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SEGURANÇA
alimentar e nutricional

Antibacterial Potential of Essential Oils Against Microorganisms Isolated from Ground Beef

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The objective of this study was to evaluate the antibacterial activity of different EOs against *Escherichia coli*, *Salmonella* spp., and coagulase-positive Staphylococcus isolated from ground beef. The EOs had values varying from 0.78µl/mL to 100µl/mL, and the essential oil of *Citrus lemon* did not present any bacterial activity against the studied strains. For *E. coli*, the essential oil of *Cinnamomum zeylanicum* presented the best inhibitory activity (MIC = 0.78µl/mL to 1.56µl/mL). In *Salmonella* spp., the EOs of *Cymbopogon citratus* (MIC = 12.5µl/mL and 25.0µl/mL), *C. zeylanicum* (MIC = 25.0 µL/mL) and *Ocimum basilicum* (MIC = 6.25µl/mL, 12.5µl/mL and 50.0µl/mL) exhibited similar results. The EOs of *C. zeylanicum* and *Eugenia caryophyllata* demonstrated the lowest average values of study against coagulase-positive *Staphylococcus* (MIC = 3.12µl/mL, 6.25µl/mL and 12.5µl/mL). With the data obtained in the study, it can be observed the potential of essential oils of cinnamon, oregano and cloves, to control pathogenic strains of food, as well as validate future researches on the proprieties and active compounds of these products, besides the toxicity of these compounds and the possible alterations that can be caused on the food.

Keywords: Minimum Inhibitory Concentration, meats, spices, bacterial

Potencial Antibacteriano de Óleos Essenciais Contra Micro-organismos Isolados de Carne Moída

O objetivo do presente estudo foi avaliar a atividade antibacteriana de diferentes óleos essenciais frente a *Escherichia coli*, *Salmonella* spp. e *Staphylococcus* coagulase positiva isoladas de carne moída. Os óleos essenciais apresentaram valores de inibição variando de 0.78 µl/mL a 100 µl/mL, sendo que o óleo essencial de *Citrus lemon* não apresentou atividade antibacteriana frente a cepas estudas. Para *E. coli*, o óleo essencial de *Cinnamomum zeylanicum* apresentou a melhor atividade inibitória (MIC = 0.78 µl/mL a 1.56 µl/mL), já para *Salmonella* spp., os óleos essenciais de *Cymbopogon citratus* (MIC = 12.5 µl/mL e 25.0 µl/mL), *C. zeylanicum* (MIC = 25.0µl/mL) e *Ocimum basilicum* (MIC = 6.25 µl/mL, 12.5 µl/mL e 50.0 µl/mL) exibiram resultados semelhantes. Os óleos essenciais de *C. zeylanicum* e *Eugenia caryophyllata* demonstraram os menores valores de médias do estudo frente a *S. coagulase* positiva (MIC = 3.12

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µl/mL, 6.25 µl/mL e 12.5 µl/mL). Com os dados obtidos neste estudo, pode-se observar o potencial dos óleos essenciais de canela, orégano e cravo, para o controle de cepas patogênicas de alimentos, assim como validar pesquisas futuras a cerca das propriedades e compostos ativos dos respectivos produtos, além de testes de toxicidade destes compostos e possíveis alterações que possam vir a ser causadas nos alimentos.

Palavras-chave: Concentração Inibitória Mínima, cárneos, condimentos, bactérias

INTRODUCTION

Pathogenic microorganisms are the main agents that cause foodborne illnesses.[1] The techniques of food conservation have as central objective to prevent microbial spoilage, thus increasing shelf life period, preventing economic losses, and to avoid contamination by foodborne pathogens.[2]

Chemical conservation remains one of key issues in food safety, making necessary the development of alternatives for conservation that can provide quality and increasingly safe foods under the microbiological and toxicological point of view along with already existing technologies.[3] The use of aromatic plants that have essential oils (EOs) and usually present flavoring agents, besides a combination of herbs and spices with proven antibacterial and antioxidant action, may be used for preserving food, decreasing the concentration of synthetic additives.[4]

Over the last decades, there has been interest in the therapeutic potential of medicinal plants that contain EOs.[5, 6] This interest aims towards natural products with antimicrobial potential combined to antioxidant potential, different EOs that act in synergy when combined.[7] In addition to these, some researchers propose the use of treatments with mild temperatures in combination with other substances, such as natural antimicrobial barriers.[8, 9]

Therefore, this study was made on the effectiveness in vitro and to assess the inhibitory effects of cinnamomum, lemongrass, lemon, clove, basil, oregano and rosmarinus EOs against samples of *Escherichia coli*, *Salmonella* spp. and coagulase-positive *Staphylococcus* isolated from ground beef.

