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Inhibitory effect and disinfectant activity of *Syzygium aromaticum* L. and *Ocimum gratissimum* L. essential oils against *Escherichia coli* and *Staphylococcus aureus* isolated from sheep carcasses

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Abstract

Syzygium aromaticum L. and *Ocimum gratissimum* L. essential oils were tested for their effectiveness in reduction the counts of inoculated *Staphylococcus aureus* and *Escherichia coli* in sheepmeat. The inhibitory effect was analysed by disk diffusion and broth macrodilution method with four strains and seven concentrations. Disinfectant activity of the oils was assessed using the suspension test with two strains and two concentrations. The inhibition was observed at concentrations 400, 200 and 100 μL/mL. *Syzygium aromaticum* L. oil was better than *Ocimum gratissimum* L. against bacteria isolated from sheep carcasses. The minimum bactericidal concentration of the essential oils aginst inocula ranged from 50 to 200 μL/mL. The suspension test showed that after 5 minutes of contact, *Syzygium aromaticum* L. disinfectant at 400 μL/mL deactivated the inoculants completely. The results demonstrated that essential oils exerted a significant bactericidal and bacteriostatic action against pathogens.

Keywords: "Alfavacão". Antiseptic. Clove. Food safety. Microorganisms.

Efeito inibitório e atividade antisséptica de óleos essenciais de *Syzygium aromaticum* L. e *Ocimum gratissimum* L contra *Escherichia coli* e *Staphylococcus aureus* isolados de carcaça de ovinos

Resumo

Verificou-se a efetividade dos óleos essenciais de *Syzygium aromaticum* L. e *Ocimum gratissimum* L. em reduzir a contagem de *Staphylococcus aureus* e *Escherichia coli* inoculadas em carne de ovino. Foram utilizadas quatro cepas e sete concentrações dos óleos nas técnicas de disco-difusão e macrodiluição em caldo empregadas na análise do efeito inibitório. No teste de suspensão, trabalhou-se com duas cepas e duas concentrações na determinação da atividade desinfetante. A inibição foi observada nas concentrações de 400, 200 e 100 μL/mL. O óleo de *Syzygium aromaticum* L. foi melhor em relação ao alfavacão para inativar as bactérias isoladas das carcaças de ovinos. A concentração bactericida mínima dos óleos contra os inóculos variou de 50 a 200 μL/mL. O teste de suspensão mostrou que após 5 minutos de contato com o desinfetante de cravo na concentração de 400 μL/mL os inóculos foram completamente inativados. Os resultados mostraram significativa atividade bactericida e bacteriostática dos óleos essenciais frente aos patógenos.

Palavras-chave: Alfavacão. Antisséptico. Cravo da índia. Segurança alimentar. Microrganismos.

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Introduction

Foods are excellent vehicles for the dissemination of pathogens such as bacteria, viruses and parasites (Newell *et al.*, 2010). Many of them, such as *E. coli* and *S. aureus* cause diseases.

In the last twenty years, different cases of diarrhea have been attributed to the consumption of food containing toxin producing *E. coli* (Hussein and Bollinger, 2005; Newell *et al.*, 2010), *Shigella* sp. or *Salmonella sp.* (Borowsky *et al.*, 2006; Brasil, 2005). Staphylococci are often related to outbreaks of foodborne illness due to the ability of some strains to produce enterotoxins (Viçosa *et al.*, 2010).

Many pathogenic organisms are resistant to traditionally used antibiotics due to the indiscriminate and inappropriate use of these drugs (Sartoratto *et al.*, 2004). Moreover, there are products on the market with moderate or even poor antiseptic effect (Pitten *et al.*, 2003), the need of finding new substances with antimicrobial action becoming of utmost importance (Duarte *et al.*, 2007).

Foodstuffs are subject to microbial spoilage during their useful life. In the Brazilian semiarid, meat sheep of the small producers are slaughtered and sold, often without proper hygienic conditions, affecting the sensory and microbiological quality of the meat (Sá *et al.*, 2007). Sheep meat, lamb or mutton, is tender and juicy, with a characteristic aroma has considerable economic importance (Costa *et al.*, 2008) and the consumer market for this product is constantly growing (Barros *et al.*, 2003).

Lately, there has been increasing demand for safe products (Rajkovic *et al.*, 2010; Todd, 2004), natural and free from chemical preservatives, leading the industry to investigate elements capable of maintaining and improving the sensory characteristics while maintaining the nutritional properties of the food (Goñi *et al.*, 2009).

