ARTIGO ORIGINAL

Chemical composition and antibacterial activity of essential oils

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ABSTRACT: Essential oils have been used as a natural alternative in the prevention and treatment of diseases that affect human health. The aim of this work was to determine the chemical composition and antibacterial activity of the essential oils of *Lavandula angustifolia, Pogostemon cablin, Rosmarinus officinalis, Thymus vulgaris, Hedyosmun brasiliense, Psidium guajava, Baccharis dracunculifolia* and *Schinus terebinthifolius* against bacteria *Staphylococcus aureus, Salmonella enteritidis, Escherichia coli* and *Pseudomonas aeruginosa*. 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 (MIC) and minimum bactericidal concentration (MBC). The chemical composition of the evaluated oils was based on monoterpenes and sesquiterpenes. The essential oil of *Thymus vulgaris* was highlighted with MIC of 0.195 and MBC of 1.56 µl/ml against *S. aureus*, 0.195 and 50 µl/ml for *S. enteritidis*; 0.390 µl/ml and 0.780 µl/ml for *E. coli* and 0.780 and 12.5 µl/ml against *P. aeruginosa*, respectively. The variability of specific components of the essential oils is very interesting and of economic importance, due to the therapeutic and aromatizing potential of these oils. The oils of *L. angustifólia, T. vulgaris, R. officinalis, B. dracunculifolia, P. guajava* and *S. terebinthifolius* showed activity *in vitro* against *S. aureus*, *E. coli*, *S. enteritidis* and stronger for *P. aeruginosa*. The oil of *Thymus vulgaris* presented the best results when its antibacterial activities were evaluated. The evaluated oils had an inhibitory effect on the microorganisms studied and the gram-positive bacteria were more sensitive to the effects of the essential oils when compared to the gram-negative bacteria. The essential oils evaluated in this study represent an important alternative for controlling bacterial infections.

Keywords: Lamiaceae, essential oil, bacteria, minimum inhibitory concentration, minimum bactericidal concentration.

RESUMO: Composição química e atividade antibacteriana de óleos essenciais. Os óleos essenciais vêm sendo utilizados como alternativa natural na prevenção e tratamentos de enfermidades que afetam a saúde humana. Objetivou-se determinar a composição química e a atividade antibacteriana dos óleos essenciais de *Lavandula angustifolia, Pogostemon cablin, Rosmarinus officinalis, Thymus vulgaris, Hedyosmun brasiliense, Psidium guajava, Baccharis dracunculifolia* e *Schinus terebinthifolius* contra as bactérias *Staphylococcus aureus, Salmonella enteritidis, Escherichia coli* e *Pseudomonas aeruginosa.* Os óleos essenciais foram analisados por cromatografia gasosa acoplada a espectrometria de massa. A atividade antibacteriana foi determinada por microdiluição em caldo, onde determinou-se a concentração inibitória mínima (CIM) e concentração bactericida mínima (CBM). A composição química dos óleos avaliados foi baseada em monoterpenos e sesquiterpenos. O óleo essencial de *Thymus vulgaris* destacou-se com CIM de 0,195 e MBC de 1,56 µl/ml contra *S. aureus*, 0,195 e 50 µl/mL para *S. enteritidis*; 0,390 µl/ml e 0,780 µl/ml para *E. coli* e 0,780 e 12,5 µl/ml contra *P. aeruginosa*, respectivamente. Os resultados da variabilidade em termos de componentes específicos dos óleos essenciais são muito interessantes e de importância econômica, devido ao potencial terapêutico e aromatizante desses óleos. Os óleos de *L. angustifólia, T. vulgaris, R. officinalis, B. dracunculifolia, P. guajava* e *S. terebinthifolius* mostraram atividade *in vitro* contra *S. aureus, E. coli, S. enteritidis* e mais estritamente para *P. aeruginosa.* O óleo de tomilho apresentou os melhores resultados quando as atividades antibacterianas foram avaliadas. Concluiu-se que os óleos avaliados possuem efeito inibitório sobre os microrganismos em estudo e que as bactérias gram-positivas foram

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mais sensíveis aos efeitos dos óleos essenciais, quando comparadas com as bactérias gram-negativas. Os óleos essenciais avaliados no estudo representam uma importante alternativa no controle às infecções bacterianas.
Palavras-chave: Lamiaceae, óleo essencial, bactérias, concentração inibitória mínima, concentração bactericida mínima.

