Chemical Composition, Antimicrobial and Antioxidant Activities of Essential Oils of Four Species of the Lamiaceae Family

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Authors' contributions

This work was carried out in collaboration among all authors. Authors DP and LAL designed the study and wrote the first draft of the manuscript. Authors DP, LAL, CB and MTF performed the experiments. Authors DP, LAL, CB, MTF and PJBO participated in laboratory analysis. Authors DP, LAL and CB managed the analyses of the study. Authors DP, LAL, CB, MTF and PJBO managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The biological properties of essential oils represent possible therapeutic alternatives, with antimicrobial and antioxidant activities, and application in many areas of the industry. The objective was to determine the yield, chemical composition, antibacterial and antioxidant activities of the essential oils of *Lavandula angustifolia*, *Pogostemon cablin, Rosmarinus officinalis*, and *Thymus vulgaris* against *Staphylococcus aureus*, *Salmonella enteritidis*, *Escherichia coli* and *Pseudomonas aeruginosa*.
Place and Duration of Study: The experiment was conducted at the microbiology laboratory of the Federal University of Technology - Paraná, Brazil, in the period between June 2016 to May 2017.

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Methodology: The essential oils were analyzed by gas chromatography coupled to mass spectrometry. The antibacterial activity was determined by microdilution in broth, showing minimum inhibitory concentration and minimum bactericidal concentration. The antioxidant activity was evaluated by scavenging of 2,2-diphenyl-1-picryl hydrazyl radical (DPPH).

Results: The average yields of essential oils from *L. angustifolia*, *P. cablin, R. officinalis*,and T. vulgaris were (%) 0.85; 2.0; 1.20, and 1.19, respectively. The major components of lavender essential oil were linalyl acetate (40.1%) and linalool (35.2%); for *P. cablin* - patchoulol (31.5%), seichelene (13.6%) and α-bulnesene (15.6%); for rosemary - camphor (32.5%), 1.8-cineole (13.6%) and α-pinene (9.8); for *T. vulgaris* - thymol (47%), o-scimene (21.6%), and carvacrol (11.4%). Thyme oil showed the best results for antibacterial activity, and low values (0.195 µL mL^{-1}) of minimum inhibitory concentration were needed to inhibit *S. aureus* and *S. enteritidis*, and for *L. angustifolia, P. cablin,* and *R. officinalis* higher concentrations of essential oil were required. The essential oils of *P. cablin* and *T. vulgaris* had the strongest antioxidant properties (12.08 and 10.2 µmol trolox mL^{-1}).

Conclusion: The essential oils evaluated have an inhibitory effect on the microorganisms under study and also interesting antioxidant activity, which could be used by medicine to control bacterial infections, with potential application as natural food preservatives and as nutraceuticals.

1. INTRODUCTION

Bacterial contamination is a well-known public health problem. Microorganisms such as *Staphylococcus* sp, *Escherichia coli* and *Salmonella sp* are responsible respectively for 7%, 5% and 28% bacterial contaminations in food. As control measures for microorganisms are traditionally used, products based on synthetic, which compromises their acceptance and effectiveness, due to the ability of microorganisms to adapt to develop resistance to antibiotics [1].

Considering the possible effects of essential oils as antifungal and antibacterial agents and the growing demand for natural products and alternative ways to combat resistant bacteria [2], essential oils and herbal extracts are gaining much recognition as a potential source of natural resources, safer and bioactive antioxidants [3]. Among essential oils that are widely used in the food, pharmaceutical and cosmetic industries, compounds from *L. angustifolia*, *P. cablin, R. officinalis*, and *T. vulgaris* of the family Lamiaceae, are also used as flavoring and preservative substances. The bioactivities reported include antioxidant, bactericidal, fungicidal and insecticidal bioactivities [4,5].

Spice and aromatic plants are rich in compounds with antioxidant properties such as vitamins (E and C), enzymes and phenolic compounds, and these compounds could be used in functional foods, pharmaceuticals, plant products and food preservation, as they are easy to find and non-toxic species, with great potential for use in the food industry [6].

Biological activity of essential oils depends on their chemical composition which is determined by the genotype and influenced by environmental and agronomic conditions [7]. Species of the Lamiaceae family are widely cultivated in Brazil, but there is little research on the chemical composition and biological properties of essential oils.

