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Actividad Antibacteriana y Antioxidante de los Aceites Esenciales Comerciales de Romero, Clavo de Olor, Orégano y Salvia

bacterial and Antioxidant Activity of Commercial Essential Oils of Rosemary, Clove, Oregano and Sage

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Resumen

Este estudio tuvo como objetivos comparar la composición química y actividad antioxidante y antibacteriana *in vitro* de los aceites esenciales comerciales de romero, clavo de olor, orégano, salvia y combinación binaria de clavo y orégano. Fueron identificados como componentes principales, eugenol (89,58%) para el clavo, el carvacrol (60,71%) para el orégano, el acetato de bornilo (39,64%) de romero, linalol (39,26%) de la salvia y el eugenol (56,42%) para la combinación binaria. En la evaluación de la actividad antibacteriana el orégano mostró las zonas de inhibiciones más altas y la concentración inhibitoria mínima más pequeña. La combinación binaria con IC₅₀ de 6,40 µg/mL, seguido de clavo de olor con IC₅₀ de 11,79 µg/mL tiene un excelente potencial antioxidante.

Palabras clave: Carvacrol; Aceites esenciales comerciales; Eugenol; Actividad antibacteriana; Actividad antioxidante.

Abstract

This study aimed to compare the chemical composition and *in vitro* antibacterial and antioxidant activity of commercial essential oils of rosemary, clove, oregano, sage and binary combination of clove and oregano. Eugenol (89.58%) for clove, carvacrol (60.71%) for oregano, bornyl acetate (39.64%) for rosemary, linalool (39.26%) for sage and eugenol (56.42%) for binary combination were identified as the main components. In evaluation of antibacterial activity, the oregano showed the highest inhibition zones and the smallest minimum inhibitory concentration. The binary combination with IC₅₀ of 6.40 µg/mL, followed by clove with IC₅₀ of 11.79 µg/mL had excellent antioxidant potential.

Keywords: Carvacrol; Commercial essential oils; Eugenol; Antibacterial activity; Antioxidant activity.

Introduction

Essential oils (EOs) are volatile aromatic liquids extracted by many parts of plants, i.e. flowers, leaves, bark, seeds, roots and resins. These natural compounds are defined as secondary metabolites of plants and have relevant function in defense of producing organism acting as antimicrobial, antiviral, antifungal and insecticides. Although the food industries use the EOs mainly as flavorings, they represent an important alternative source of natural antimicrobial and antioxidant and can be used for the preservation of food products. In Brazil, EOs and extracts are included within the class of additives such as natural flavoring (1).

The EOs are formed by several organic compounds of low molecular weight, with different antimicrobial activities and may be divided into four groups according to their chemical structure: terpenes, terpenoids, phenylpropenes

and others(2). The presence of those compounds justifies

its antimicrobial and antioxidant properties. The EOs of rosemary, clove, oregano and sage have antimicrobial and antioxidant activities described in the literature (3-6). However, there are few studies comparing these effects, mainly for commercial EOs, which can be easily used in the food industry.

Given the importance of the search of natural antimicrobials and antioxidants with application in food industry, this study aimed to determine comparatively the chemical composition and *in vitro* antibacterial and antioxidant activity of commercial EOs of Rosemary (*Rosmarinus officinalis* L.), clove (*Eugenia caryophyllata* L.), oregano (*Origanum vulgare* L.), sage (*Salvia sclarea* L.) and of binary combination of EOs of clove and oregano, in proportion 1:1.ation of EOs of clove and oregano, in proportion 1:1.

Materials and methods

To determine the chemical composition and *in vitro* antimicrobial and antioxidant activities, commercially EOs obtained by (Ferquima®) Rosemary (*Rosmarinus officinalis* L.), clove (*Eugenia caryophyllata* L.), oregano (*Origanum vulgare* L.) and sage (*Salvia sclarea* L.) were used.