INTRODUCTION

Bacterial contamination is a worldwide health problem. Microorganisms such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aerugi*nosa, and *Salmonella enteritidis* are responsible for the majority of bacterial contaminations in food. The problem is that many of these strains are resistant to some type of antibiotic and thus become a health hazard (Kariminik et al. 2017).

The medicinal plants are widely used in different areas of health, with antimicrobial, antioxidant, and antifungal activities, due to the diversity of active phytochemicals. In this sense the pharmaceutical and cosmetic industries started to use the herbal compounds in their products. Herbal antimicrobials are considered of global importance because they have low toxicity to humans and are less likely to induce bacterial resistance (Oliveira et al. 2019), being ecologically correct and safer to use.

Considering that Brazil has approximately 20% of the world's plant diversity (Modolo and Foglio 2019), which represents a great potential for obtaining new biomolecules, with antioxidant and antimicrobial properties. It is necessary to evaluate the activity of oils from medicinal, seasoning or aromatic plants of the family Lamiaceae and native. Especially Brazil is rich in plants of this family, however, these are only rarely studied.

The presented work is based on the hypothesis that the antibacterial activity of essential oils has been explained by the possible ability to inhibit bacterial growth through mechanisms related to both structural and functional damage to the cytoplasmic membrane of bacteria (Burt 2004).

The objective of this work was to evaluate the chemical composition and the bioactivity of essential oils of the species of the family Lamiaceae *L. angustifolia* L., *P. cablin* (Blanco) Benth, *R. officinalis* L., *T. vulgaris* L. and native species *H. brasiliense* Mart. Ex Miq., *P. guajava* L., *B. dracunculifolia* DC. and *S. terebinthifolius* Raddi as antibacterial agents against strains of *S. enteritidis*, *S. aureus, P. aeruginosa* and *E. coli.*

MATERIALS AND METHODS

Extraction of essential oils

The essential oils of *R. officinalis*, *B. dracunculifolia*, *H. brasiliense*, *T. vulgaris*, *P. guajava* and *S. terebinthifolius* were acquired in an organic farm, called Harmonia Natural®, located in Santa Catarina - Brazil. The oils were extracted by steam distillation from the leaves of the fresh plants. The distiller has a capacity of 300 liters and the distillation time was 3 h, and plant material was standardized to 10 kg and temperatures below 40 °C to obtain the essential oil.

The essential oils of *L. angustifólia* and *P. cablin* were acquired from the company Chamel®, in Paraná – Brazil. They were obtained by hydro-distillation with a Clevenger device from fresh and milled flowers of *L. angustifólia* and leaves of *P. cablin*. The distillation time was 1 h, and extraction temperatures were below 40 °C, using 10 kg of plant material.

Chemical analyses of the essential oil

A sample of essential oil from each plot was diluted in chloroform (1%) and then 1 µl of each solution was injected into 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 an 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 H2 (3 ml/min). 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. The software used for acquiring the data was 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). The carrier gas was He (1 ml/min). 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 (Nikolic et al. 2014).

Identification of the compounds

The compounds were identified by comparing the mass spectra fragmentation patterns with those of a computer library (Adams 2007; National Institute of Standards and Technology 2010), 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

(Adams 2007). Relative amounts of individual components were calculated based on the GC peak areas without FID response factor correction.

Bacterial strains

The analyses of antibacterial activities were carried out in the microbiology laboratory at the Federal University of Technology - Paraná, Campus Dois Vizinhos, Brazil, in the period from June 2016 to May 2017.