Studies have confirmed the bactericidal and/or bacteriostatic action of spices, seasonings and extracts or essential oils. Essential oils, plant secondary metabolites isolates, have won over the market and consumer tastes, owing to their health benefits and lower environmental impact (Pereira *et al.*, 2008). Among the plants and condiments currently researched and used are clove (*S. aromaticum*), an oriental spice used in cooking (Bruneton, 1995) and the "Alfavacão" (*O. gratissimum*), or wild basil, an aromatic plant known for its medicinal aspects (Di Stasi *et al.*, 2002) and use in cooking (Pereira and Maia, 2007).

The objective here in was to evaluate the inhibitory effect, the minimum bactericidal concentration and disinfectant activity of essential oils of *S. aromaticum* and *O. gratissimum* on the bacteria *S. aureus* and *E. coli,* isolated from sheep carcasses.

Material and methods

Sample collection

Samples were collected from 36 carcasses, gathered during three visits to a butcher in Montes Claros - MG. The slaughter happened informally, at a different location from the butcher shop, according to the owner.

We used a 5-point surface smear technique (neck, shoulder, ribs, loin and leg), and the swabs were transported in of 50 mL bottles with 0.1% peptone water (Himedia®) (Midura and Bryant, 2001). The volumes of bottles with the swabs (solution 10^{-1}) were homogenized in 0.1% peptone water and then underwent dilution to obtain the other two solutions (10^{-2} and 10^{-3}) (Swanson *et al.*, 2001).

Upon collection, the samples were taken in a cooled container to the laboratory in the same city, and cultivation being conducted on the same day.

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These strains were identified by microbiological analyses conducted previously (QUINN *et al.*, 2005) and were stored in glycerol at -18°C.

Plant material and essential oil extraction

The extraction of essential oils was conducted by hydrodistillation in a Clevenger apparatus. There were five extractions with 188 g each of fresh of *O. gratissimum*, leaves, collected in the of Medicinal Plant Garden of the Institute of Agricultural Sciences, Federal University of Minas Gerais, and two extractions, each using 118 g of *S. aromaticum*, inflorescences, acquired in the commerce of Montes Claros. The samples were placed separately into 1 liter flasks and kept boiling for 3 hours, at constant temperature. The content was expressed in volume of oil per dry matter of the material used in the extraction (Souza *et al.*, 2010).

The *Ocimum gratissimum* specimen collected was identified by Professor Alexandre Salino and a voucher specimen deposited in the BHCB Herbarium at the Federal University of Minas Gerais, under voucher number 150123.

Chromatographic analysis of essential oils

The qualitative analyses to identify the essential oil constituents were carried out at the Natural Products Laboratory - Centro de P&D Recursos Genéticos Vegetais, at the Agronomic Institute of Campinas in Campinas - SP.

Separation and quantification (area normalization method) of the substances was performed by gas chromatography with a flame ionization detector (GC--FID, Shimadzu GC-2010) under the following analytical conditions: DB-5 capillary column (J & W Scientific; 0.25 mm x 30.0 x 0.25 mM); Injector and detector temperatures maintained respectively at 220°C and 230°C; helium carrier gas at a flow rate of 1.0 mL/min); split: 1/20; Injection volume: 1 μL of essential oil solution (1 μL of essential oil/1 mL of ethyl acetate); oven temperature program: Initial temperature 60°C up to 240°C at 3°C/min.

The identification of compounds was performed on a gas chromatograph coupled to mass spectrometer (GC-MS, Shimadzu QP-5000) operating at an electron impact (70 eV) under the same chromatographic conditions described above, using capillary column: OV -5 (Ohio Valley Specialty Chemical, Inc.; 30.0 x 0.25 mm x 0.25 um.). The identification of the substances was performed by comparing their mass spectra with the database of the GC-MS system (Nist. 62 lib.) and the retention ratio (Adams, 2007). To obtain the retention index (RI), we used a standard hydrocarbon mixture (C9-C24) injected under the same operating conditions as the samples, by applying the Van den Dool; Kratz equation (1963).

Evaluation of inhibitory activity and bactericidal oils

For *in vitro* evaluation of the antimicrobial activity of *S. aromaticum* and *O. gratissimum* oils against strains of *S. aureus* and *E. coli* isolated from sheep carcasses and *S. aureus* ATCC 25923 and *E. coli* ATCC 8733, we used the broth macrodilution and diffusion methodologies in Clinical and Laboratory Standards Institute plates - CLSI (2018a).

In plate diffusion, one aliquot of each strain was placed in BHI broth (Himedia®) and taken for activation to an oven at 37°C for 24 hours. Activated cultures were transplanted on TSA agar (Triptic Soy Agar Himedia®) and incubated under the same conditions. The inoculum was prepared by placing five isolated colonies from the plates in 5 mL of saline solution. to the Turbidity was adjusted to the 0.5 McFarland standard solution equivalent (CLSI, 2018b).