Based on the hypothesis that the chemical composition of the essential oils interferes in the cytoplasmic membrane of the bacterium, generating an increase of the permeability and that larger concentrations of essential oil have greater antibacterial activity [4]. The objective was to evaluate the yield, chemical composition, antioxidant activity and bioactivity of essential oils *L. angustifólia* L., *P. cablin, R. officinalis* L., and *T. vulgaris* L., as antibacterial agents against strains of *S. enteritidis*, *S. aureus*, *P. aeruginosa* and *E. coli*. Research on the chemical composition, antibacterial and antioxidant properties of essential oil could contribute to access to new technologies and natural antimicrobial products with applications in the food, pharmaceutical and cosmetics industries.

2. MATERIALS AND METHODS

2.1 Extraction of Essential Oil

Samples of two-year-old plants of *L. angustifólia*, *P. cablin, R. officinalis*, and *T. vulgaris* L., were collected in February 2016, in the medicinal plant garden of the Federal University of Technology (UTFPR) (latitude, 25242′S; longitude, 53206′W; average altitude, 520 m) - Paraná, Brazil. The essential oil was extracted from the fresh leaves and from flowers of L. angustifólia, using the hydrodistillation method and a Clevenger device modified for 3 h [8]. A voucher specimen of *L. angustifólia*, *P. cablin, R. officinalis*, and *T. vulgaris* L., were deposited at the UTFPR Herbarium (code number DVPR 5513, DVPR 5514, DVPR 5510, and DVPR 5512, respectively).

2.2 Essential oil Analysis

A sample of essential oil was diluted in chloroform (1%) and then 1 µL of each solution was injected in to split-mode gas chromatography (1:50). High resolution gas chromatography was performed using GC Agilent Technologies 7820 A apparatus, equipped with the split-splitless injector attached to a HP-5 column (30 m × 0.32 mm), with a film thickness of 0.25 µm (Agilent), and fitted to a flame-ionization detector (FID). The carrier gas was H_2 (3 mL min^{-1}). Temperatures were set as follows: injector at 240 °C (split: 1/30), FID detector at 250 °C, while the column temperature was linearly programmed starting from 70 °C at 0 min and reaching 200 °C at an increasing rate of 3°C min^{-1}. Data acquisition software used was the EZChrom Elite Compact (Agilent).

The GC–MS was performed on HPG 1800 C Series II GCD analytical system equipped with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm). Carrier gas was He (1 mL min^{-1}). Other chromatographic conditions were the same as those for GC-FID. The transfer line was heated at 260 °C. All mass spectra were acquired in electron impact (EI) mode with an ionization voltage of 70 eV, in a range of m/z 40–450 [9].

2.3 Identification of the Compounds

The compounds were identified by comparing the mass spectra fragmentation patterns with those of a computer library [10,11], and the linear retention indices (RI), based on a homologous series of C8–C32 n-alkanes, compared with those of authentic products included in the laboratory database, and/or literature data [10]. Relative amounts of individual components were calculated based on the GC peak areas without FID response factor correction.

2.4 Bacterial Strains

The strains of *S. aureus* INCQS 00015; *E. coli* INCQS 00033; *P. aeruginosa* INCQS 00025; *S. enteritidis* INCQS 00035 were provided by the Oswaldo Cruz Foundation in Rio de Janeiro - Brazil, and were kept in Mueller Hinton agar in microbiological refrigerator at temperature of 2 to 8°C. Petri® dishes were prepared with Mueller Hinton agar of each bacterial strain tested and incubated at 37°C in a microbiological incubator for 24 hours. The strains were inoculated with Mueller Hinton broth and taken to a Shaker-type orbital homogenizer at 100 rpm at 37°C for 12 hours [12].

2.5 Determination of Minimum Inhibitory Concentration (MIC)

Antibacterial activity analyses were performed in triplicate, at the microbiology laboratory of the Federal Technological University of Paraná, Brazil. The sensitivity of the bacterial strains to the essential oils was determined in vitro by microdilution in broth, standardized according to the Clinical and Laboratory Standards Institute [9] - M07-A6 volume 23, number 2, adjusting the inoculum to the 0.5 MacFarland scale (2x108 cfu/mL) and 625 nm wavelength [13].