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 a 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 h. The strains were inoculated with Mueller Hinton broth and taken to a Shaker-type orbital homogenizer at 100 rpm at 37 °C for 12 h.

Determination of minimum inhibitory concentration (MIC)

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 (CLSI) - M07-A6 volume 23, number 2, adjusting the inoculum to the 0.5 MacFarland scale ($2x10^8$ cfu/ml) and 625 nm wavelength (Clinical and Laboratory Standards Institute 2003).

The oils in the volume of 400 µl were initially emulsified with 20 µl of polysorbate 80 in a sterile Eppendorf-type tube, also adding 580 µl of Mueller Hinton broth. After 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 (Clinical and Laboratory Standards Institute 2003).

We also used positive control with ampicillin at a concentration of 12 µg/ml 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 identified and placed in a bacteriological incubator at 37 °C for 24 h. 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 (Moussa et al. 2013). The experiment was performed in triplicate.

Determination of minimum bactericidal concentration (MBC)

For the 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 factors for MBC (Clinical and Laboratory Standards Institute 2003).

Statistical analysis

The MIC and MCB values were calculated from the arithmetic mean of the triplicates on each plate. The MIC and MCB values were calculated and expressed as mean ± standard deviation.

RESULTS AND DISCUSSION

Chemical composition of the essential oils

Results obtained by the chromatographic analysis of *R. officinalis, T. vulgaris, L. angustifolia* and *P. cablin* are presented in Table 1. In total, 39 compounds were identified, being 25 monoterpenes, 10 sesquiterpenes and four esters. The monoterpenes were the compounds most frequently present in the essential oils of *T. vulgaris* (96.2%) and *R. officinalis* (81.2%). Referring to the sesquiterpene concentration, the highest concentration was in the oil of *P. cablin*, and in the oil of *L. angustifolia*, the constituents that stood out in these oils were the esters.

The chemical composition of the oils of native species presented as major components 18.8% β-selinene, 16.2% α-selinene, 13.0% α-caryophyllene, 13.9% β caryophyllene. The minor compounds were identified as curzerene, caryophyllene oxide and humulene (Table 2).

The oil of B. dracunculifolia presented as major components 17.6% cis-trans nerolidol, 15.0% gamma-elemene, 10.5% D-limonene, 9.6% β-pinene, 9.8% caryophyllene, and also presenting 10 other compounds such as 1.6% α-caryophyllene and 8.5% spathulenol, which represent the minor components.

For the species *H. brasiliense* the essential oil presented mainly monoterpenes and sesquiterpenes, being 19.59% sabinene, 31.6% germacrene B, 5.67% pinocarvone, in addition to

TABLE 1. Chemical composition of the essential oils of the family Lamiaceae.

		Family Lamiaceae			
Compound		***Rosmarinus officinalis***	***Thymus vulgaris***	***Lavandula angustifólia***	***Pogostemon Cablin***
Monoterpenes	RI[a]	%	%	%	%
1,8-cineole	1035	13.6	0.6	2.8	-
Borneole	1161	0.7	-	-	-
Camphene	981	7.9	2.1	-	-
Camphor	1133	32.5	-	4.7	-
Carvacrol	1322	-	11.4	-	-
E-β-ocimene	1050	-	-	1.2	-
Linalool	1102	4.6	1.4	35.2	-
Myrcene	1008	1.2	0.5	0.5	-
O-cimene	1030	-	21.6	-	-
Patchoulol	1645	-	-	-	31.5
p-cimene	1025	-	-	0.4	-
Seychellene	1435	-	-	-	13.6
Terpinolene	1082	1.2	3.3	-	-
Thymol	1308	-	47	-	-
Thymol methyl ether	1193	-	1.2	-	-
Z-β-ocimene	1042	-	-	1.3	-
α-phellandrene	1008	-	-	0.5	-
α-pinene	973	9.8	1.8	-	0.1
α-terpinene	1015	-	0.4	-	-
α-terpineol	1198	3.2	-	-	-
α-thujone	967	-	0.2	-	-
β-pinene	997	2.1	0.6	-	0.3
Terpinene	1057	1.0	1.4	-	-
Limonene	1033	3.4	1.8	0.9	-
Isoborneol	1170	-	0.9	-	-
Sesquiterpenes					
Copaene	1396	-	-	-	0.7
Espathulenol	1666	-	-	-	2.2
α-bulnesene	1502	-	-	-	15.6
β-caryophyllene	1411	0.6	0.6	1.7	3.3

Continua...