Chemical composition

EOs samples were prepared by diluting in hexane (Merck®) (10.000 mg mL⁻¹). For identification of the volatile compounds of EOs were made analyses by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu QP5050A) using aDB-WAX capillary column (30m, 0.25mm, 0.25µm). The column temperature was programmed at 50°C for 3 minutes, increased 5°C per min until 130°C and then 1°C per min until 210°C per 5 min. Helium was used as carrier gas and the temperatures of the detector and injector were 250°C. This device was operated with a flow rate of 1 mL/min with an electronic impact of 70 eV and split mode (split ratio 1:3). The volume injected was 1.0 µL. The peaks were integrated by manual mode, and the compounds were identified by comparing their mass spectra with those available in the Wiley mass spectral database (330,000) and by comparing the retention times of standard compounds (eugenol and linalool). 90% was adopted as a minimum percentage of similarity between the mass spectra of the compounds of the samples and the library of the equipment for identification of them.

Antimicrobial activity

Twelve microorganisms were selected for the analysis of antibacterial activity, six Gram-positive bacteria *Enterococcus faecalis* (ATCC 29212), *Micrococcus luteus* (ATCC 10240), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus mutans* (ATCC25175), *Listeria monocytogenes* (ATCC 7644) and six Gram negative bacteria *Aeromonas* sp. (microorganism obtained from the Biological Institute, Campinas-São-Paulo), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella choleraesuis* (ATCC 107008), *Klebsiella pneumoniae* (ATCC 10031), *Proteus mirabilis* (ATCC 25933), previously grown in Luria Bertani (LB) (10 g L⁻¹ of tryptone, 5g L⁻¹ of yeast extract and 5 g L⁻¹ of NaCl) for 24 h at 36 ± 1°C.

To evaluate the antimicrobial activity by the formation of the inhibition halo, Petri plates were used with culture medium Müller-Hinton Agar (Merck®) and Whatman paper disks number 3, with 7 mm of diameter (7). The cultures of bacteria were inoculated by scattering on plates with the aid of a Drigalski hook, in a volume of 200 µL (108 UFC mL⁻¹). For each bacterium and EO tested, a plate was prepared in which three discs have been deposited with the volume being tested (5, 10 and 15 µL of pure oil), a negative control disk (white) and a positive control

disk (30µg of chloramphenicol antibiotic). These volumes were determined from the previous tests, where the maximum volume of EO used was that the paper disc absorbed without overflowing. After incubation of the plates at 36 ± 1°C for 48 hours, the results were analyzed by measuring the diameter of the inhibition halo of bacteria's growth, including the diameter of the paper disk.

Results were expressed in millimeters (mm) by the arithmetic averages of the values of halos obtained in three replicates (per volume used), and the averages were subjected to Analysis of Variance (ANOVA) and compared by Tukey test (p < 0.05), using the ASSISTAT® program.

To determine the Minimum Inhibitory Concentration (MIC), the indirect method for bacterial growth by optical density in liquid culture (8) was used. After the results of the antibiogram analysis on solid media, eleven selected bacteria were grown in culture medium LB broth at 37°C for 24 h. The bacterium *E. faecalis* was not used in this step in view of the impossibility of its reactivating. After a period of growth, the cultures were inoculated into microtubes (Eppendorf) 10 µL of pre-inoculum (10⁸ UFC mL⁻¹), 1 mL of LB broth, plus 1% of emulsifier dimethylsulfoxide (DMSO) (Nuclear®) containing different concentrations of EOs (0.01 to 2.50 mg mL⁻¹) and the control without EO. All concentrations of each EO, with different bacteria were evaluated in triplicate. Subsequent to inoculation process, the microtubes were incubated in an electromagnetic stirrer (60 Hz) for 24h at 32°C. Before and after the incubation period, 0 and 24 h respectively, aliquots of 100 µL of bacterial culture were transferred to flat-bottom micro plates; three readings for each repetition were performed. There were problems of turbidity of the sample that contained only oregano EO on the concentration of 2.50 mg mL⁻¹ which cannot be used.