The oils (400 µL) were initially emulsified with 20 µL of polysorbate 80 in sterile Eppendorf-type tube, also adding 580 µL of Mueller Hinton broth. Subsequent to the dilutions, 100 µL of Mueller Hinton broth were distributed in the wells of the 96-well round bottom cell culture microplate. Then we added 200 µL of the emulsified oil dilution in the column named A of the microplate and performed the serial dilutions. After that, we inoculated 100 µL of the already standardized microorganisms [13].

We also used positive control with ampicillin at a concentration of 12 µg mL^{-1} and in serial dilutions against all bacterial strains under study. As negative control, we incubated pure Mueller Hinton broth and polysorbate 80.

The plates were placed in a bacteriological incubator at 37°C for 24 hours. We added 20 µL of 2,3,5-triphenyltetrazolium chloride (TTC) aqueous solution at 0.5% (m/v) with 1-hour

incubation for readings of minimum inhibitory concentrations, taking into consideration the lowest concentration of essential oil that inhibited bacterial growth, through observation of dye color [14].

2.6 Determination of Minimum Bactericidal Concentration (MBC)

For determination of minimum bactericidal concentration (MBC), we used the minimum inhibitory concentration (MIC), collecting 10 µL individually from the wells in which bacterial growth was not observed, from the MIC well and a well following this one (with bacterial growth), seeding on Mueller Hinton agar plate with the addition of 100 µL of Mueller Hinton broth and spreading with Drigalski loop. The plates were incubated at 37°C for 24 h for subsequent reading of MBCs, considering the bacterial growth on the plates as determining factor for MBC [13].

2.7 Antioxidant Activity

Antioxidant activities of essential oils were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) spectrophotometric method, which is based on the capture of the DPPH radical by antioxidants, leading to decreased absorbance at 517 nm [15]. DPPH radical scavenging assays were performed as described by Brand-Williams et al. [15] with the modifications reported by Mensor et al. [16]. Briefly, 0.5 µM DPPH solution was prepared by dissolving 0.0232 g of DPPH in 100 mL flat-bottomed flask containing ethanol, and was protected from light by wrapping in opaque paper. The 2000 µM trolox standard curve was then prepared by dissolving 0.025 g of troloxin ethanol in 50 mL flat-bottom flasks.

Subsequently, 500 µL aliquots of trolox were added to 3 mL aliquots of ethanol and containing 300 µL of 0.5 µM DPPH solution. Blank solutions were prepared by adding 500 µL aliquots of trolox dilutions to 3.3 mL aliquots of ethanol. Control solutions contained 300 µL of 0.5 µM DPPH solution and 3.5 mL of ethanol.

Absorbance values of analytes were determined in triplicate at 517 nm after storage in the dark for 30 min.

2.8 Data Analysis

The MIC and MCB values were calculated from the arithmetic mean of the triplicates on each plate and expressed as mean ± standard deviation and the results of antioxidant activity were submitted to an analysis of variance paired with the F test *(P <0.01)*. The variances of the treatments were tested for homogeneity by the Bartlett test and the means were compared by the Tukey test at 5% probability using Statistical Analysis System® software [17].

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Essential Oil

The average yield of *L. angustifolia* essential oil was 0.85% (Table 1) and is in accordance with Blažeković et al. [18]. Results obtained by the chromatographic analysis of *L. angustifolia* revealed the presence of 13 compounds (Table 2), which were classified as mono (47.5%), sesquiterpenes (3.2%), and esters (41.8%). The major components were linalyl acetate (40.1%) and linalool (35.2%). According to International Organization for Standardization (ISO 3515) [23], for the *L. angustifolia* chromatographic standard, the essential oil must have a minimum of 25% of linalool and linalyl acetate, 2% of lavandulyl acetate and terpineol, and 4% of cis-β-ocimene. The oil evaluated in the present study presented 40.1% of linalyl acetate and 35.2% of linalool, being in accordance with the standards required by the legislation. Linalyl acetate, linalool, 1.8-cineole and camphor are the compounds that strongly influence the aroma and quality of the essential oil of *L. angustifolia* [24]. The high content of linalyl acetate and linalool and the low content of 1,8-cineole (2.8%) and camphor (4.7%) resulted in essential oil of quality and with a very pleasant aroma.