TABLE 1. *Continuação*

Compound		Family Lamiaceae			
		Rosmarinus officinalis	***Thymus vulgaris***	***Lavandula angustifólia***	***Pogostemon Cablin***
α-guaiene	1429	-	-	-	7.2
α-humulene	1444	-	-	-	5.7
α-patchoulene	1447	-	-	-	3.0
β-patchoulene	1370	-	-	-	2.2
β-elemene	1389	-	-	0.6	0.9
β-guaiene	1495	-	-	-	2.9
Esters					
Lavandulyl acetate	1256	-	-	1.8	-
Linalyl acetate	1256	-	-	40.1	-
3-octanone	1006	8.6	-	-	-
Bornyl acetate	1279	2.1	-	-	-
Monoterpenes		81.2	96.2	47.5	45.5
Sesquiterpenes		0.6	0.6	3.2	43.7
Esters		10.7	-	41.8	-
Others*		7.6	2.3	0.5	10.9
Total identified		100	99.1	93.1	100

[a] Retention index *others: percentage of active substances not identified in the oil. Caption: (-) substance not present in the oil;

7.23% eucalyptol, 5.44% α-pinene and compounds at lower concentrations such as spathulenol.

The oil extracted from the leaves of *P. guajava* presented as major components 18.84% β-selinene, 16.16% α-selinene, 13.03% α-caryophyllene, 13.92% β-caryophyllene. The minor compounds were curzerene, caryophyllene oxide, and humulene. We also detected traces of other substances, which complement the composition of the oil.

The oil from the leaves of *S. terebinthifolius* presented in the composition 18.66% α-bergamotene, 11.94% (+)-aromadendrene, 14.04% α-pinene, 14.34% β-caryophyllene, 10.89% α-terpinene, and other minor compounds such as β-pinene and α-caryophyllene that composed the oil.

Antibacterial activity

The antibacterial activity of the essential oils evaluated in vitro showed that against *S. aureus* the oils of *R. officinalis*, *T. vulgaris*, *B. dracunculifolia*, *P. guajava* and *S. terebinthifolius* presented bactericidal effect already at low concentrations (0.19 to 7.25 µl/ml) (Table 3).

We also observed that the oils of *L. angustifolia* and *P. cablin* showed a bactericidal effect, but at concentrations of 50 µl/ml and 12.5 µl/ml, respectively (Table 3). On the other hand, the essential oil of H. brasiliense showed no activity against the bacteria in all concentrations evaluated, which can be justified by the volatilization of compounds, degradation or oxidation of mainly monoterpenic active substances (Xiang et al. 2017) although its composition presents 31.6% germacrene B, 19.59% sabinene, 5.44% α-pinene, 7.23% eucalyptol, 6.41% carotol and other compounds that compose the oil.

TABLE 2. Chemical composition of the essential oils from the native plants.