To evaluate the bacterial growth (optical density) and to determine the MIC of EO on bacterium, reading of the micro plate was performed using the automated micro plate reader (Bio-Tec Instruments Inc., Model EL800), coupled to one computer with Kc junior program, with preset length of 490 nm. Growth inhibition was determined by the difference between the readings taken at 24 and 0 h. The average optical density values were statistically analyzed by analysis of variance (ANOVA) and compared by Tukey's test (p < 0.05), to determine the MIC, using the ASSISTAT® program.

Antioxidant activity by free radicals capture with DPPH test

The methodology for evaluation of antioxidant activity, based on the measurement of the extinction of absorption of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical at 515 nm, was performed in triplicate by spectrophotometric method (9). The technique consisted of incubation for 30 min of 500 µL of at 0.1m Methanolic solution of DPPH with 500 µL of solutions containing increasing concentrations of EOs of rosemary, clove, oregano and sage (1.0; 2.5; 7.5; 10; 25; 50; 75; 100; 250; 500; 750 and 1000 µg mL⁻¹)

in ethanol. We proceeded similarly to the preparation of the solution called “control”, replacing 500 µL of sample per 500 µL of ethanol. A solution called “white” was prepared with solutions at different concentrations of EOs and ethanol, without DPPH. The antioxidant compound BHT (3,5-di-*tert*-4-butylhydroxytoluene) was used as a positive control.

The percent uptake of the DPPH radical was calculated in terms of percentage of antioxidant activity (AA %), according to Equation 1.

$$AA\% = 100 - \left\{ \frac{(Abs_{sample} - Abs_{white}) \times 100}{Abs_{control}} \right\} \quad (1)$$

The concentration of EO needed to capture 50% of the free radical DPPH (IC₅₀) was calculated by linear regression analysis of the points plotted graphically (10). For the plot points, the average values obtained from triplicates performed for each concentration were used. The antioxidant capacity was calculated according to the equation of the curve of the antioxidant activity, where y is replaced by 50 and x is the value of IC₅₀. Results were expressed as the arithmetic average of the values obtained in three replicates, where the averages were statistically analyzed by analysis of variance (ANOVA) and compared by the Tukey test ($p < 0.05$), using the ASSISTAT® program.

Results and discussion

Chemical composition

The contents of the major components found in the EOs evaluated, including the binary 1:1 combination of EOs of clove and oregano, determined by analysis by GC-MS, are shown in Table 1.

Table 1: Principal volatile compounds (% of area) found in rosemary (*R. officinalis*), clove (*E. caryophyllata*), oregano (*O. vulgare*), sage (*S. sclarea*) commercial essential oils and binary combination of clove/oregano 1:1.

Compound	I.K.*	rosemary	clove	oregano	sage	clove/oregano 1:1
<i>p</i> -cymene	1026	-	-	10.06	-	2.98
1.8 cineole	1033	22.52	-	-	0.25	-
γ -terpinene	1062	-	-	6.73	-	3.34
Linalool	1098	-	-	3.57	39.26	1.73
Camphor	1143	16.72	-	0.94	0.15	2.03
α -terpineol	1189	7.21	1.12	0.54	16.14	1.03
Linalyl acetate	1257	-	7.75	-	26.17	2.22
Bornyl acetate	1285	39.64	-	-	-	-
Thymol	1290	-	-	4.50	-	2.79
Carvacrol	1298	-	-	60.71	-	15.39
Eugenol	1356	-	89.58	-	-	56.42
Geraniol acetate	1383	-	-	-	1.65	-
Trans-caryophyllene	1404	-	-	4.35	1.67	3.80

I.K.* = Kovats index (11).