With the aid of a sterile swab 200 μL of the inoculum was seeded on the surface of Petri dishes containing Mueller-Hinton agar (Himedia®). Whatman filter paper discs number one, 6 mm in diameter and impregnated with 10 μL of each test concentration were uniformly distributed across the plate with the aid of a sterile tweezer.

For the first concentration we used, 400 μL of essential oil, 27 μL of Tween 80 and the volume was completed to 1 mL with sterile distilled water according to the methodology proposed by Oliveira *et al.* (2006). The final volume was vortexed for 5 minutes. The concentrations evaluated were 400; 200; 100; 50; 25; 12.5 and 6.25 μL/mL, obtained by the serial dilution method at a ratio of 2 (CLSI, 2018a). The paper disc impregnated with Tween 80 represents the negative control, while commercial antibiotic disks served as positive control, using 10 μg Gentamycin and 1 μg Oxacillin on the plates with *Staphylococcus* sp. and 5 μg Ciprofloxacin and 1 μg Oxacillin the plates with *E. coli*, all DME® brand. The plates were placed in an oven for 24 hours at 37°C. After the incubation period, the microbial growth inhibition zone diameter was measured with a ruler and recorded in millimeters.

The experiment was conducted according to a randomized block design. The factorial design was 4 x 2 x 7, four bacteria (*S. aureus* and *E. coli* isolated from sheep carcass and *S. aureus* ATCC 25923 and *E. coli* ATCC 8733), two oil-seven concentrations, totaling 56 treatments with two replications. Data were subjected to analysis of variance, the SAEG 9.1 program, and the averages compared by Scott-Knott test at 5% significance level.

In the minimum bactericidal concentration test, was employed the broth macrodilution technique described by Chanwitheesuk *et al.* (2007) with modifications. The seven concentrations used in the diffusion test were tested, using the same principles as in serial dilution, but replacing the distilled water by BHI broth (Himedia®), preserving the volumes. At the end, we added 2.5 μL of each inoculum obtained from isolates of the sheep meat adjusted in a manner equivalent to the McFarland 0.5 standard solution and maintained the tubes incubated for 24 hours at 37°C. After incubation, an aliquot of each tube was plated on agar plates (TSA Triptic Soy Agar Himedia®) which were taken to an oven under the same conditions. The absence of bacterial growth was defined as the MBC. The test was conducted in duplicate.

Verification of oil efficiency disinfectant

In determining the disinfectant activity, the methodology described by Medeiros *et al.* (2009) was employed with suspension test techniques defined by the Ministry of Agriculture, Livestock and Supply (Brasil, 1993). Concentrations of 400 and 200 μL/mL were tested, the most effective in diffusion test plates and the minimum bactericidal concentration of each essential oil against *S. aureus* ATCC 25923 and *E. coli* ATCC 8733. Bacterial suspensions were prepared in 0.85% saline, equivalent to a 0.5 McFarland standard solution with approximately $1.5x10^8$ CFU/mL.

A 900 μL volume of disinfectants - used in item 2.5, prepared with the essential oil, Tween 80 and distilled water - was distributed aseptically into tubes and added to 100 μL of sterile organic matter, being 10 g of sheep meat, homogenized with 100 ml of distilled water and tyndallized once for 30 minutes. Subsequently, 10 μL of the bacterial suspension was added and exposure durations

timed. After 5, 15, 30 and 60 minutes, via bacteriological loop, an aliquot was transferred to tubes containing 5 mL Brain Heart Infusion broth (Himedia®), shaken and incubated at 37°C for 96 hours. Also at 5 minutes, aliquots were placed on Baird-Parker agar (Himedia®) and CP (Oxoid®) for counting the number of colonies after this of contact period between disinfectant solution, the organic substance and inoculum. After inoculation, the plates went into an oven at 37°C for 24 hours.

At 24, 48, 72 and 96 hours, presence of turbidity of tube contents, surface film formation and/or precipitate on the bottom of the glassware was examined. Turbidity, or not, was considered, respectively as active bacteria (negative) or inactive (positive). The tubes classified as positive were transferred to solid media for specific for *S. aureus*, (Baird-Parker Himedia®) and *E. coli* (EMB, Oxoid®), incubated at 37°C for 24 hours, to confirm the effectiveness of the disinfectant by the absence of bacterial growth on the plates.

The test with each concentration was performed in duplicate and we used descriptive statistical analysis.