Compounds		Native species			
		Baccharis dracunculifolia	***Hedyosmum brasiliense***	***Psidium guajava***	***Schinus Terebinthifolius***
Monoterpenes	RT[a]	%	%	%	%
Aromadendrene	23609	1.60	-	-	11.94
Camphor	3343	-	-	7.92	-
D-limonene	6631	10.54	-	-	-
Eucalyptol	6667	-	7.23	-	-
Pinocarvone	1193	-	5.67	-	-
Sabinene	4930	-	19.59	-	-
α-pinene	3934	4.03	5.44	-	14.4
α-terpinene	2705	-	-	-	10.9
α -tujeno	3816	-	-	-	7.96
β-myrcene	5403	1.56	-	-	-
β-pinene	4934	9.57	-	-	3.08
β -selinene	25716	-	-	19.84	
Delta-elemeno	19624	-	-	-	7.31
Sesquifelandrene	27330	-	-	-	7.11
Sesquiterpenes					
Carotol	2981	-	6.41	-	-
Cis;trans_nerolidol	2910	17.58	-	-	-
Caryophyllene	22899	9.76	-	-	-
Curzerene	2622	-	-	4.2	-
Germacrene B	2618	-	31.6	-	-
Germacrene D	2539	8.24	6.55	-	-
Globulol	2940	3.79	-	-	-
Caryophyllene oxide	2903	-	-	6.99	-
Humulene oxide	3031	-	-	5.24	-
Spathulenol	2913	8.49	4.08	-	-
α-amorphene	2629	1.76	-	-	-
α-cadinene	2720	4.04	-	-	-
α-bergamotene	2633	-	-	-	18.66
α-caryophyllene	2434	1.56	-	14.03	4.69
β-caryophyllene	2294	-	-	13.92	14.34

Continua...

TABLE 2. *Continuação*

Compounds		Native species			
		Baccharis dracunculifolia	***Hedyosmum brasiliense***	***Psidium guajava***	***Schinus Terebinthifolius***
α -tujeno					
α-selinene	1616	-	-	16.16	-
gama-elemene	2611	15.03	-	-	-
L-4-terpineol	12542	-	5.89	-	-
Monoterpenes		27.5	37.93	31.96	62.7
Sesquiterpenes		70.25	54.53	60.54	37.69
Others		-	7.55	4.44	-
Total identified		97.7	100	96.94	100

[a] Retention time. Caption: (-) substance not present in the oil; *others: percentage of active substances not identified in the oil.

TABLE 3. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils of *R. officinalis, T. vulgaris, L. angustifólia, P. cablin, H. brasiliense, B. dracunculifolia, P. guajava* and *S. terebinthifolius* against strains of *S. aureus, S. enteritidis, E. coli,* and *P. eruginosa.*

	Microorganisms			
	S. aureus	***S. enteritidis***	***E. coli***	***P. aeruginosa***
Oils	**MIC±DP** **MBC±DP** **µl/ml**	**MIC±DP** **MBC±DP** **µl/ml**	**MIC±DP** **MBC±DP** **µl/ml**	**MIC±DP** **MBC±DP** **µl/ml**
Rosmarinus officinalis	7.25±0.00 12.5±0.00	50±0.00 50±0.00	12.5±0.00 25±0.00	200±0.00 400±0.00
Thymus vulgaris	0.19±0.00 1.6±0.00	0.19±0.00 50±0.00	0.39±0.00 6.25±0.00	0.78±0.00 12.5±0.00
Lavandula angustifólia	50±0.00 100±0.00	25±0.00 50±0.00	25±0.00 50±0.00	R* R*
Pogostemon cablin	12.5±0.00 25±0.00	25±0.00 50±0.00	25±0.00 25±0.00	R* R*
Hedyosmum brasiliense	R* R*	R* R*	R* R*	R* R*
Baccharis dracunculifolia	0.19±0.00 0.39±0.00	R* R*	R* R*	R* R*
Psidium guajava	0.19±0.00 1.6±0.00	R* R*	400.0±0.00 R*	R* R*
Schinus terebinthifolius	0.39±0.00 0.78±0.00	25±0.00 50±0.00	12.5±0.00 50±0.00	100±0.00 200±0.00
Ampicillin (positive control)	12.5±0.00 25±0.00	12.5±0.00 25±0.00	12.5±0.00 25±0.00	50±0.00 100±0.00
Polysorbate 80 (negative control)	N.I.**	N.I.**	N.I.**	N.I.**

Caption: *R: Resistant at the concentrations tested. **N.I.: no inhibition of bacterial growth. Tests performed in triplicate.