Several publications have presented data on the chemical composition of different EOs, which may comprise

more than sixty individual components (3). The major compounds may constitute up to 85% of the EO, while others are present only as trace elements, the latter have a key role to play in biological activities, possibly producing a synergistic effect between other components (2).

In the present study, bornyl acetate was identified as the major compound (39.64%) for the rosemary EO (Table 1). Some studies report the isolation and identification of different compounds with antioxidant and antimicrobial activity present in the rosemary EO, which consists of monoterpenichydrocarbons, terpenicesters, linalool, verbinol, terpineol, 3-octanone and iso-bornylacetate, among other compounds (12). Pintore *et al.* (13) identified for the rosemary EO, percentages ranging from 3-89% of bornylacetate, 2-25% of 1,8-cineole, camphor 2-14% and 2-25% α -pinene, like in this study.

The clove EO presented phenylpropene eugenol as the major volatile compound (89.58%) (Table 1), where in similar result was obtained by Silvestri *et al.* (7) with eugenol representing 90.30% of volatile compounds.

The monoterpenoid carvacrol was the major compound found in oregano EO (60.71%) (Table 1), which presents known antimicrobial activity (3). Silva *et al.*, (14) evaluated five distinct trademarks of EOs of oregano, and all chromatograms showed a single large peak at the same retention time. When compared to a standard, it was identified as carvacrol, in percentages ranging between 61.70 and 93.40% of the total volatile detected in each oil. Busatta *et al.* (15) found much lower percentage of carvacrol (11.67%), evaluating oregano EO obtained from leaves originating from Chile.

Sage EO had linalool as main volatile compound (39.26%) (Table 1). Pierozan *et al.* (8) determined the chemical composition of different species of sage, where the majority of volatile compounds obtained were α -thujone (40.37%), *Salvia sclarea*, β -thujone (19.96%) in the *S. lavandulifolia*, linalool (29.36%) in *S. sclarea* and α -thujone (22.39%) in *S. triloba*. The binary combination of clove and oregano EOs (1:1) qualitatively presented sum of both oils, but with quantitative changes, as expected.

Antibacterial activity

The average values of inhibition zone (mm) obtained by disc diffusion are shown in Table 2.

Table2: Average values of inhibition zone (mm) of essential oils (5, 10 and 15 μ L) of clove (*Eugenia caryophyllata* L.), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis* L.) and sage (*Salvia sclarea* L.) front Gram-positive and Gram-negative bacteria.