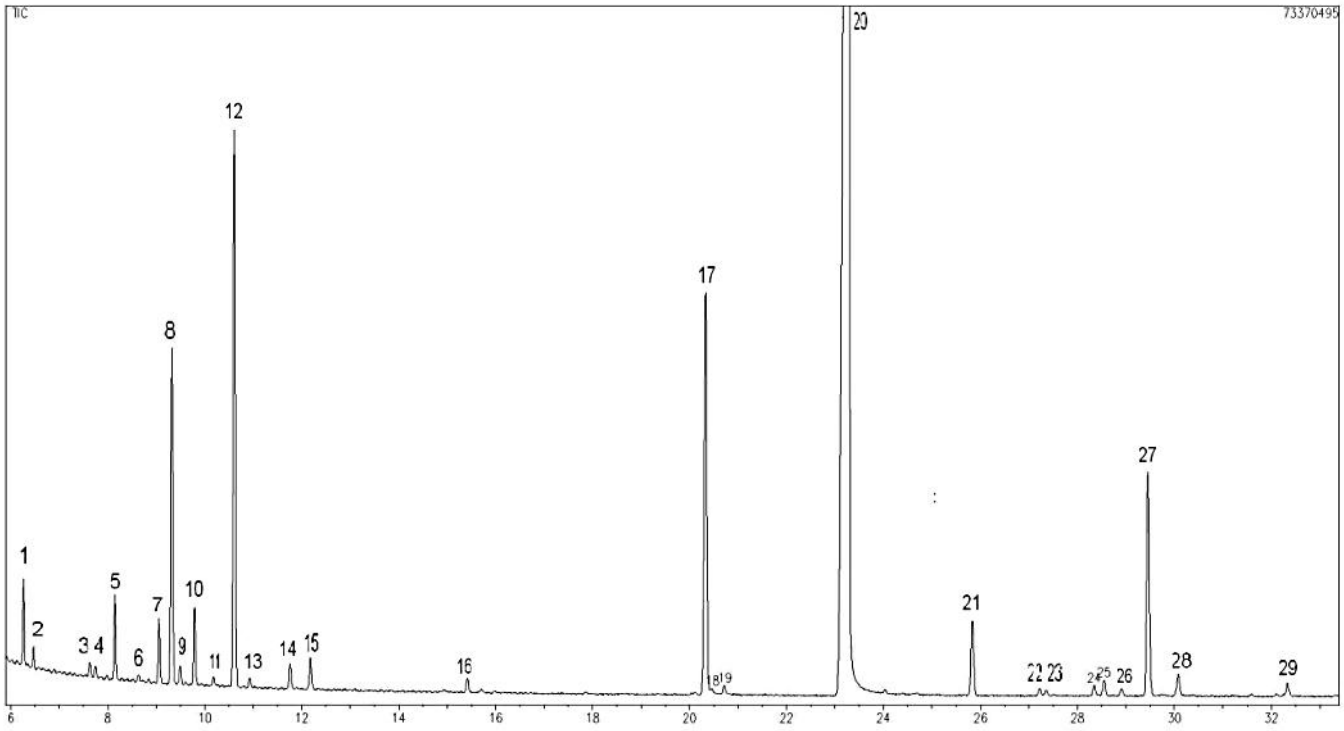
Result and discussion

Yield and chemical characterization of essential oils

The essential oil yields, 2.77% for *Ocimum gratissimum* and 5.04% for *S. aromaticum*, was higher than that found in the literature. Pereira (2006) obtained a yield of 2.32% from *S. aromaticum* inflorescences, and the literature reports a range from 0.29% to 1.12% in the yield of *O. gratissimum* (Craveiro *et al.*, 1981; Faria *et al.*, 2006; Ngassoum *et al.*, 2003). According to Cimanga *et al.* (2002), these variations can be attributed to factors such as climate, plant age, harvest time and the extraction method. Silva *et al.* (1999) demonstrated the sunlight influence on eugenol production. The content of this aromatic substance ranged from 98.0% in the oil extracted from the leaves of *O. gratissimum* harvested at 12:00 p.m., to 11.4% in the collection at 5 p.m.

According to the literature, the essential oil of *O. gratissimum* has two chemical types: thymol and eugenol (Silva *et al.*, 1999; Silva *et al.*, 2010). Freire *et al.* (2006) added geraniol to the list. According to the chromatographic analysis, the essential oil of this present study is the eugenol type, with 61.28% of the substance (Figure 1).

