone or in combination

As shown in **Table 1**, for antibacterial activity, the search resulted in 11 articles, of which, only three performed the disk diffusion test. The Broth Microdilution Method (BMM) with the results expressed as MIC and defined as the lowest concentration at which no growth is observed, was the most performed test. Two studies performed only the Agar Plating Technique (APT) for the determination of the MBC, defined as the lowest concentration without bacterial growth assay, and six performed both BMM and APT with the results expressed as MIC and MBC. Two studies were performed with the disk diffusion test, BMM and APT assays; and only one study performed both the disk diffusion test and BMM assay, recent literature has shown that the disc diffusion method is no longer a commonly used antimicrobial method to determine the antimicrobial activity of medicinal plants, and when used, it is usually complemented by the MIC assay[10]. The most frequently used method for antimicrobial screening according to the 2008 review, was the MIC assay.

**Table 1.** Antibacterial activity of essential oil of *L. sidoides*

| Local and part of the plant used | Microorganism | Volume oil per disk** inhibition zone (mm) | MIC | MBC | MIC Score[9]*** (ug/mL) | Reference |
|---|---|---|---|---|---|---|
| Manaus, Amazonas (dried leaves and inflorescences) | *A hydrophila* | Not done | 1.250 ug/mL | 1.250 ug/mL | +*** | Majolo et al[11] |
| Fortaleza, Ceará (leaves) | | **10 uL** | | | | Botelho et al[12] |
| | *S. mutans* | 18.7 | 5.000 ug/mL | 20.000 ug/mL | - | |
| | *Streptococcus miti* | 10.0 | 10.000 ug/mL | 40.000 ug/mL | - | |
| | *Streptococcus salivarius* | 8.5 | 10.000 ug/mL | 40.000 ug/mL | - | |
| | *Strep sanguis* | 12.0 | 10.000 ug/mL | 40.000 ug/mL | - | |
| Montes Claros, Minas Gerais (NMPPU*) | | **20 uL** | | | | Castro et al[13] |
| | *S. aureus* | 26.0 | 13 uL/mL | 25 uL/mL | Not applicable**** | |
| | *E. coli* | 23.0 | 13 uL/mL | 25 uL/mL | | |
| Campinas, São Paulo (fresh leaves) | *Streptococcus mutans* | Not done | 62.5-125 ug/mL | 125-250 ug/mL | ++++ to +++ | Galvão et al[14] |
| João Pessoa, Paraíba (NMPPU*) | | **20 uL** | | | | Oliveira et al[15] |
| | *S. aureus* (12 strains) | 15.0-21.0 | 0.4 uL/mL all the strains | Not done | Not applicable | |
| Crato, Ceará (fresh leaves) | *S. aureus* | Not done | 128 ug/mL | Not done | +++ | Veras et al[16] |
| | *Streptococcus mutans* | | 256 ug/mL | | +++ | |
| | *Enterococcus faecalis* | | 512 ug/mL | | ++ | |
| | *Escherichia coli* | | 512 ug/mL | | ++ | |
| | *Enterobacter cloacae* | | 256 ug/mL | | +++ | |
| | *Klebsiella pneumoniae* | | 256 ug/mL | | +++ | |
| | *Pseudomon aeruginosa* | | 512 ug/mL | | ++ | |
| | *Providencia retinerai* | | 256 ug/mL | | +++ | |
| Manaus, Amazonas (dried leaves and inflorescences or rhizomes) | *Streptococcus agalactiae* | Not done | 312.5 ug/mL | 416.7 ug/mL | +++ | Majolo et al[17] |

MIC (Minimum Inhibitory Concentration)
MBC (Minimal Bactericidal Concentration)
* NMPPU = Not Mention the Part of the Plant Used
** Disks with 6 mm diameter
*** + weak activity; ++ moderate activity; +++ strong activity; ++++ very strong activity and – no activity
**** Not applicable: measures different from those used in the reference