MATERIAL AND METHODS

Essential oils: It has been used for the study of EOs from *Cinnamomum zeylanicum* (L.) Blume. (Cinnamomum), *Cymbopogon citratus* (DC.) Stapf (Lemongrass), *Citrus lemon* (L.) Burm (Lemon), *Eugenia caryophyllata* (L.) Thumb. (Clove), *Ocimum basilicum* (L.) (basil), *Origanum vulgare* (L.) (oregano) and *Rosmarinus officinalis* (L.) (rosmarinus). All EOs used in the study are commercially available through Florananda Ind. and Com. de Cosm. and Prod. Nat. Ltda., except for the EO of *O. vulgare* belonging to Universidade Federal de Pelotas – Rio Grande do Sul/Brazil. All essential oil's were extracted by the steam distillation technique in a Clavenger-type apparatus.[10]

Microorganisms: The strains of *Escherichia coli* (nine), *Salmonella* spp. (eleven) and coagulase-positive Staphylococcus (twelve) used in this study were previously isolated from ground beef, collected in Pelotas city, Rio Grande do Sul, Brazil. The samples were collected from different establishments randomly chosen using the methodology of Silva et al. [11], and maintained in nutritional medium agar (Brain Heart Infusion - Acumedia®) until the antibacterial test.

Preliminary evaluation of the antibacterial activity: For the initial antibacterial activity analysis of the EOs, the bacterial were picked by depletion in a medium plate (BHI agar- Acumedia®) in order to obtain isolated colonies. After, the disk diffusion was realized following the methodology of CLSI M02-A11.[12] A suspension of 1.5 x 10^8 CFU.mL^{-1} of each bacterial strain, performed through MacFarland Scale, was inoculated onto Muller Hinton agar using a swab. Filter paper disks (6 mm) with 10 μL of each EO (in duplicate for each test) and a filter paper disc containing 10 μL of sterile distilled water as negative control were used. The Broth Microdilution Test was performed for all EOs that showed visible inhibition halo against the bacterial strains.

Determination of Minimum Inhibitory Concentrations (MIC): The MICs were determined using 96-well microtiter plates. The bacterial suspension was adjusted with sterile saline to a concentration of 1.5 x 10^8 cfu/ml, after subsequent dilution in BHI Broth (Acumedia®) plus Tween 80 1% (T80-Vetec®) surfactant agent, achieving a bacterial population of 1.5 x 10^5 cfu/ml.[13] The highest concentration of EO used was 100 μl.mL^{-1}, from it was serially diluted up to 0.781 μl.mL^{-1}. It was included negative controls containing only BHI broth and another containing the pure EO, besides positive control containing BHI medium with microorganisms. The microplates were incubated for 24h at 36ºC ±2. The lowest concentrations were defined as concentrations that inhibited bacterial growth, demonstrated by no changing the color of the medium from transparent to red when exposed to the reagent used (2, 3, 5 triphenyl-tetrazolium chloride - 0.5%). The change in color from the medium of transparent to red indicates bacterial growth.

Statistical analysis: Data was subjected to T-Test analysis paired for mean comparisons using SPSS statistic program (Version 20.0).

RESULTS AND DISCUSSION

According to the diffusion disk tests, all EOs showed some antibacterial activity (inhibitory halo formation), and the MIC was evaluated for all of these. After the MIC assays, only the lemon EO did not show any antibacterial effect.

Previous studies have shown antibacterial activity of Lemon on molds like *Aspergillus niger*, *A. flavus*, *Penicillium* spp. and *Fusarium* spp..[14, 15] On the other hand, there are few reports on the bacteriostatic and bactericidal activity of this EO and compounds thereof against bacterial species, such as *Salmonella* spp., *E. coli*, *Staphylococcus aureus* and others.[16, 17] Espina et al.[18] showed great activity of this oil on *E. coli* and *Salmonella enteretidis* only in combination with mild heat treatment (54ºC/10min).