Bacteria	Inhibition halo EO of clove (mm) *			Inhibition halo EO of oregano (mm) *			Inhibition halo EO of rosemary (mm) *			Inhibition halo EO of sage (mm) *		
Gram-positive	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L
<i>E. faecalis</i>	11.0 ^a ±1.00	11.3 ^a ±0.58	11.3 ^a ±1.15	15.3 ^c ±0.58	19.7 ^b ±0.58	23.3 ^a ±0.58	9.0 ^a ±1.00	9.6 ^a ±0.58	11.3 ^a ±1.53	23.3 ^b ±1.53	27.0 ^a ±0.01	29.0 ^a ±1.73
<i>M. luteus</i>	18.6 ^c ±0.58	25.3 ^b ±0.58	27.7 ^a ±0.58	NS	9.3 ^b ±0.58	11.0 ^a ±0.01	8.7 ^c ±0.58	10.0 ^b ±0.01	11.3 ^a ±0.58	NS	NS	NS
<i>L. monocytogenes</i>	13.7 ^b ±1.15	15.0 ^{ab} ±0.01	17.3 ^a ±1.15	11.7 ^c ±1.53	17.3 ^b ±0.58	23.0 ^a ±1.00	9.0 ^a ±0.01	9.33 ^a ±0.58	10.7 ^a ±1.15	10.7 ^b ±0.58	13.0 ^{ab} ±1.00	15.0 ^a ±1.73
<i>S. aureus</i>	17.0 ^b ±1.00	19.3 ^b ±0.58	23.7 ^a ±1.15	26.7 ^b ±0.58	30.3 ^a ±0.58	31.0 ^a ±0.01	8.3 ^a ±1.15	9.0 ^a ±0.01	10.0 ^a ±1.00	16.7 ^c ±1.15	23.67 ^b ±1.53	27.0 ^a ±0.01
<i>S. epidermidis</i>	11.0 ^c ±1.00	13.0 ^b ±0.01	15.3 ^a ±0.58	28.3 ^c ±1.15	32.3 ^b ±1.15	37.3 ^a ±1.15	8.6 ^b ±0.58	11.0 ^{ab} ±1.73	12.7 ^a ±1.53	10.67 ^b ±1.15	13.0 ^{ab} ±1.00	15.0 ^a ±2.00
<i>S. mutans</i>	11.3 ^b ±0.58	13.7 ^a ±0.58	14.0 ^a ±1.00	27.0 ^c ±1.00	37.7 ^b ±2.08	47.3 ^a ±1.15	9.0 ^b ±0.01	10.33 ^{ab} ±0.58	12.0 ^a ±1.73	NS	NS	NS
Gram-negative	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L	5 μ L	10 μ L	15 μ L
<i>Aeromonas</i> sp.	11.0 ^a ±1.00	13.0 ^a ±0 ^a	13.0 ^a ±1.00	18.7 ^c ±0.58	25.3 ^b ±1.53	30.0 ^a ±1.73	13.3 ^a ±2.05	15.0 ^a ±1.41	17.0 ^a ±1.41	NS	NS	NS
<i>E. coli</i>	13.0 ^b ±1.73	15.0 ^a ±1.0 ^{ab}	16.67 ^a ±1.15	17.0 ^a ±1.00	17.0 ^a ±1.00	19.33 ^a ±1.53	12.7 ^b ±0.94	16.7 ^a ±1.25	19.3 ^a ±1.25	NS	NS	NS
<i>K. pneumoniae</i>	25.0 ^b ±0.01	27.33 ^a ±0.58 ^b	33.33 ^a ±1.53	NS	NS	NS	18.6 ^b ±0.94	21.3 ^b ±0.47	31.3 ^a ±1.25	NS	NS	NS
<i>P. aeruginosa</i>	11.0 ^b ±1.00	15.0 ^a ±0 ^a	15.0 ^a ±1.00	9.0 ^b ±1.00	11.0 ^{ab} ±1.00	12.67 ^a ±1.15	NS	9.0 ^b ±0.01	10.7 ^a ±0.47	NS	NS	NS
<i>P. mirabilis</i>	11.0 ^b ±0.01	13.33 ^a ±0.58 ^b	17.67 ^a ±1.53	24.7 ^b ±1.53	27.0 ^{ab} ±0.01	30.67 ^a ±2.52	10.7 ^c ±0.47	13.7 ^b ±0.94	19.3 ^a ±1.25	NS	NS	NS
<i>S. choleraesuis</i>	13.3 ^b ±0.58	14.67 ^a ±0.58 ^a	15.0 ^a ±0.01	12.7 ^b ±1.15	13.0 ^b ±1.00	17.67 ^a ±1.53	13.0 ^b ±0.01	15.3 ^b ±1.25	18.7 ^a ±0.94	8.0 ^a ±0.01	8.0 ^a ±1.00	9.3 ^a ±1.15

NS=not sensitive; *Means followed by the same letter in the same row for the same EO, do not differ by Tukey test at 5% probability.

The clove EO showed action over all the bacteria tested, with the highest activity observed (33.33 mm) on *K. pneumoniae*. Other authors found that the clove EO showed strong antimicrobial activity when tested for microorganisms *S. aureus*, *E. coli*, *C. jejuni*, *S. enteritidis*, *L. monocytogenes* and *S. epidermidis* (5).