Figure 1 – Chromatogram of total ions of *O. gratissimum* essential oil



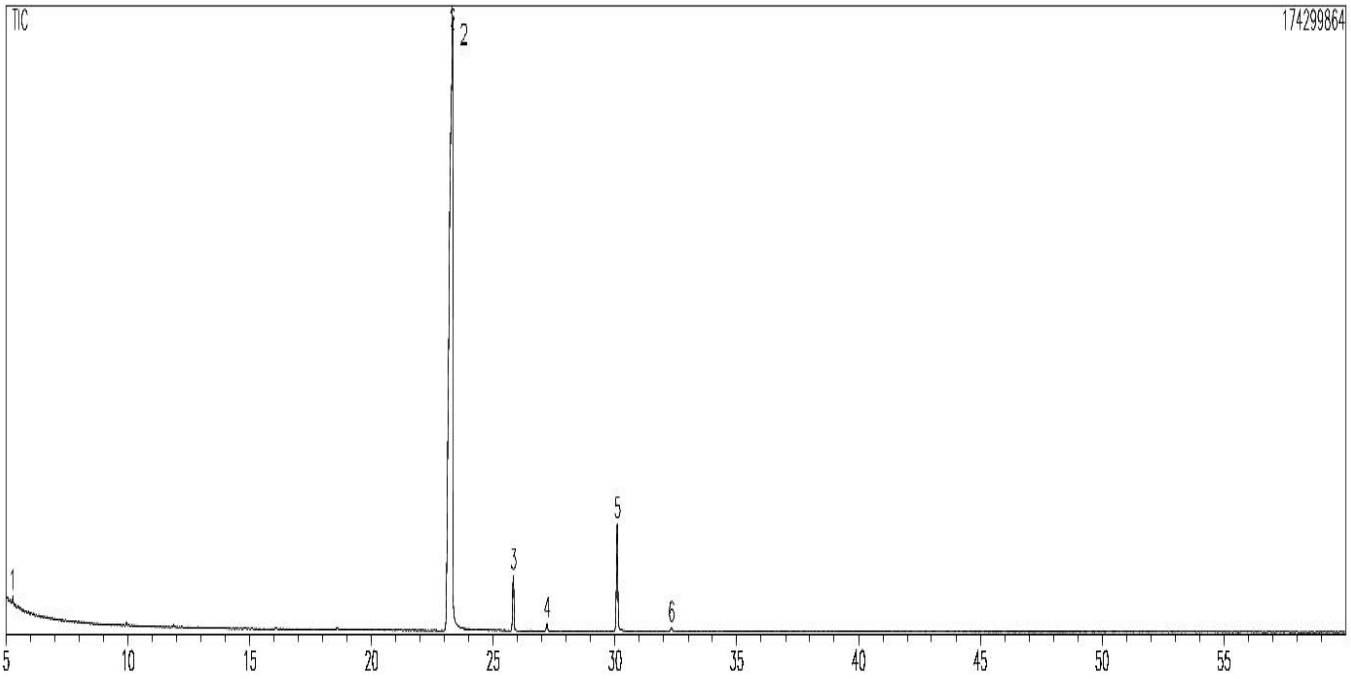
1- tricyclene (1.07%), 2- α-pinene (0.30%), 3- sabinene (0.21%), 4- β-pinene (0.17%), 5- myrcene (1 21%), 6- α-phellandrene (0.18%), 7- α-terpinene (1.02%), 8- p-cymene (5.23%), 9- limonene (0.33%) 10- *cis*-β-ocimene (1.14%), 11- *trans*-β-ocimene (0.17%), 12- γ-terpinene (8.88%), 13- unidentified compound (0.19%), 14- m-cymene (0.45%), 15- linalool (0.58%), 16- unidentified substance (0.30%), 17- thymol (8.07%), 18- o-methoxyacetophenone (0.22%), 19- carvacrol (0.27%), 20- eugenol (61.28%), 21- *trans*-caryophyllene (1.60%), 22- α-humulene (0.20%), 23- β-farnesene (0.15%), 24- germacrene D (0.25%), 25- β-selinene (0.38%), 26- α-selinene (0.19%), 27- β-bisabolene (4.98%), 28- eugenyl acetate (0.60%), 29- unidentified substance (0.40%).

Eugenol is the main bacterial agent present in the oil extracted from *Ocimum gratissimum* leaves, but the presence of thymol (8.07%), a recognized antimicrobial (Burt, 2004) was also found as one of the components. Cimanga *et al.* (2002) speculate on the involvement of less abundant constituents, such as thymol, carvacrol,

linalool and γ-terpinene, in the biological activity of the essential oils. For Maciel *et al.* (2002), numerous plant constituents or classes of compounds with different structures, contribute to the same activity. This responsibility does not lie only among the substances present in higher proportions, the synergistic effect among the components also being suggested. Bakkali *et al.* (2008) corroborate this statement. For them, it is more significant to study the oils as a whole than just some of their components, because it is the complex mixtures whose biological effects can be the result of key molecules present or the synergy among all the molecules identified by chromatographic analysis.