It can be observed the inhibition averages of the EOs that exhibited antibacterial activity by the Broth Microdilution Test against the bacterial samples used in the study. The EOs that showed the best antibacterial against *E. coli* and coagulase-positive *Staphylococcus* potential were Cinnamomum and Clove. Their MIC averages for isolates of *E. coli* were in 0.90 µl/mL and 7.20 µl/mL, respectively. Both having antibacterial activity against coagulase-positive *Staphylococcus*, with values of $\bar{x}$ = 7.50 µl/mL to Cinnamomum and $\bar{x}$ = 5.90 µl/mL to Clove. The activities of other essential oils are shown in Figure 1, wherein the essential oil of rosemary, showed weak activity against isolates of *E. coli* and *Salmonella* sp.

The major compound present in both EO, eugenol, representing between 78% and 88% of components.[19, 20, 21] On their studies, Catherine et al.[22] analyzed the antibacterial activity of eugenol against different bacteria in vitro system, and achieved MIC values of 2.00µl/mL as well as *S. aureus* as *E. coli*, the findings of this study may be associated with the usual presence of high rates of eugenol in both EO's.

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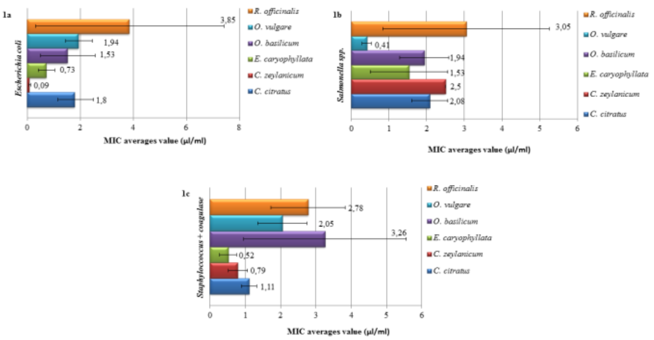

Figure 1. Averages of antibacterial activity of essential oils (µl/ml), by the broth microdilution test, against the bacterial samples of *Escherichia coli* (1a), *Salmonella* spp. (2a) and *Staphylococcus* positive coagulase (3a).

Oregano EO presented a more intense inhibitory effect in *Salmonella* spp. isolates ($\bar{x}$ = 3.90 µl/mL). The high antimicrobial activity of oregano EO could be explained by their high percentage of phenol components, such as carvacrol, which is able to disintegrate the outer membrane of gram-negative bacteria, releasing lipopolysaccharides (LPS) and increasing the permeability of cytoplasmic membrane to ATP.[23, 24]

Table 1. Paired differences of the minimum inhibitory concentration (%) and their confidence intervals showed by essential oils against isolates of *Escherichia coli* and *Salmonella* spp.

EO/bacterial species compared	Paired differences		
	Mean (%)	IC95%	*p* value
Lemongrass	0.27889	1.18254 - 0.62476	0.497
Cinnamomum	2.40333	2.43408 - 2.37259	< 0.001
Clove	0.8	2.015 - 0.415	0.167
Basil	0.41667	1.40754 - 0.57421	0.361
Oregano	1.53111	1.02955 - 2.03267	< 0.001
Rosmarinus	0.79667	4.02238 - 5.61571	0.713

In this study, the EO of oregano has significant antibacterial activity averages for *E. coli* and *S. aureus*, and lemongrass showed a low antibacterial activity against the three bacterial species tested. Several studies demonstrate the excellent effect of Lemongrass EO against bacterial pathogens.[25, 26, 27] However, variations in the components may occur in different samples from the same plant species which might lead to conflicting results among different studies. For large-scale production, it is important to note that the composition of the EOs of a particular species of plant may differ according to the method of drying and the season of the year in which the crop was made, because of genetic factors.[28, 29] Tables 1, 2 and 3 describe the statistical matching between MIC results obtained on the bacterial samples.