Table 1. Average yield of *L. angustifolia, P. glabin, R. officinalis* and *T. vulgaris* essential oil compared to the average cited in the literature

Essential oils	Yield (%)	Yield cited in literature (%)
L. angustifolia	0.85	0.90 [18]
P. glabin	2.00	1.50 to 3.50 [19]
R. officinalis	1.20	0.75 [20] and 3.33 [21]
T. vulgaris	1.19	1.00 [22]

Table 2. Chemical components identified in the essential oil extracted from flowers of
Lavandula angustifólia

Compounds[a]	RI[b]	Composition (%)	Identified method[c]
Monoterpenes			
1,8-cineole	1035	2.8	RI; MS
Camphor	1133	4.7	RI; MS
E-β-ocimene	1050	1.2	RI; MS
Linalool	1102	35.2	RI; MS
Myrcene	1008	0.5	RI; MS
p-cimene	1025	0.4	RI; MS
Z-β-ocimene	1042	1.3	RI; MS
α-phellandrene	1008	0.5	RI; MS
Limonene	1033	0.9	RI; MS
Sesquiterpenes			
β-caryophyllene	1411	1.7	RI; MS
β-elemene	1389	0.6	RI; MS
Esters			
Lavandulyl acetate	1292	1.8	RI; MS
Linalyl acetate	1256	40.1	RI; MS
Monoterpenes		47.5	
Sesquiterpenes		3.2	
Esters		41.8	
Others		6.8	
Total identified		93.1	

Note: [a]Results obtained using the CG-FID chromatogram; [b]Retention index relative to n-alkanes on HP – 5 ms column; [c]Identification methods: Retention index (RI), Gas chromatography – mass spectrophotometry (GC – MS), Comparison of retention time and mass with standards and known essential oils.

The average yield of *P.cablin* essential oil was 2.0% (Table 1) and is in accordance with the average yield cited by Blank et al. [19] for this species. Chemical analyses of essential oil revealed the presence of 14 compounds, which were classified as mono and sesquiterpenes. The major components were patchoulol (31.5%), seichelene (13.6%) and α-bulnesene (15.6%; Table 3). Patchoulol is the major component of *P.cablin* essential oil and is important for the duration of the essential oil odor, is used as an indicator of quality [25], can state that the essential oil is of quality and meets the commercial standards. According to International Organization for Standardization (ISO 3757) [26], chromatographic standards of *P.cablin* essential oil must contain at least 1.8% β-patchulene, traces of copaene, 11% α-guianene, 2% β-caryophyllene, 13% bulnesene, 27% patchoulol and 1% pogostol. In comparisons with the standard, our sample oils contained various concentrations of seichelene (13.6%), α-bulnesene (15.6%) and patchoulol (31.5%).

The average yield of rosemary essential oil was 1.20% (Table 1) and is in accordance with [20, 21]. The major components were camphor (32.5%), 1.8-cineole (13.6%) and α-pinene (9.8%; (Table 4). When comparing the essential oil of *R. officinalis* to the Brazilian

Pharmacopoeia [27], it was observed that the content of 32.5% camphor content was above the required minimum of 5%, the other components were in acceptable concentrations according to the chromatographic standards of the legislation. Camphor is of great commercial importance, with applications in the cosmetic, pharmaceutical and food industries, due to its antioxidant, antimicrobial and antifungal properties [6].

For *T. vulgaris* essential oil, an average yield of 1.19% was obtained (Table1). This yield is in accordance with to International Organization for Standardization (ISO 6754) [22]. The major components of the essential oil were thymol (47%), o-scimene (21.6%), and carvacrol (11.4%) (Table 5). The composition of the essential oil was in agreement with the components predicted in the Brazilian Pharmacopoeia [27]. The concentration of 47% thymol was adequate with that mentioned by Borugă et al. [28] which obtained a concentration of 47.59% thymol. This component is of great commercial importance, considered a quality parameter and the antimicrobial activities of *Thymus vulgaris* oil is mostly believed to be related to the thymol and carvacrol contents of the oil [29].