The bacterium *K. pneumoniae* showed up not sensitive to oregano EO, which showed the highest activity on *S. mutans* (47.33 mm). Busatta et al. (15) obtained mean halos of 19.50 mm of oregano EO against *K. pneumoniae*. Sahin et al., (16) evaluating oregano EOs from Turkey, using 10 μ L per disc, also did not obtain action against *K. pneumoniae*, corroborating this research.

The rosemary EO did not act on *P. aeruginosa*, when used 5 μ L and presented highest activity on *K. pneumoniae* (31.33 mm) at 15 μ L. High sensitivity of Gram-positive bacteria to EOs of rosemary and sage from Egypt was reported, including *S. aureus*, *Micrococcus* sp. and *Sarcina* sp. as well as *S. cerevisiae*. However, no or very little effect was observed against Gram-negative bacteria *P. fluorescens*, *E. coli* and *S. marcescens* (17).

The sage EO showed the lowest performance among the oils tested, with activity on ly against *S. choleraesuis*, among Gram-negative bacteria. Pozzo et al. (18) evaluated the antimicrobial activity of EOs of condiments against *Staphylococcus* spp. and did not observe antibacterial activity of EOs of ginger, basil, rosemary and sage. In contrast to these authors, Delamare et al., (19) observed activity of sage EO against some isolates of *Staphylococcus* sp.

There was a tendency to increase the efficiency of EOs when it was increased the volume used from 5 to 15 μ L,

but this effect was not statistically significant ($p > 0.05$) for all microorganisms and EOs tested (Table 2).

Although there are exceptions in the literature, in general EOs are more effective against Gram-positive than Gram-negative bacteria, this low efficiency can be attributed to the fact that Gram-negative bacteria possess an outer membrane, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (3). This fact can be observed in EOs of oregano and sage tested in this study, for all concentrations used. The clove EO showed similar behavior against the Gram-positive and negative bacteria, while the rosemary EO was more effective in relation to Gram-negative bacteria.

The plates methodology has great importance to provide initial data of the antimicrobial action of natural products, by its easy and quick execution (20). However, it is considered essential to continue the studies to obtain values of MIC, which was performed in the second part of this study.

In the evaluating of MIC, it was observed that the oregano EO exhibited the best performance among the tested oils, being effective against all microorganisms evaluated (Table 3), reaching an average of 0.016 mg mL⁻¹ for Gram-positive bacteria and 0.020 mg mL⁻¹ for Gram-negative bacteria. Busatta et al. (15) evaluated *in vitro* antimicrobial activity of oregano EO found average values for MIC of 0.460 mg mL⁻¹, i.e., lower performance than that found in the present study.

There are reports that the carvacrol and thymol are the main components and those responsible for the antimicrobial activity of the oregano EO (14). Furthermore,

there are evidences that some components present in small amounts, such as γ -terpinene and p -cymene, affect in the antimicrobial activity by producing a synergistic effect between the others components (3,21).

The clove EO was also effective in inhibiting all microorganisms (Table 3), with mean values for 0.70 mg mL^{-1} for Gram-positive bacteria and 0.49 mg mL^{-1} for Gram-negative bacteria. Silvestri *et al.* (7), obtained values of MIC similar to clove EO, 0.50 mg mL^{-1} for Gram-positive bacteria and 0.58 mg mL^{-1} for Gram-negative bacteria. The clove EO has eugenol as major substance, responsible for the analgesic, anti-inflammatory and antioxidant activity (6).

The Eos of rosemary and sage had the worst performance, with a mean value of MIC of 2.5 mg mL^{-1} for all microorganisms used in the study (data not found). Fu *et al.* (22) found activity of rosemary EO against *S. epidermidis* (ATCC 12228) and *S. aureus* (ATCC 6538). Delamare *et al.*, (19) observed the activity of sage EO against some isolates of *Staphylococcus* spp.