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| Local and part of the plant used | Microorganism | Volume oil per disk** inhibition zone (mm) | MIC | MBC | MIC Score[9]*** (ug/mL) | Reference |
|---|---|---|---|---|---|---|
| Campinas, São Paulo (leaves and branches) | Two *S. aureus* strains (S8 and S10) | Not done | Not done | 0.025% v/v for S8 0.050% for S10 | Not applicable | Vázquez et al[18] |
| Campinas, São Paulo (leaves and branches) | Two *L. monocytogenes* strains (L2 and L8) | Not done | Not done | v/v = 0.1% for L2 and 0.5% for L8) | Not applicable | Vázquez et al[19] |
| Hidrolândia, Goiás (dried leaves) | *Bacillus cereus* | Not done | 1.000 ug/mL | Not done | ++ | Morais et al[6] |
| | *B. subtilis* | | 1.000 ug/mL | | ++ | |
| | *Micrococcus roses* | | 1.000 ug/mL | | ++ | |
| | *M. luteis* | | 1.000 ug/mL | | ++ | |
| | *Staphylococcus epidermis* | | 1.000 ug/mL | | ++ | |
| | *P. aeruginosa* | | 1.000 ug/mL | | ++ | |
| | *Salmonellaspp* | | 1.000 ug/mL | | ++ | |
| | *Serratiamarcenscens* | | 1.000 ug/mL | | ++ | |
| | *S. aureus* | | 500 ug/mL | | +++ | |
| | *S. aureus* | | 500 ug/mL | | +++ | |
| | *Enterobacter aerogenes* | | 2.000 ug/mL | | + | |
| | *E. cloacae* | | 2.000 ug/mL | | + | |
| | *E. coli* | | 2.000 ug/mL | | + | |
| | *Pseudomonasaeruginosa* | | 2.000 ug/mL | | + | |
| Campinas, São Paulo (fresh leaves) | *Streptococcus sanguis* | Not done | 125 ug/mL | 500 ug/mL | +++ | Bersan et al[20] |
| | *Streptococcus mitis* | | 250 ug/mL | >1.000 ug/mL | +++ | |
| | *Porphyromonas gingivalis* | | 250 ug/mL | 250 ug/mL | +++ | |
| | *Fusobacterium nucleatum* | | 125 ug/mL | 125 ug/mL | +++ | |

MIC (Minimum Inhibitory Concentration)
MBC (Minimal Bactericidal Concentration)
* NMPPU = Not Mention the Part of the Plant Used
** Disks with 6 mm diameter
*** + weak activity; ++ moderate activity; +++ strong activity; ++++ very strong activity and – no activity
**** Not applicable: measures different from those used in the reference

One aspect of antimicrobial analysis from the natural product which is generally lacking is the reporting of the MBC. It consists in a simple addition to the MIC assay but yields information that demonstrates the killing effect rather than just the inhibitory effects[10].

It has been well established that the chemistry of the plant has an impact on the antimicrobial activity, and as the chemistry may vary from season to season; as well as between geographical populations, the antimicrobial effects may also vary[10].

According to the results, most studies evaluated the effect of the LSEO against *Staphylococcus aureus*. Most of the LSEOs showed a strong antibacterial activity as described by Freires et al[9]. Only one demonstrated no activity and negative MIC score. Four studies were excluded because the MIC was not evaluated or the concentration of the was in a different unit (uL/mL) than that specified by Freires et al[9] (ug/mL). The plants originated from the North, Northeast, and Southeast of Brazil.

Articles describing the antimicrobial activity of LSEO were found more often in the Web of Science and Scopus databases (n = 9) than Medline (n = 5), Lilacs (n = 3), and Science Direct (n = 3).

All these studies were published in Brazilian scientific journals and most of them were originated from the state of Ceará (n = 19), followed by São Paulo (n = 9) and Sergipe (n = 8).

The Federal University of Sergipe-SE was the one that published the most articles (n = 8), followed by the State University of Ceará-CE (n = 7), the Federal University of Ceará Fortaleza-CE (n = 5) and the Regional University Cariri Crato-CE (n = 5).

**Chemical composition of LSEO**

Several constituents can be obtained from leaves or others parts of *L. sidoides*, including: α-Thujene, α-Pinene, β-Pinene, 2-β-Pinene, Mircene, β-Mircene, α-Terpinene, γ-Terpinene, *p*-Cymene, o-Cymene, Limonene, (E)-β-Ocimone, Thymol methyl ether, Thymol, Methyl thymol, α-Copaene, δ-Cadinene, Ar-curcumene, (E)-Caryophyllene, α-Caryophyllene, Caryophyllene oxide, β-Caryophyllene, Aromadendrene, α-Humuleno, Ipsdienol, Umbelulone, α-Terpineol, Sylvestrene, Deidro-aromadendreno, α-Felasadreno, Carvacrol, Carvacrol-methyl ether, Ether ethyl carvacrol, Ethyl-methyl-carvacrol, Methyl carvacrol, δ-(3)-Carene, Cis-thujopsene, α-Himachalene, Terpinen-4-ol, α-Copaene, 1,8-Cineole, α-Selinene, β-Selinene, Sabinene, Cis-sabinene hydrate, Camphor, Borneol, Isoborneol, Bornyl acetate, α-Cedrene, Artemisia triene, Cis-calamenene, Zierone, Rosifoliol, Cytronellyl pentanoate, Alo-himachalol, Octen-3-ol, β-Elemene, γ-Elemene, *Trans*-β-caryophillene, Iso-caryophillene, Cyclohexanone, Camphene, α-Fenchene, Eucalyptol, Thyone, Benzene, Anisole, carvone, Iedene, Verbene, P-menth-2,4(8)-diene, α-Guaiene, Germacrene a, Tricyclene, 3-Octanone, α-Phelandrene, β-(Z)-ocimene, β-(E)-ocimene, Terpinolene, β-(Z)-farnese, α-Terpineol, Neral, γ-Muuroleno, Amorfa-4,7-(11)-dieno, 7-Epi-alfa-selineno, Bicyclo [3.1.1]hept-2-ene, Bicyclo [3.1.0]hex-3-en-2-one, 2,6,6-Trimethyl, 1-Phellandrene, 1-Methyl-4-(1-methylethyl), Bornylene, 1,3,6-Octatriene, 3,7-Dimethyl-, (Z)-(CAS), 3,7-Dimethyl-, (E)-(CAS), Linalyl acetate, 2-(Chloromethyl) tetrahydropyran, 4-Methyl, 3-Cyclohexen-1-ol, 4-Methyl-1-(1-methylethyl), and Iedene[6,21-25].