The phenolic compound, eugenol, is also the principal component of the essence of *Syzygium aromaticum* (Figure 2). Studied as a food preservative (Pereira, 2006), it is found in *Syzygium aromaticum* and other plants. Attributed to this substance are antifungal (Faria *et al.*, 2006), anti-mycotoxigenic (Dambolena *et al.*, 2010), antibacterial (Santurio *et al.*, 2007) and antioxidant activity (Pereira and Maia, 2007). Wenqiang *et al.* (2007) and Oliveira *et al.* (2009) also found eugenol as the most abundant principle component in *S. aromaticum* oil.

Figure 2 – Chromatogram of total ions of *S. aromaticum* essential oil



1- unidentified substance (0.29%), 2- eugenol (88.93%), 3- *trans*-caryophyllene (3.23%), 4- α-humulene (0.49%), ethyl 5- eugenyl (6.80%), 6- caryophyllene oxide (0.26%).

In vitro antimicrobial activity of essential oils on *E. coli* and *S. aureus*

In the diffusion plate tests, strains react differently to oils, independent of both having eugenol as the principle component. Halo diameter averages are shown in Table 1. Inhibition zones were observed in only three of the seven concentrations (400, 200, and 100 μL/mL), which lowered the average of the results, but did not interfere with their interpretation. The inhibition zone is the area with no detectable growth of microorganisms with the naked eye (CLSI, 2018b) and is directly related to the sensitivity of the bacterial sample (Brasil, 2008).

The means 4.00 mm and 5.14 mm show the best *S. aromaticum* oil action, compared to the *Ocimum gratissimum* (2.79 and 3.43 mm), against the bacteria isolated from sheep meat. The same was not observed with the standard ATCC microorganisms that statistically responded analogously to both oil activities. The results reported by Oussalah *et al.* (2007) and Pereira (2006) confirm these data, because the former tested 28 plants against *E. coli* O157: H7, *S. Typhimurium*, *S. aureus* and *L. monocytogenes* and seven of them, *S. aromaticum* among them, showed strong antimicrobial activity against the four bacteria, and the latter, also assigned the best *E. coli* and *S. aureus* inhibition results *S. aromaticum*.

Table 1 – Inhibition halo diameters (mm) of the essential oils of *S. aromaticum* and *O. gratissimum* against *S. aureus* and *E. coli*

Oil	***S. aureus* from sheep carcass**	***S. aureus* ATCC 25923**	***E. coli* from sheep carcass**	***E. coli* ATCC 8733**
	Halo diameter (mm)			
O. gratissimum	2.57 Bb	2.36 Ab	3.71 Ba	4.79 Aa
S. aromaticum	4.00 Ab	2.79 Ab	5.14 Aa	3.43 Ab

Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level. Uppercase letters compare the means in columns and lowercase, on the lines.

The essential oil of *Ocimum gratissimum* was more effective against Gram-negative bacteria, with average diameters of 3.71 and 4.79 mm. In the work of Kotzekidou *et al.* (2008), among the tested species, Gram-negative were the most sensitive to oils and plant extracts and Gram-positive, more resistant, confirming the results. In general, gram-negative bacteria such as *E. coli* and *Salmonella* sp., are less sensitive to the action of essential oils. This is probably due to the difficulty of the compounds to act on the complex cell wall structure of these microorganisms (Cimanga *et al.*, 2002; Holley and Patel, 2005). Burt (2004) cites other studies that strengthen this proposition. The cell wall of Gram-positive bacteria is typically permeable and does not restrict the passage of antimicrobial agents. The outer membrane of the Gram-negative favors resistance and therefore does not allow, or at least complicates the introduction of toxic molecules into the cytoplasm or other destination (Denyer and Maillard 2002; Lambert, 2002).

The diffusion plate method (disk diffusion and agar diffusion) is simple, reliable (Brasil, 2008) and one of the most used in verification testing of essential oil antimicrobial activity (Tajkarimi *et al.*, 2010), as it ascertains, quickly, the susceptibility of microorganisms to many substances (Ponce *et al.*, 2003). In addition to the structural diversity of the cell, the metabolic differences between microorganisms should be considered (Holley and Patel, 2005), since the antimicrobial action of the constituents vary considerably according to species lineage (Silva *et al.*, 2009).