DISCUSSION

Chemical composition of the essential oils

When comparing the essential oil of *R. officinalis* to the Brazilian Pharmacopoeia (2017), it was observed that the content of 13.6% of 1.8-cineol was 2.4% below that recommended. In contrast, the 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.

The composition of the essential oil of *R. officinalis* was similar to the components obtained by Fonseca et al. (2015) that verified a higher concentration of monoterpene compounds in *R. officinalis* oil, the majority composition being 28.5% cineol, 27.7% camphor and 21.3% α-pinene.

The composition of the essential oil of *T. vulgaris* was in agreement with the components predicted in the Brazilian Pharmacopoeia (2017). According to Bakkali et al. (2008), *T. vulgaris* oil presented thymol as the main component (45.6%), a value similar to that found in the present study. Thymol showed to have antimicrobial activity against microorganisms such as *Streptococcus* spp., *E. coli* (Packer and Luz 2007). The high percentages of thymol help explain the antimicrobial activity of this essential oil (Ferronatto et al. 2007).

According to ISO 3515 (2002), 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. It was verified that the oil evaluated in this study presented 40.1% of linalin acetate and 35.2% of linalool, being in accordance with the legal standards required.

Analyzing the composition of the oil of *P. cablin* following ISO 3757 (2002), it was verified that the content of 11% of α-guanene was 3.8% lower than recommended, the other components were in adequate concentrations.

When comparing our results of the composition of the *B. dracunculifolia* oil with those determined by Fabiane et al. (2008), β-pinene was 17.8% smaller in this study, while the other contents were similar. Although extracted from the same plant species, the chemical composition of the essential oil can vary significantly depending on factors such as collection time, climatic conditions and soil conditions (Jakiemiu et al. 2010). The essential oil of *H. brasiliense* presented mainly monoterpenes and sequiterpenes, being similar to those reported in the literature by Vido (2009).

For *P. guajava* oil the composition was similar to the components obtained by Silva et al. (2016) with leaves of *P. guajava* harvested at 14:00 h.

Regarding the chemical composition of the oil of *S. terebinthifolius*, observed α-pinene, β-pinene and β-caryophyllene components were similar to those reported in the literature by Moyna et al. (2007) with a composition of 20.89% α -pinene, 5.65% β pinene and 20.69% β-caryophyllene. On the other hand, the aromadendrene constituent (11.94%) had a composition different from the mentioned study (0.76%).

Essential oils have various chemical compositions and effects, and these factors may be related with the plant and its environment. Biotic and abiotic factors including rainfall, luminosity, stage of development, soil nutrition, herbivory, temperature, time of harvest interfere directly with plant metabolism and consequently with the composition of the essential oil extracted (Souza et al. 2017). According to Souza et al. (2017), other factors are directly related with the oil such as extraction method, extraction time and temperature, plant material used, solvents, among others, are also chemical differentiation factors.

Active substances classified as monoterpenes are highly volatile when compared with sesquiterpenes, due to the lower number of carbon chains (Bakkali et al. 2008). According to Bakkali et al. (2008), the complexity of the essential oils' chemical composition and oil-related factors are just a few of the items that can be referred to as factors interfering with the composition and action of these oils. It is not determined if the action shown by the oils is due to synergism between the substances or due to compounds isolatedly.

Antibacterial activity

We observed that the MICs and MBCs of *T. vulgaris* oil were more effective than the positivie control (ampicillin), requiring low concentrations of this oil to inhibit the evaluated bacteria. The antimicrobial activity of *T. vulgaris* essential oil can be explained by the main component, the monoterpene thymol (47%). Although the activity of oils is generally attributed to their major components, synergistic or antagonistic effects may occur among compounds that are present in lower concentrations, with likely contributions of all components to the biological activities exerted by the oil (Wang et al. 2017).

Oils with higher concentrations of phenolic compounds such as thymol and carvacrol are used in industries for a production of oral hygiene products, such as peppermint, spearmint and eucalyptus (Souza et al. 2017). Active substances based on monoterpenes such as linalool, limonene, 1,8-cineole are highlighted against bacteria and so they can be used in liquid soaps or bar soaps with antibacterial and antiseptic properties (Packer and Luz 2007).