According to Bakkali et al[1], EOs are complex natural mixtures that can contain 20–60 components at different concentrations and are characterized by the presence of two or three major components at higher concentrations (20–70%) compared to the other components present in trace amounts.

Among the selected studies, the main chemical-element in most cases was Thymol, and its concentration ranged from 6.0 to 84.95%, followed by *p*-Cymene (1.82–34.1%), y-Terpinene (1.32–16.60%); Myrcene (1.1–6.5%), and Caryophillene (0.19–30.20%), as shown in **Table 2**.

**Table 2.** Data of *L. sidoides* essential oil

| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Crato, Ceará (fresh leaves) | Hydrodistillation | CG-MS/comparison the spectra with NIST/Wiley | Thymol (84.9%)<br>*p*-Cymene (5.33%)<br>Ethyl-methyl-carvacrol (3.01%)<br>1,8-Cineole (1.68%)<br>γ-Terpinene (1.32%) | Veras et al[7] |
| Manaus, Amazonas (dried leaves and inflorescences) | Hydrodistillation | GC-MS/comparison the spectra and retention indices with Wiley and literature data | Thymol (76.6%)<br>o-Cymene (6.3%)<br>β-Caryophyllene (5.0%)<br>γ-Terpinene (2.0%)<br>Myrcene (1.1%) | Carvalho et al[26] |
| Fortaleza, Ceará (leaves) | Hydrodistillation | GC-MS/comparison of the spectra and retention indices with computer data | Thymol (56.67%)<br>Carvacrol (16.73%)<br>*p*-Cymene (7.13%)<br>Thymol methyl ether (5.06%)<br>Aromadendrene (2.79%) | Botelho et al[12] |

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| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Campinas, São Paulo (aerial fresh leaves) | Hydrodistillation | GC-MS/comparison of retention indices with data in the literature/NIST | Thymol (65.8%)<br>*p*-Cymene (17.3%)<br>*Trans*-caryophyllene (10.5%)<br>Cyclohexanone (6.5%) | Galvão et al[14] |
| Manaus, Amazonas (dried leaves and inflorescences or rhizomes) | Hydrodistillation | GC-MS/comparison of retention indices with Wiley and literature data | Thymol (76.6%)<br>*p*-Cymene (6.3%)<br>β-Caryophyllene (5.0%)<br>γ-Terpinene (2.0%)<br>Myrcene (1.1 %) | Majolo et al[17] |
| Hidrolândia, Goiás (dried leaves) | Hydrodistillation | GC-MS/use of digital libraries of mass spectral data and by comparison of their retention indices and authentic mass spectra, relative to C8–C32 n-alkane series in a temperature-programmed run | 1,8-Cineole (26,67%)<br>Isoborneol (14,60%)<br>Bornyl acetate (10.77%)<br>Camphene (6.19%)<br>α-Humulene (5.66%) | Morais et al[25] |
| São Gonçalo do Abaeté, Minas Gerais (powdered leaves) | Hydrodistillation | GC-MS/comparison the spectra and retention indices to literature | Isoborneol (14.66%)<br>Bornyl acetate (11.86%)<br>α-Humulene (11.23%)<br>α-Fenchene (9.32%)<br>1,8-Cineole (7.05%) | Morais et al[6] |
| São Cristovão, Sergipe (dried leaves) (4 different types of *L. sidoides*) | Hydrodistillation | GC-MS/comparison of the spectra with NIST data library and retention indices (RI) to literature | Thymol (7.25-70.36%)<br>Carvacrol (0.30-46.09%)<br>*p*-Cymene (8.36-15.06%)<br>β-Caryophyllene (0.19-8.81%) | Cavalcanti et al[23] |
| Itumirim, Minas Gerais (leaves) | Hydrodistillation | GC-MS/comparison the spectra and retention indices with Wiley and literature data | Carvacrol (26.44%)<br>1,8-Cineole (22.63%)<br>*p*-Cymene (9.89%)<br>γ-Terpinene (7.27%)<br>Sabinene (3.57%) | Guimarães et al[24] |
| Fortaleza, Ceará (leaves and branches) | Steam distillation | GC-MS/performed by computer library search, retention indices and visual interpretation of the mass spectra | Thymol (80.8%)<br>*p*-Cymene (8.6%)<br>*Trans*-caryophyllene (5.1%)<br>γ-Terpinene (1.6%)<br>1,8-Cineole (1.3%) | Cavalcanti et al[22] |
| Commercial sample (not specified) | Not specified | GC-MS comparison the spectra with Wiley electronic library and Kovats index (IK) with literature | Thymol (68,22%)<br>*p*-Cymene (9.43%),<br>*Trans*-caryophyllene (7.72%)<br>β-Myrcene (2.84%)<br>γ-Terpinene (2.71%) | Baldim et al[21] |
| Campinas, São Paulo (leaves) | Hydrodistillation | GC-MS/comparing of linear retention index (LRI) and spectra with literature, NIST or Wiley | Thymol (53.54%)<br>*p*-Cymene (13.29%)<br>β-Caryophyllene (7.16%)<br>γ-Terpinene (6.75%)<br>Thymol methyl ether (3.05%) | Vázquez et al[19] |