The biological effect of phenolic compounds such as eugenol and essences in general, is easily observed in the experiments, but the action mechanism has not been fully elucidated. Normally, it is considered as the start of the cell wall degradation mechanism, causing damage to protein and cytoplasmic membrane, interrupting the proton motive force, the flow of electrons and active transport, favoring coagulation of the cytoplasm (Kotzekidou *et al.*, 2008; Silva *et al.*, 2010). According to the results, 400μL/mL was the most effective concentration for the strains two, three and four, with halos of 10.25, 12.25 and 13.75 mm (Table 2), respectively, being more efficient for *S. aromaticum* oil, with a 13.38 mm inhibition zone (Table 3). Strain one reacted similarly to 400 and 200 μL/mL. The research of Duarte *et al.* (2007) differed from this present work. When testing the activity of essential oils extracted from Brazilian medicinal plants against different *E. coli* serotypes they observed *Ocimum gratissimum* inhibitory activity at concentrations from 600 to > 1000 μL/mL, or at least 50% higher. Pereira (2006), with the essence of *S. aromaticum* tested on *S. aureus* and *E. coli*, observed inhibition zone formation at virtually all concentrations, but the most effective for *S. aureus* was 50%, higher than that of the present work, and 10% for *E. coli*, a more satisfactory result. In analogy, it appears that 400 μL/mL represents 40% oil in solution.

The lower the inhibitory concentration, the better for use in foods because these compounds have outstanding flavor and aroma and can alter the organoleptic characteristics of foodstuffs (Nedorostova *et al.*, 2009; Tajkarimi *et al.*, 2010). The use of essential oils to promote food safety is a viable technique (Goñi *et al.*, 2009), but having determined the concentration capable of preventing microbial growth, any change can modify the nature of the inhibitory effect (Holley and Patel, 2005), compromising the microbiological quality of the product.

The concentration used is also important in the verification of the inhibitory activity of essential oils (Ponce *et al.*, 2003), because together there can be a cytotoxic effect on the living cell (Bakkali *et al.*, 2008). Hardly only one natural antimicrobial, however potent, could be used effectively in a single concentration in all kinds of food and against all microorganisms (Holley and Patel, 2005; Yossa *et al.*, 2010).

The biological activity of essential oils of *E. coli* and *S. aureus* is represented by regression equations represented in Figure 3. The results were discussed in the text and confirm the action and the power of the tested antimicrobials. The straight lines show the halo diameter behavior in relation to the interaction of the bacteria with the oils and concentrations tested. The inhibitory

effect, that is, the antibiotic sensitivity represented by the inhibition zone increased as the concentration increased, but varied according to the oil applied and test-microorganisms used. Concentrations below 100 μL/mL showed no biological activity.

Table 2 – Relationship between *O. gratissimum* and *S. aromaticum* essential oil concentrations and Gram-positive and negative bacteria

Bacteria	Concentration (μL/mL)						
	400	200	100	50	25	12,5	6,5
	Halo diameter (mm)						
1	11.50 Aa	9.25 Aa	2.25 Bb	0 Ab	0 Ab	0 Ab	0 Ab
2	10.25 Aa	6.00 Bb	1.75 Bc	0 Ac	0 Ac	0 Ac	0 Ac
3	12.25 Aa	10.25 Ab	8.50 Ab	0 Ac	0 Ac	0 Ac	0 Ac
4	13.75 Aa	11.00 Ab	4.00 Bc	0 Ad	0 Ad	0 Ad	0 Ad

Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level. Uppercase letters compare the means in columns and lowercase on the lines.
1- *S. aureus* from sheep carcasses; 2- *S. aureus* ATCC 25923; 3- *E. coli* from sheep carcasses; 4- *E. coli* ATCC 8733.

Table 3 – Relationship between *O. gratissimum* and *S. aromaticum* essential oil concentrations

Oil	Concentration (μL/mL)						
	400	200	100	50	25	12,5	6,5
	Halo diameter (mm)						
O. gratissimum	10.50 Ba	8.25 Ab	4.75 Ac	0 Ad	0 Ad	0 Ad	0 Ad
S. aromaticum	13.38 Aa	10.00 Ab	3.50 Ac	0 Ad	0 Ad	0 Ad	0 Ad

Means followed by the same letter do not differ by the Scott-Knott test at 5% significance level. Uppercase letters compare the averages in columns and lowercase on the lines.

The results obtained by Nakamura *et al.* (1999) are consistent with those of this study, since the bactericidal concentration of the *Ocimum gratissimum* essence for *S. aureus* was lower, 1.5 μg/mL, compared to that obtained for *E. coli*, 12 μg/mL. In contrast, Silva *et al.* (2010) found antagonistic results, i.e., lower MBC for Gram-negative bacteria and higher for Gram-positive; however, the researchers used the oil from the inflorescences and not the leaves.