P. aeruginosa was not inhibited by the

oils of *L. angustifolia*, *P. cablin*, *H. brasiliense*, *B. dracunculifolia* and *P. guajava*. The high concentration required for the inhibition of this microorganism may be explained by the possible versatility of this bacterium. Citing the existence of intrinsic resistance genes that may confer low permeability of the bacterial cell wall, a mechanism through which possibly the oils perform their effect (Loureiro et al. 2016).

The bacteriostatic and bactericidal effects presented by the tested oils (except for *H. brasiliense*) may have occurred due to their lipophilicity so they eventually accumulate in the lipid bilayer of the plasma membrane of the bacteria, generating increased permeability (Fonseca et al. 2015). This permeability depends, then, on the effect that the solutes of the medium in which the bacteria may be inserted, in this case, on the partition of the components of the essential oil itself in the membrane lipidic phase, for triggering effect or not against the strains.

The mechanism of membrane permeabilization increase is closely associated with the dissipation of the proton-motive force, generating a reduction of ATP, internal pH, electrical potential and loss of ions and metabolites, for example, phosphate and potassium. This cascade of structural damage in the cytoplasmic membrane leads to the impairment of functions that are essential for bacterial survival, such as generation of energy and loss of selectivity (Bakkali et al. 2008).

Bacteria characterized as gram-negative have a cell wall composed of a layer of peptidoglycans, lipoproteins, outer membrane and lipopolysaccharides. Peptidoglycans are responsible for the formation of the cell and protection of the cytoplasm from different osmotic pressures; thus, they confer rigidity to the membrane (Silva et al. 2010). The outer membrane functions as a molecular barrier, hindering the loss of proteins and the action of hydrolytic enzymes, in addition to having receptors for bacteriophages and bacteriocins, and participation in bacterial nutrition (Nguefack et al. 2004).

In general, gram-positive bacteria are more sensitive to the effects of essential oils when compared to gram negative. we tested eight essential oils, and seven (*R. officinalis*, *T. vulgaris*, *B. dracunculifolia*, *P. guajava*, *S. terebinthifolius*, *L. angustifolia* and P. cablin) showed activity against *S. aureus* at low concentrations, while *H. brasiliense* showed no effect at the concentrations tested, against all microorganisms.

The susceptibility of gram-positive bacteria and relative tolerance of gram-negative bacteria to essential oils can be explained due to the presence of a hydrophilic outer layer (Holetz et al. 2002). Penetration of hydrophobic components into Gram-negative microorganisms is presumed to be more difficult due to the presence of a second physical barrier formed by cellular wall composed of a layer of peptideoglycans, lipoproteins, outer membrane and lipopolysaccharides, which make up a molecular barrier (Kalemba and Kunicka 2003).

According to Lahmar et al. (2016) the oils that have greater amount of phenolic compounds can sensitize the lipid bilayer of the cell membrane, altering the activity of calcium channels and causing increased permeability and release of intracellular constituents that are essential for maintaining microbial activity. Damage to the enzyme system of the microorganism involved in the production of energy and synthesis of structural components can also occur, as well as the destruction or inactivation of the genetic material (Lahmar et al. 2016).

Essential oils from medicinal, seasoning and aromatic plants have diverse chemical compositions, with some constituents being monoterpenes, sesquiterpenes, esters and phenolic compounds, which are possibly responsible for different activities and results presented. Moreover, oils that present phenolic hydroxyl groups are quite reactive and possibly act through the formation of hydrogen bonds with active sites of target enzymes, through their inactivation (Xiang et al. 2017).

It was concluded that the essential oils presented terpenes as the major component, classified as monoterpenes and sesquiterpenes. The essential oil of *T. vulgaris* presented the best results when evaluated its antibacterial activities against *S. aureus*, *E. coli* and *S. enteritidis*, being an important alternative for medicine in the fight against bacterial infections.

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