The EO of basil and rosmarinus has a weak antibacterial activity over the bacterial isolates. The Basil EO exhibited better effect on gram-negative bacteria, *E. coli* ($\bar{x}$ = 15.20 µl/mL) and *Salmonella* spp. ($\bar{x}$ = 20.40 µl/mL), whereas *R. officinalis* has better action on gram-positive used, coagulase-positive *Staphylococcus* ($\bar{x}$ = 24.20 µl/mL).

Table 2. Paired differences of the minimum inhibitory concentration (%) and their confidence intervals showed by essential oils against isolates of *Escherichia coli* and *Staphylococcus* coagulase-positive.

EO/bacterial species compared	Paired differences		
	Mean (%)	IC95%	*p* value
Lemongrass	0.69444	0.18804 - 1.20085	0.013
Cinnamomum	0.69889	0.97143 - 0.42635	< 0.001
Clove	0.20889	0.24187 - 0.65965	0.316
Oregano	1.73667	4.39832 - 0.92499	0.171
Basil	0.10333	0.66749 - 0.46083	0.684
Rosmarinus	1.07444	2.99078 - 5.13967	0.559

Table 3. Paired differences of the minimum inhibitory concentration (%) and their confidence intervals showed by essential oils against isolates of *Salmonella* spp. and *Staphylococcus* coagulase-positive.

EO/bacterial species compared	Paired differences		
	Mean (%)	IC95%	*p* value
Lemongrass	1.02364	0.52252 - 1.49475	0.001
Cinnamomum	1.73636	1.51765 - 1.95507	< 0.001
Clove	0.88273	0.00249 - 1.76795	0.051
Oregano	1.59091	3.81623 - 0.63441	0.142
Basil	1.62182	1.07479 - 2.16884	< 0.001
Rosmarinus	0.14182	1.67265 - 1.95628	0.865

According to the results obtained by statistical analysis, it can be observed a discrepancy between MIC values of some EOs in bacterial strains. The EOs of Cinnamomum and Oregano showed significant differences in their antibacterial activities in both gram-negative bacteria of the present study, *Salmonella* spp. and *E. coli* (Table 1), demonstrating the restricted antibacterial action of these oils, probably because of their variation, even against strains with similar bacterial walls.

When comparing, gram positive and gram negative bacteria analyzed, significant variation can be observed in the antibacterial action of EOs in Cinnamomum and Lemongrass (Tables 2-3), result expected because of the biochemical differences in the bacterial walls. However, the EOs of Clove, Basil and Rosmarinus showed no statistically significant variation in their MIC values in the researched bacterial species, what can infer that these could generate compounds of broad antimicrobial spectrum. In contrast, the EO Cinnamomum showed statistical difference between all species, attribute to restricted spectrum characteristic of antimicrobial action.

The importance of EOs and extracts as flavonoid action products in foods has been known scientifically for many decades [30], however, some considerations must be made. When analyzing a plant extract as an antibacterial product to be used in food, for example, the necessary concentration for inhibiting a microorganism should be taken into account, since the in vitro MIC of the compound will be different from that required for the preservation of the food *in vivo*.[31]

When it is necessary to increase the concentrations of the EO to obtain antibacterial activity, it is important to observe the established rules in some countries. Some examples are the ban of the addition of the sassafras and fava-tonca EOs in Brazil, and substances with maximum values established in the European Union.[32, 33] Other aspects should be emphasized before the application of EOs in foods, such as the interaction of these with matrix components of food (eg. fats), and the alteration in product aroma, which can be a negative aspect to the consumer.[34, 35]

For these considerations, some alternatives can be put into practice, such as the study of components and extracts considered safe for human consumption, such as clove, oregano, thyme, basil and cinnamon EOs.[36] Also, further studies with nanoemulsion-encapsulated oils, which may minimize organoleptic effects and associate the type of EO with a particular food, where the flavoring action of the compound can enhance the flavor/aroma of the food.[37]

CONCLUSION

The essential oil of cinnamon presented the best in vitro activity against *Escherichia coli* isolated from ground beef, followed by the essential oil of clove. For the samples of *Salmonella* sp., Oregano essential oil showed better antibacterial efficiency, while for *Staphylococcus* coagulase-positive isolates, clove essential oil showed satisfactory results.

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