Table 3: Minimum Inhibitory Concentration (MIC) of oregano EO (*Origanum vulgare* L.), clove EO (*Eugenia caryophyllata* L.) and mixing of oregano and clove (1:1).

Gram-positive bacteria	MIC (mg mL ⁻¹)		
	Oregano	Clove	Oregano/Clove 1:1
<i>Micrococcus luteus</i>	0.010	0.500	0.500
<i>Listeria monocytogenes</i>	0.025	0.750	0.075
<i>Staphylococcus aureus</i>	0.010	0.750	0.075
<i>Staphylococcus epidermidis</i>	0.010	0.750	0.025
<i>Streptococcus mutans</i>	0.025	0.750	0.500
Gram-negative bacteria			
<i>Aeromonas</i> sp.	0.010	0.750	0.50
<i>Escherichia coli</i>	0.025	0.750	0.50
<i>Klebsiella pneumoniae</i>	0.025	0.100	0.075
<i>Pseudomonas aeruginosa</i>	0.025	0.750	0.075
<i>Proteus mirabilis</i>	0.025	0.500	0.075
<i>Salmonella choleraesuis</i>	0.010	0.100	0.025

Given the better performance of Eos of clove and oregano in the MIC evaluation, it was decided to perform a binary combination of both, in the proportion 1:1, against to the same microorganisms. There was a reduction of MIC presented by clove EO individually for all bacteria tested, except for *M. luteus*, which remained unchanged (Table 3). Evaluating the chemical composition of the binary combination 1:1 of clove and oregano EOs (Table 1), can be observed that the eugenol, present in the clove essential oil, is the major component (56.42%), and the other six components present in the oregano essential oil presented minor percentual area, suggesting synergist effect of these compounds, which can justify the improvement of the MIC when compared to the clove EO with the mixture of oils (Table 3). The *Origanum vulgare* EO has demonstrated good bactericidal and fungicidal activity against different pathogens, due to the compounds carvacrol and thymol, which are phenolic components present in greater quantity (23).

Antimicrobial activity of an EO can depend only on one or two of the major components that constitute it. However, an increasing amount of evidence indicates that

the inherent activity of the EOs may be a function of the interaction between its minor constituents. Several synergistic antimicrobial activities were reported to components or fractions of EOs, using binary or tertiary combinations (3).

Doing a parallel between the results obtained in the diffusion plates and MIC, of the EOs tested, it can be observed that the EOs of clove and oregano remained as the best performers, with some particularities. In disc diffusion test, the oregano EO was ineffective against the bacteria *K. pneumoniae*, whereas a value of 0.025 mg mL^{-1} was obtained when performing their MIC (Table 3). This may be due to a possible difficulty of the oil to migrate to the agar in the disc diffusion test, whereas when performed the determination of MIC, the oil is in direct contact with the microorganism.

Antioxidant activity by capture radicals with DPPH test

The antioxidant activity (AA%) of the oils tested was calculated from the percent uptake of DPPH radical. The EO of clove showed the most AA%, 89.38% at the concentration of $50 \mu\text{L mL}^{-1}$, followed by rosemary oil 77.90% at a concentration of $750 \mu\text{L mL}^{-1}$ and 75.11% at a concentration of $250 \mu\text{L mL}^{-1}$ to oregano EO. With the BHT, used as a reference synthetic antioxidant, was observed an activity of 89.92% at a concentration of $100 \mu\text{L mL}^{-1}$.

Silvestri *et al.* (7), evaluating the clove EO in different concentrations, obtained a maximum AA% of 95.6% at a concentration of $10,000 \mu\text{L mL}^{-1}$, which confirms the high antioxidant activity of this oil. Scherer *et al.* (5), analyzed the volatile composition of the clove EO where eugenol was identified as the major compound, with 83.75% of the total area. The same was observed in the present study (Table 1), which is a phenolic compound with strong antioxidant action that has been proven both in vitro and in vivo (24).