**Table 3. Chemical components identified in the essential oil extracted from aerial parts of
*P. glabin***

Compounds[a]	RI[b]	Composition (%)	Identified method*
Monoterpenes			
Patchoulol	1645	31.5	RI; MS
Seychellene	1435	13.6	RI; MS
α-Pinene	973	0.1	RI; MS
β-Pinene	997	0.3	RI; MS
Sesquiterpenes			
β-Patchoulene	1370	2.2	RI; MS
β-Elemene	1388	0.9	RI; MS
Copaene	1396	0.7	RI; MS
β-Caryophyllene	1411	3.3	RI; MS
α-Guaiene	1429	7.2	RI; MS
α-Humulene	1444	5.7	RI; MS
α-Patchoulene	1447	3.0	RI; MS
β-Guaiene	1495	2.9	RI; MS
α-Bulnesene	1502	15.6	RI; MS
Spathulenol	1666	2.2	RI; MS
Monoterpenes		28.6	
Sesquiterpenes		71.4	
Others		10.9	
Total identified		100	

Note: [a]Results obtained using the CG-FID chromatogram; [b]Retention index relative to n-alkanes on HP – 5 ms column;
[c]Identification methods: Retention index (RI), Gas chromatography – mass spectrophotometry (GC – MS), Comparison
of retention time and mass with standards and known essential oils

**Table 4. Chemical components identified in the essential oil extracted from aerial parts of
*R. officinalis***

Compounds[a]	RI[b]	Composition (%)	Identified method[c]
Monoterpenes			
1.8-cineole	1035	13.6	RI; MS
Borneole	1161	0.7	RI; MS
Camphene	981	7.9	RI; MS
Camphor	1133	32.5	RI; MS
Linalool	1102	4.6	RI; MS
Myrcene	1008	1.2	RI; MS
Terpinolene	1082	1.5	RI; MS
α-pinene	973	9.8	RI; MS
α-terpineol	1198	3.2	RI; MS
β-pinene	997	2.1	RI; MS
y-Terpinene	1057	1.0	RI; MS
Limonene	1033	3.4	RI; MS
Sesquiterpenes			
β-caryophyllene	1411	0.6	RI; MS
Esters			
3-octanone	1006	8.6	RI; MS
Bornyl acetate	1279	2.1	RI; MS
Monoterpenes		81.2	
Sesquiterpenes		0.6	
Esters		10.7	
Others		7.6	
Total identified		100	

Note: [a]Results obtained using the CG-FID chromatogram; [b]Retention index relative to n-alkanes on HP – 5 ms column;
[c]Identification methods: Retention index (RI), Gas chromatography – mass spectrophotometry (GC – MS), Comparison
of retention time and mass with standards and known essential oils

The biological activity of essential oils depends on their chemical composition, determined by the genotype, harvesting time, the collection location, environmental conditions, drying method, essential oil extraction method, and the growth stage [30].

3.2 Antibacterial Activity

The antibacterial activity of the essential oils of *R. officinalis*, *L. angustifolia*, *T. vulgaris* and *P. glabin* were evaluated against the strains of *S. aureus*, *S. enteritidis*, *P. aeruginosa* and *E. coli* in vitro (Table 6). The data presented summarize the results of the microdilutions, showing that against *S. aureus* the oils of *R. officinalis*, and *T. vulgaris* presented bactericidal effect already at low concentrations (0.195 to 6.250 µL mL^{-1}).

We observed that the highest MIC was obtained with the essential oil of *R. officinalis* against *S. enteritidis*, and *P. aeruginosa*. The antibacterial activity was similar to Teixeira et al. [31] who obtained MIC of 90.8 mg mL^{-1} against *B. thermosphacta* and *S. Typhimurium* for that same species.

The oils of *L. angustifolia* and *P. cablin* showed bactericidal effect, but higher concentrations of essential oil were needed to inhibit the microorganisms evaluated (Table 5). Another point refers to *P. aeruginosa*, which was not inhibited by the oils of *L. angustifolia* and *P. cablin,* this high concentration that could be required for the inhibition of this microorganism with these essential oil could be justified by the possible versatility of this bacterium. Citing the existence of intrinsic resistance genes that may confer low permeability of the bacterial cell wall, mechanism through which possibly the oils perform their effect [32]. The results of the antimicrobial activity of lavender essential oil were similar to those obtained by Blažeković et al. [18], where the highest concentrations of essential oil were needed to control *P. aeruginosa*.