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| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Fortaleza, Ceará (fresh leaves) | Hydrodistillation | GC-MS/not reported | Caryophyllene (30.2%)<br>*p*-Cymene (28.7%)<br>γ-Terpinene (9.8%)<br>Eucalyptol (7.5%)<br>Thymol (6.3%) | Mota et al[27] |
| Mossoró, Natal (dried leaves) | Steam distillation | GC-MS/comparison the massa spectruns with GC-MS system data bank and retention index with literature | Thymol (22.37%)<br>1,8-Cineole(18.69%)<br>(E)-β-Ocimene (9.86%)<br>α-Humulene (7.84%)<br>Aromadendrene (5.35%) | Sousa et al[28] |
| Crato, Ceará (fresh leaves) | Hydrodistillation | LC-MS/comparison of MS with data bank and literature | Thymol (43.5%)<br>α-Felandreno (22.4%)<br>β-Caryophyllene (9.7%)<br>*p*-Cymene (8.6%)<br>Myrcene (6.5%) | Costa et al[29] |
| Horizonte, Ceará (leaves) | Steam distillation | GC-MS/comparison of spectra and retention indeces with computer data library | Thymol (59.65%)<br>E-Caryophyllene (10.60%)<br>*p*-Cymene (9.08%)<br>Myrcene (5.43%)<br>γ-Terpinene (3.83%) | Fontenelle et al[30] |
| Commercial sample PRONAT – Natural Products (not specified) | Not specified | GC-MS/comparison of spectra and retention indices with computer data library | Thymol (59.65%)<br>E-Caryophyllene (10.60%)<br>*p*-Cymene (9.08%)<br>Myrcene (5.43%)<br>γ-Terpinene (3.83%) | Camurça et al[31] |
| Fortaleza, Ceará (fresh leaves) | Steam distillation | GC-MS/by comparison with library data, retention indices | Thymol (66.67%)<br>E-Caryophyllene*<br>(β-Caryophyllene) (11.73%)<br>*p*-Cymene (7.13%)<br>γ-Terpinene (4.06%)<br>Carvacrol (γ-Elemene*) (1.81%) | Girão et al[32]<br><br>*Monteiro et al[4] |
| Teresina, Piauí (fresh leaves) | Hydrodistillation | GC-MS/comparison of the spectra with Willey library | Thymol (68.3%)<br>*p*-Cymene (14.4%) | Oliveira et al[33] |
| Lavras, Minas Gerais (fresh leaves) | Hydrodistillation | GC-MS/comparison the mass spectra with spectra library Wiley and the Kovats retention index to literature | Carvacrol (31.68%)<br>*p*-Cymene (19.58%<br>1,8-Cineole (9.26%)<br>γ-Terpinene (9.21%)<br>Sabinene (5.26%) | Lima et al[34] |
| Teresina, Piauí (fresh aerial parts) | Hydrodistillation | GC-MS/comparison of the experimental gas chromatographic retention indices and mass spectrum with literature and Willey library | Thymol (78,37%)<br>*p*-Cymene (6.32%)<br>E-Caryophyllene (6.17%)<br>(−) Caryophyllene oxide (1.68%)<br>1,8-Cineole (1.60%) | Medeiros et al[35] |
| Teresina, Piauí (not specified) | Hydrodistillation | GC-MS/comparison of the spectra obtained with the data bank (Willey) and the retention index (RI) to literature | Thymol (78.4%)<br>E-Caryophyllene (6.2%)<br>1,8-Cineole (1.6%)<br>Thymol methyl ether (1.4%)<br>γ-Terpinene (1.1%) | Borges et al[36] |