The sage EO presented the lowest performance among the oils analyzed, with an antioxidant activity of 19.70% at a concentration of $1,000 \mu\text{L mL}^{-1}$, although this condiment was extensively studied and recognized for its antioxidant capacity related to their phenolic compounds (25).

From the correlation between the antioxidant activity (AA%) and the concentration of the oils used, linear equations were obtained, which provide the data to calculate the IC_{50} , which corresponds to the sample required to reduce by 50% the initial concentration of DPPH (Table 4).

Table 4: Linear equations and IC_{50} of EOs tested.

Essential Oil	Linear equation	$\text{IC}_{50} (\mu\text{g mL}^{-1})$
Clove	$y = 933.93x + 38.99$ ($R^2 = 0.76$)	11.79
Oregano	$y = 306.5x - 2.853$ ($R^2 = 0.98$)	172.44
Rosemary	$y = 99.899x + 2.5384$ ($R^2 = 0.98$)	475.14
Sage	$y = 14.414x + 2.9161$ ($R^2 = 0.87$)	> 1,000
Clove/Oregano 1:1	$y = 7954.9x + 0.9575$ ($R^2 = 0.96$)	6.40
BHT	$Y = 501.4x + 45.63$ ($R^2 = 0.86$)	8.00

As shown in Table 4, the clove EO presented an IC_{50} of $11.79 \mu\text{L mL}^{-1}$, which can be considered good compared with antioxidant of excellence as ascorbic acid ($IC_{50} = 2.15 \mu\text{L mL}^{-1}$) and BHT ($IC_{50} = 5.37 \mu\text{L mL}^{-1}$) (26). Pérez-Rosés *et al.* (27) reached an IC_{50} of $13.20 \mu\text{g mL}^{-1}$ for the clove EO obtained from the leaves of the plant.

Sahin *et al.* (16), evaluating EOs of oregano from plants of Turkey, had IC_{50} values greater than those obtained in this study with average values of $8,900 \mu\text{g mL}^{-1}$, where the major compounds present in the oils were caryophyllene (14.40%) and spathulenol (11.60%). Zaouali *et al.* (28) evaluating rosemary EOs from Tunisian, had IC_{50} values ranging between 6.00 and $28.50 \mu\text{L mL}^{-1}$, higher than those found in this study, and this activity being associated with high levels of camphor, linalool acetate and α -tujene found in the oil. For BHT, the IC_{50} obtained was $8.00 \mu\text{L mL}^{-1}$ (Table 4).

Aiming future application in food products, the binary combination (1:1) of clove and oregano EOs was also evaluated with respect to antioxidant activity. This combination showed a maximum AA% of 85.16% at the concentration of $100.00 \mu\text{L mL}^{-1}$ and a IC_{50} of $6.40 \mu\text{L mL}^{-1}$ (Table 4), higher than that obtained for the reference substance, the BHT, used in this research as well as for both EOs when used in isolated, possibly as a result of different chemical composition obtained from this combination (Table 1). Together, the different compounds present in the EOs, produce an array of antioxidants that may act by different mechanisms to confer an effective system of defense against the attack of free radicals (29).

The eugenol and carvacrol were the major volatile compounds found in binary combination of EOs of clove and oregano (1:1) (Table 1), which justifies the IC_{50} obtained of the mixture (Table 4).

Conclusion

From the assessed parameters, it can be concluded that the clove EO and oregano EO and the binary combination of EOs of clove and oregano (1:1) showed excellent potential to be used as antioxidant and antibacterial, which are justified by the major volatile compounds identified, carvacrol (60.71%), eugenol (89.58%), and, carvacrol (15.39%)/eugenol (56.42%), for oregano, clove and their combination, respectively. Evaluating the overall performance achieved by EOs *in vitro* tests, it can be inferred that they present a promising alternative in food preservation, such as component of bioactive packaging.

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