Table 5. Chemical components identified in the essential oil extracted from aerial parts of *Thymus vulgaris*

Compound [a]	RI [b]	Composition (%)	Identified method [c]
Monoterpenes			
1,8-cineole	1035	0,6	RI; MS
Camphene	981	2,1	RI; MS
Carvacrol	1322	11,4	RI; MS
Linalool	1102	1,4	RI; MS
Myrcene	1008	0,5	RI; MS
O-cimene	1030	21,6	RI; MS
Terpinolene	1082	3,3	RI; MS
Thymol	1308	47	RI; MS
Thymol methyl ether	1193	1,2	RI; MS
α-pinene	973	1,8	RI; MS
α-terpinene	1015	0,4	RI; MS
α-thujone	967	0,2	RI; MS
β-pinene	997	0,6	RI; MS
Terpinene	1057	1,4	RI; MS
Limonene	1033	1,8	
Isoborneol	1170	0,9	RI; MS
Sesquiterpenes			
β-caryophyllene	1411	0,6	RI; MS
Monoterpenes		96,2	
Sesquiterpenes		0,6	
Others*		2,3	
Total identified		99,1	

Note: [a]Results obtained using the CG-FID chromatogram; [b]Retention index relative to n-alkanes on HP – 5 ms column; [c]Identification methods: Retention index (RI), Gas chromatography – mass spectrophotometry (GC – MS), Comparison of retention time and mass with standards and known essential oils

Table 6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils of
***R. officinalis*, *T. vulgaris*, *L. angustifól*ia, and *P. cablin* against strains of *S. aureus*, *S. enteritidis*, *E. coli* and *P. aeruginosa**

Essential oils	Microorganisms							
	S. aureus		*S. enteritidis*		*E. coli*		*P. aeruginosa*	
	MIC and MBC ((µL mL-1)							
R. officinalis	6.25±0.2*	12.5 ±0.1	50 ±0.1	50 ±0.1	12.5±0.1	25±0.3	200±0.3	400±0.6
T. vulgaris	0.195±0.1	1.56 ±0.1	0.195 ±0.1	50 ±0.1	0.39±0.2	6.25±0.2	0.78±0.2	12.5±0.4
L. angustifólia	50±0.2	100 ±0.4	25 ±0.3	50±0.1	25 ±0.4	50 ±0.5	R**	R**
P. cablin	10.5±0.3	25±0.3	25±0.1	50±0.1	25±0.1	25±0.2	R**	R**
Ampicillin	12.5±0.1	25±0.3	12.5±0.1	25±0.2	12.5±0.1	25±0.1	50±0.2	100±0.5
Polysorbate 80	N.I.***	N.I.***	N.I.***	N.I.***	N.I.***	N.I.***	N.I.***	N.I.***

*Note: *Tests performed in triplicate and mean ± standard deviations;**R: Resistant at the concentrations tested; ***N.I.: no inhibition of bacterial growth*

On the other hand, lower concentrations of essential oil from thyme were needed to inhibit the growth of some important human pathogens such as *P. aeruginosa*, as well as bacteria that causing food-borne diseases, such as *S. aureus*, *S. enteritidis*, and *E. coli* which are also the most common cause of microbial contamination of food and cosmetic products [33].

By comparing the MICs and MBCs of the essential oils used as treatments and the positive control (ampicillin), we observed that the oil of *T. vulgaris* showed higher bactericidal effect than ampicillin, considering that all bacteria under study were inhibited at lower concentrations when treated with this essential oil. This fact could be associated with the composition of the oil, which presented as major component 47% of the monoterpene thymol. Most of the antimicrobial activities of *Thymus vulgaris* oil could be associated with the phenolic compounds thymol and carvacrol [29]. These results agree with those reported by other authors [31,34].

The MIC results obtained with the essential oil of thyme are in agreement with Fani et al. [25] who obtained MIC 1.9 ± 0.2 μL mL^{-1} for *Thymus vulgaris* against strains of *Streptococcus pyogenes*. According to Kryvtsova et al. [34] the highest antimicrobial activity was registered against the typical and clinic strains of *Staphylococcus aureus* and microscopic Candida genus fungi.