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| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Poço Redondo, Sergipe (dried and powdered leaves) *L. sidoides* LSD102 | Hydrodistillation | CG-MS/comparison of the spectra to NIST data library and from the literature | *p*-Cymene (34.1%)<br>γ-Terpinene (6.8%)<br>Methyl thymol (9.4%)<br>Thymol (38.7%)<br>Myrcene (3.3%) | Farias et al[37] |
| Poço Redondo, Sergipe (dried and powdered leaves) *L. sidoides* LSD104 | Hydrodistillation | CG-MS/comparison the spectra to NIST data library and from the literature | *p*-Cymene (17.8%)<br>γ-Terpinene (16.6%)<br>Methyl thymol (4.1%)<br>Carvacrol (43.7%)<br>Thymol (6.0%) | Farias et al[37] |
| Fortaleza, Ceara (fresh leaves) | Hydrodistillation | CG-MS/comparison the spectra with the literature and NIST data base, as well the retention indices with literature | Thymol (67.60%)<br>Carvacrol (6.3%)<br>E-Caryophyllene (2.36%)<br>1,8-Cineole (2.35%)<br>*p*-Cymene (1.82%) | Gomes et al[38] |
| Cariri, Ceará (fresh leaves) | Hydrodistillation | CG-MS/based on spectral fragmentation, using Wiley data library, plus: retention index and comparison with literature data | Thymol (84.90%)<br>*p*-Cymene (5.33%)<br>Carvacrol-methyl ether (3.01%)<br>1,8-Cineole (1.68%)<br>*γ*-Terpinene (1.32%) | Marco et al[39] |
| Crato, Ceará (dry leaves) | Hydrodistillation | CG-MS/based on spectral fragmentation, using Wiley data library, retention indices and comparison with literature data | Thymol (84.87%)<br>*p*-Cymene (5.33%)<br>Thymol methyl ether (3.01%)<br>Limonene (1.68%)<br>α-Terpinene (1,32%) | Mota et al[40] |
| Commercial sample (not specified) | Not specified | GC-MS/comparison of retention indices and mass spectra to a database from literature | Thymol (59.65%)<br>β-Caryophyllene (10.60%)<br>Cymene (9.08%) | Rondon et al[41] |
| São Cristóvão, Sergipe (dried leaves) | Hydrodistillation | GC-MS/comparison of their spectra, retention rates and NIST with literature | Thymol (42.33%)<br>*p*-Cymene (11.97%)<br>β-Caryophyllene (11.03%)<br>Methyl thymol (9.35%)<br>γ-Terpinene (5.44%) | Carvalho et al[42] |
| Commercial sample (not specified) | Not specified | GC-MS/comparison of the spectra with computer data and retention indices | Thymol (83.24%)<br>*Trans*-Caryophyllene (5.77%)<br>*p*-Cymene (4.46%)<br>Caryophyllene oxide (4.07%)<br>*β*-Myrcene (1.69%) | Lima et al[43] |
| Montes Claros, Minas Gerais (fresh leaves) | Steam distillation | CG-MS/comparison of the spectra with Wiley, literature and injection of standard substances and indice Kovats | Thymol (30.24%)<br>Benzene (14.49%)<br>*Trans*-β-caryophyllene (11.82%)<br>Borneol (11.38%)<br>γ-Terpinene (8.05%) | Aquino et al[44] |

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| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Commercial sample (not specified) | Steam distillation | GC-MS/comparison of mass spectra with literature and NIST, and retention indices with literature | Thymol (69.91%)<br>o-Cymene (14.84%)<br>E-Caryophyllene (4.04%)<br>Myrcene (3.57%)<br>Thymol methyl ether (0.84%) | Gomes et al[45] |
| Commercial sample (leaves) | Steam distillation | GC-MS/based on the information of analytic methods, and comparison of the data with NMR 1 H of the oil | Thymol (69.91%)<br>o-Cymene (14.84%)<br>E-Caryophyllene (4.04%)<br>Myrcene (3.57%)<br>Thymol methyl ether (0.84%) | Monteiro et al[5] |
| Commercial sample (not specified) | Not specified | GC-MS/performed by computer-based library search, retention indices and visual interpretation of the spectra | Thymol (70.97%)<br>Caryophyllene (8.30%)<br>*p*-Cymene (7.51%)<br>Myrcene (2.12%)<br>Caryophyllene oxide (1.59%) | Oliveira et al[46] |
| Campinas, São Paulo (leaves) | Hydrodistillation | GC-MS/comparison of Kovats Indexes/ spectra with literature, NISTand WiIley | Thymol (49.46%)<br>Cymene (11.40%)<br>Iso-caryophyllene (8.80%)<br>γ-Terpinene (8.06%)<br>Anisole (4.08%) | Zillo et al[47] |
| Campinas, São Paulo (fresh parts) | Hydrodistillation | GC and GC-MS/comparision of retention indices with reference data, and spectra with NIST | Thymol (65.76%)<br>*p*-Cymene (17.28%)<br>α-Caryophyllene (10.46%)<br>Cyclohexanone (6.50%) | Bersan et al[20] |
| Crato, Ceará (fresh leaves) | Hydrodistillation | GC-MS/comparison of the spectra with NIST, retention indices and literature | Thymol (84.95%)<br>*p*-Cymene (5.33%)<br>Éter metil carvacrol (3.01%)<br>1,8-Cineole (1.68%)<br>ɣ-Terpinene (1.31%) | Brito et al[48] |
| Fortaleza, Ceará (dried leaves) | Steam distillation | GC-MS not reported | Thymol/Carvacrol (93.36%)<br>Caryophyllene (3.59%)<br>Benzene (2.07%)<br>Cycloheptatriene (0.98%) | Lobo et al[49] |
| Manaus, Amazonas (dry leaves and inflorescences) | Hydrodistillation | GC-MS/comparison of the spectra and retention indices with Wiley and literature | Thymol (64.5%)*<br>*p*-Cymene (11.7%)*<br>(E)-Beta-Caryophyllene (4.9%)<br>Carvone (4.6%)<br>γ-Terpinene (3.6%) | *Chagas et al[50] and Soares et al[51]<br><br>Soares et al[51] |
| Commercial sample (leaves) | Not specified | GC-MS comparison of retention indices and spectra with literature | Thymol (68.45%)<br>*p*-Cymene (10.66%)<br>E-Caryophyllene (7.28%)<br>Myrcene (2.58%)<br>γ-Terpinene (2.12%) | Oliveira et al[52] |

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| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Commercial sample<br>(not specified) | Not specified | GC-MS and GC-FID/ comparison of retention index with literature and spectra with Wiley/NIST | Thymol (76,3%)<br>*p*-Cymene (10.0%)<br>β-Caryophyllene (6.80%)<br>Myrcene (2.09%)<br>γ-Terpinene (1.39%) | Santos et al[53] |
| Fortaleza, Ceará<br>(leaves) | Hydrodistillation | GC-MS and GC-FID/not reported | Thymol (77.8%)<br>*p*-Cymene (5.6%)<br>β-Caryophyllene (5.41%) | Pinto et al[54] |
| São Cristovão, Sergipe<br>(dry leaves) | Hydrodistillation | GC-MS/comparison of retention indices with literature | Thymol (44.55%)<br>*p*-Cymene (25.21%)<br>Thymol methyl ether (16.87%)<br>Thymol acetate (4.97%)<br>Myrcene (3.35%) | Bacci et al[55] |
| Araxá, Minas Gerais<br>(dried aerial parts) | Hydrodistillation | GC-MS/comparison the spectra with NIST data library and retention index with literature data | Thymol (68.40%)<br>*p*-Cymene (8.72%)<br>β-Caryophyllene (5.90%)<br>Caryophyllene oxide (2.55%)<br>Eucalyptol (1.46%) | Silva et al[56] |
| Jardinópolis, São Paulo<br>(dried aerial parts) | Hydrodistillation | GC-MS/comparison the spectra with NIST data library, and retention index with literature | Carvacrol (67.89%)<br>*p*-Cymene (21.76%)<br>β-Caryophyllene (3.90%)<br>Caryophyllene oxide (1.68%)<br>Ipsdienol (0.90%) | Silva et al[56] |
| Juiz de Fora, Minas Gerais<br>(fresh leaves) | Hydrodistillation | GC-MS/comparison of retention times with a standard n-alkanes solution, and the literature | Thymol (38.42%)<br>o-Cymene (30.08%) | Gomide et al[57] |
| São Cristóvão, Sergipe<br>(dried leaves) | Hydrodistillation | GC-MS comparison of the spectra with NIST and literature, and retention indices with literature | Thymol (44.55%)<br>*p*-Cymene (25.25%)<br>Methyl thymol ether (16.87%) | Lima et al[58] |
| Juiz de Fora, Minas Gerais<br>(fresh leaves) | Hydrodistillation | GC-MS comparison of retention indexes (RI) with literature | Thymol (63.20%)<br>o-Cymene (10.89%)<br>α-Humulene (5.06%)<br>γ-Terpinene (4.61%)<br>Thymol methyl ether (3.82%) | Gomide et al[59] |
| Not specified | Steam distillation | GC-MS/comparison of the spectra with literature/NIST data library, and retention indices with literature | Thymol (69.91%)<br>o-Cymene (14.84%)<br>E-Caryophyllene (4.04%)<br>Myrcene (3.57%)<br>Thymol methyl ether (0.84%) | Figueiredo et al[60] |
| Poço Redondo, Sergipe<br>(dried and powdered leaves) | Hydrodistillation | GC-MS/comparison the spectra with NIST/Wiley | Carvacrol (43.7%)<br>*p*-Cymene (17.8%)<br>γ-Terpinene (16.6%)<br>Thymol (6.0%)<br>Methyl thymol (4.1%) | Santos et al[61] |