We observed that gram-positive bacteria were more sensitive to the effects of the essential oils, when compared with the gram-negative bacteria, because the essential oils tested showed activity against *S. aureus* at low concentrations. According to Imelouane et al. [35], greater susceptibility of gram negatives against *Thymus vulgaris* oil than the gram positive bacteria. The greater resistance of gram negatives might be associated with the presence of an outer membrane hydrophilic lipopolysaccharide, which inhibits accumulation of hydrophobic plant essential oil on the cell membrane [29].

The ways the microorganisms are inhibited by the essential oils seem to be distinguished by the mode of action and also to a variation in the penetration rate of the essential oil constituent through the cell wall and cell membrane structures [36]. According to Silva et al. [37] essential oils act on bacterial cell membranes, impairing their structure and function, increasing their fluidity and permeability and, thus, inducing the leakage of intracellular materials, leading to cell damage and death. [38] state that the mechanism of action of essential oils and their constituents is not fully elucidated by the fact that in essential oils there are many phytochemical compounds and their antibacterial activity could not be attributed to a specific mechanism, but there are probably different targets in the bacterial cell.

3.3 Antioxidant Activity

The essential oils of *P. cablin* and *T. vulgaris* showed antioxidant activity superior to *L. angustifólia* and *R. officinalis* (Table 7). The antioxidant activity of the *P. cablin* could be attributed to the major components of essential oil, mainly monoterpenes (patchoulol and seychellene) and sesquiterpenes (α-bulnesene and α-guaiene) [39]. The result of the antioxidant activity of *P. cablin* oil corroborates with [40].

Table 7. Antioxidant activity of essential oils of *P. cablin*, *T. vulgaris*, *R. officinalis* and *L. angustifolia* by the DDPH method

Essential oils	DDPH (μmol trolox mL^{-1}
P. cablin	12.08 a*
T. vulgaris	10.2 a
R. officinalis	4.71 b
L. angustifolia	3.09 c
Mean	7.52
C.V. (%)	10.3

*Note. *Means followed by the same letter in the column do not differ significantly by Tukey test, at P=0.05; C.V.: Coefficient of variance*

The significant antioxidant activity of the *T. vulgaris* could be attributed to the major components of essential oil, mainly monoterpenes (thymol 47%; O-cimene 21.6% and carvacrol 11.4%) results according to Barakat and Abdel-Rahman [41].

On the other hand, [42] report that the antioxidant activity of essential oils does not depend only on the presence of major compounds, but could be due to the sum or the synergistic effect of each compound present in the essential oil.

The antioxidant activity of the *R. officinalis* could be attributed to the major components of essential oil, mainly monoterpenes (camphor 32.5%; 1.8 cineole 13.6% and α-pinene 9.8%) The result of the antioxidant activity of

R. officinalis oil corroborates with data from the literature [6].

The essential oil of *L. angustifolia* has interesting antioxidant activity. The antioxidant activity of *L. angustifolia* oil does not depend only on the presence of major compounds such as linalool and linalyl acetate, but could be due to the sum or the synergistic effect of each compound present in the essential oil [18].The result of the antioxidant activity of *L. angustifólia* oil corroborates with Kokina et al. [43].

The results of the antioxidant activity of the essential oils evaluated can be of significant importance for the food and pharmaceutical industries, with monoterpenes being frequently used as flavoring and adjuvant substances in food and drugs [42].

4. CONCLUSION

The species evaluated in the Lamiaceae family showed essential oil yield within the average cited in the literature. The essential oils presented terpenes as the major composition, classified as monoterpenes, sesquiterpenes and esters. The major components of essential oils were linalyl acetate, linalool and camphor for *L. angustifolia*; patchoulol, seichelene, and α-bulnesene for *P. cablin*; camphor, 1.8-cineole and α-pinene for *R. officinalis*; thimol, o-scimene, and carvacrol for *T. vulgaris*. The essential oil of *T. vulgaris* presented the best results when evaluated its antibacterial activities against *S. aureus*, *E. coli* and *S. enteritidis*, where lower amounts of essential oil were needed to strongly inhibit the growth of bacteria, being an important alternative for medicine in the fight against bacterial infections. The essential oils of *P. glabin* and Thyme had the greatest antioxidant activities. The results confirm the potential applicability of natural substances of plant origin as antibacterials and antioxidants, with potential application in many areas, as natural food preservatives and as nutraceuticals.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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