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*Continuation*

| Origin (part of the plant used) | Extraction method | Separation/ identication method | Compounds | Reference |
|---|---|---|---|---|
| Manaus, Amazonas (dried leaves) | Hydrodistillation | GC-MS/comparison the spectra with Wiley and retention index with literature data | Thymol (72.2%)<br>*p*-Cymene (8.15%)<br>E-Caryophyllene (4.9%)<br>γ-Terpinene (2.23%)<br>Myrcene (1.49%) | Brasil et al[62] |
| Teresina, Piauí (dried leaves) | Steam distillation | CG-MS/comparison of the elution orders with literature | Thymol (33.52%)<br>*Trans*-Caryophillene (17.47%)<br>*p*-Cymene (13.07%)<br>γ-Terpinene (4.34%)<br>Caryophilllene oxide (3.71%) | Dias et al[63] |
| Commercial sample (not specified) | Not specified | GC-MS/comparison of the spectra and Kovats indices with literature and Wiley data library | Thymol (71.8%)<br>*o*-Cymene (9.8%)<br>Caryophellene oxide (5.4%)<br>β-Myrcene (4.1%)<br>γ-Terpinene (2.4%) | Fernandes et al[64] |
| Differents locals* (dried leaves of several *L. sidoides* genotypes) | Hydrodistillation | CG-MS/comparison of the spectra with NIST and Wiley, and retention index with literature | Several compounds* | Santos et al[8] |

*Annex 1

## Annex 1.

| Origin/genotype | Compounds (%) | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | year old plants | | | | | | | | | | | | | | | | | | | |
| | Thymol | | *p*-Cymene l | | (E) Caryo-phyllene | | Caryophyl-lene oxide | | Myrcene | | γ-Terpinene | | α-Terpinene | | Thymol-methyl-ether | | Carvacrol | | Terpine-4-ol | |
| | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 | 2 | 8 |
| Mossoró, RGN* LSID002 | 77.95 | 69.33 | 7.24 | 5.07 | 4.74 | 7.92 | 1.19 | 1.93 | 0.85 | 0.57 | 0.69 | 1.58 | 0.51 | 0.49 | 0.65 | 1.39 | 0.30 | 2.04 | 0.74 | 1.76 |
| Mossoró, RGN* LSID003 | 79.30 | 72.29 | 8.44 | 5.54 | 5.23 | 7.55 | 1.13 | 2.21 | 0.83 | 0.78 | 0.63 | 2.07 | 0.39 | 0.60 | 0.36 | 1.23 | 0.42 | 2.42 | 0.67 | 1.77 |
| Quixeré, Ceará LSID004 | 79.60 | 73.56 | 8.13 | 4.13 | 4.86 | 6.12 | 0.98 | 1.80 | 0.91 | 0.45 | 0.71 | 1.25 | 0.48 | 0.38 | 0.57 | 1.07 | 0.32 | 2.57 | 0.69 | 0.94 |
| Limoneiro do Norte, Ceará LSID005 | 76.22 | 69.74 | 7.70 | 4.69 | 5.21 | 6.32 | 1.18 | 2.04 | 1.03 | 0.57 | 1.74 | 1.68 | 0.71 | 0.43 | 0.66 | 1.07 | 0.42 | 2.56 | 0.87 | 1.36 |
| Tabuleiro do Norte, Ceará LSID006 | 80.91 | 71.56 | 8.00 | 3.93 | 4.81 | 5.74 | 0.97 | 1.96 | 0.85 | 0.56 | 0.57 | 1.43 | 0.40 | 0.37 | 0.51 | 2.41 | 0.16 | 2.16 | 0.67 | 1.12 |
| Poço Redondo, Sergipe LSID102 | 56.65 | 68.80 | 23.26 | 8.82 | 0.42 | 0.79 | 0.08 | 0.22 | 2.59 | 0.58 | 3.57 | 2.34 | 0.91 | 0.32 | 0.50 | 9.82 | 0.19 | 2.39 | 0.69 | 1.05 |
| Poço Redondo, Sergipe LSID103 | 83.20 | 73.28 | 8.18 | 6.62 | 0.32 | 0.93 | 0.00 | 0.51 | 2.57 | 2.42 | 1.45 | 2.06 | 0.38 | 0.57 | 0.74 | 1.62 | 0.20 | 1.92 | 0.59 | 1.24 |
| Poço Redondo, Sergipe LSID104 | 8.41 | 8.88 | 13.81 | 4.42 | 0.08 | 0.16 | 0.00 | 0.20 | 2.44 | 0.61 | 9.49 | 3.60 | 1.46 | 0.41 | 3.96 | 3.70 | 55.39 | 72.39 | 0.81 | 0.91 |
| Poço Redondo, Sergipe LSID105 | 77.39 | 62.60 | 7.68 | 7.00 | 0.27 | 0.89 | 0.35 | 0.37 | 2.29 | 2.65 | 0.41 | 1.91 | 0.23 | 0.51 | 8.13 | 15.0 | 0.34 | 1.96 | 0.72 | 1.86 |
| Recife, Pernambuco LSID301 | 76.70 | 75.79 | 6.67 | 6.45 | 6.84 | 5.09 | 1.54 | 1.03 | 0.87 | 1.28 | 0.59 | 2.25 | 0.40 | 0.87 | 0.58 | 0.79 | 0.21 | 0.00 | 0.78 | 0.18 |

*RGN: Rio Grande do Norte

Barbosa et al[65] also showed that the major constituents of LSEO were thymol, *p*-Cymene, myrcene, and caryophyllene, and thymol was described as a major component and a strong antiseptic among the phenol group[28,54].

In contrast to Botelho et al[12], Araújo et al[66], Benelli et al[67], Costa et al[68], Gomes et al[38], this study showed *p*-Cymene instead of carvacrol as being the second major component.

Environmental factors such as the climate, fertility, temperature, luminosity, and latitude, as well as the age of the plant and harvest time have direct influences on agronomic responses and in their chemical constitution, which include EOs. Thus, knowledge of these factors subsidises the decisions of agronomic practices, favouring the content and yield of essential oil content at the moment of harvest[8,69].

The northeast of Brazil (56.92%) had the highest origin of *L. sidoides* for EO extraction, followed by the southeast (20.00%), north (6.15%), and central west (1.53%). Commercial oil of *L. sidoides* was found in 17.46% of the articles cited.

As shown in **Table 2**, thymol and *p*-Cymene were the predominant components in LSEO. Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol occurring in EOs isolated from plants belonging to the Lamiaceae family (*Thymus*, *Ocimum*, *Origanum*, and *Monarda genera*) and other plants such as those belonging to the Verbenaceae, Scrophulariaceae, Ranunculaceae, and Apiaceae families. Thymol is recognised for its positive antioxidant, anti-inflammatory, local anaesthetic, cicatrizing, antiseptic, antibacterial, and antifungal properties, as well as for its beneficial effects on the cardiovascular system[70].

The component *p*-Cymene [1-methyl-4-(1-methylethyl)-benzene] is a monoterpene found in over 100 plant species, which is used in medicine and alimentation. It shows a range of biological activities, including antioxidant, anti-inflammatory, anxiolytic, anticancer, and antimicrobial effects. This last property has been widely investigated due to the urgent need for new substances with antimicrobial properties to be used as a treatment for communicable diseases whose diffusion in developed countries has been facilitated by globalization and the evolution of antimicrobial resistance[71].

The third most common component was caryophyllene, a synonym of E-Caryophyllene, β-Caryophyllene, and *Trans*-Caryophyllene. It is a natural bicyclic sesquiterpene compound existing in the EO of many plants and has exhibited a wide range of biological activities[72].

Timol presented an average presence in the analyzed EOs of 61.86%, with a standard deviation of 21.61%, which shows heterogeneous results among the researched articles.

The other chemical compounds were found in lower average percentages as shown in **Table 3**.

**Table 3.** Statistical analysis of the main chemical components of *L. sidoides* essential oil

| | Mean (%) | Standard Deviation | Median (%) | Absolute Frequency | Relative Frequency |
|---|---|---|---|---|---|
| **Thymol** | 61.86 | 21.61 | 68.80 | *59.65%; 69.91% = 3 | *59.65%; 69.91% = 4.28% |
| ***p*-Cymene** | 10.76 | 6.66 | 8.44 | 5.33% = 3 | 5.33% = 5.08% |
| **Caryophillene** | 6.30 | 4.91 | 5.74 | *4.04%; 10.6% = 3 | *4.04%; 10.6% = 5.66% |
| **γ-Terpinene** | 3.96 | 3.96 | 2.34 | *2.0%; 2.34%; 3.6%; 3.83%; 6.6% = 2 | *2.0%; 2.34%; 3.6%; 3.83%; 6.6% = 3.92% |
| **Myrcene** | 2.11 | 1.51 | 1.63 | 3.57% = 3 | 3.57% = 9.64% |

*Each one

The differences in the chemical composition of EOs are determined by genetic factors, however, others factors can lead to significant changes in production of secondary metabolites. The secondary metabolites represent an interface between plants and the environment. The stimuli arising from the environment, in which the plant is found, can redirect the metabolic route, causing the biosynthesis of different compounds. Among these factors, we can highlight the plant/microorganisms, plant/insects and plant/plant; age and stage of development, abiotic factors such as luminosity, temperature, rainfall, nutrition, time and time of collection, as well as techniques for harvest and post-harvest[73].

## CONCLUSION

The difference in the chemical composition of *L. sidoides* essential oil described in this paper highlights the need for further studies to assess the variation in the chemical composition of this plant in different environments. The broth microdilution method with the results expressed as MIC was the most frequently used experimental method to determine antimicrobial activity, and *Staphylococcus aureus* was the most frequently tested bacteria. Unfortunately, few studies have been published showing the antibacterial activity of the LSEO, which has shown promise against most of the evaluated bacteria. To analyse it is important to standardize the concentration measurements.

### CONFLICT OF INTEREST
The Authors declare that there is no conflict of interest.

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