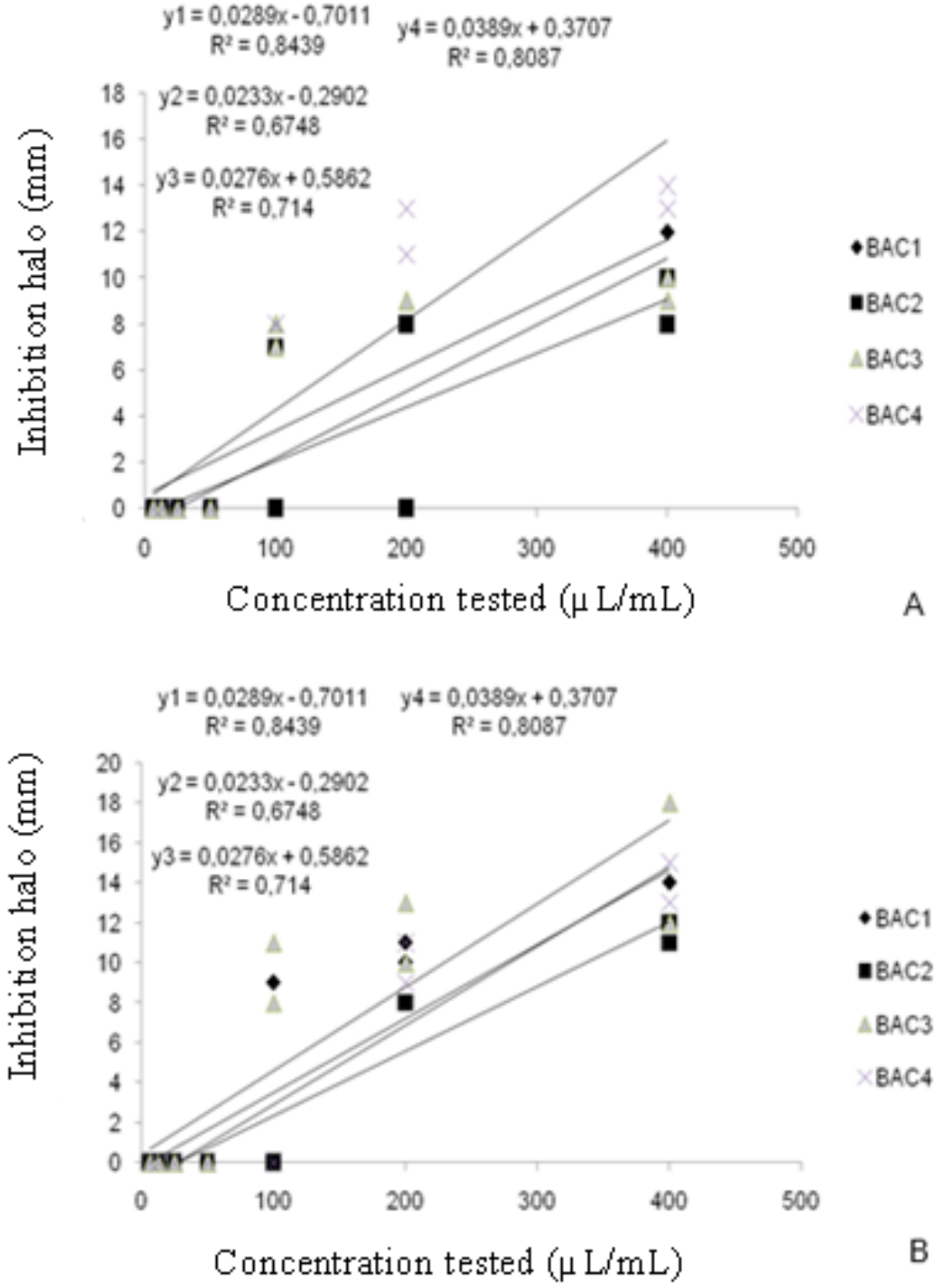
As with the *Ocimum gratissimum*, the essence of *Syzygium aromaticum* presented the lowest minimum bactericidal concentration against *S. aureus*, with 50 μL/mL. In research of Hoffmann *et al.* (1999), *S. aromaticum* oil showed to be a good antimicrobial as it completely inhibited bacteria such as *S. aureus* and S. *enteritidis*, in addition to yeast, at 10%. Moreira *et al.* (2005) in their work, observed the effectiveness of *S. aromaticum* against strains of *E. coli* O157: H7 only at concentrations 30 times higher than that found in this present study. Other favorable results for S. aromaticum, but contrary to the results obtained, i.e., with lower concentrations identified for Gram-negative bacteria, were Fu *et al.* (2007) and Silva *et al.* (2009), whose units used in dilution were different from those in the present work.

To increase the chances of complete inactivation of the colonies, Berrington and Gould (2001) suggest the application of the highest concentration used in the tests. Ponce *et al.* (2003), however, suggest small changes in low concentrations to achieve significant changes in cell behavior. Upon altering the highest values, not even the most relevant changes promoted an inactivation that justifies the use.

Inequalities in the effects of susceptibility testing can be explained by the lack of uniformity in the criteria established by the researchers (Ríos and Recio, 2005). The contradictions observed and the difficulty in comparing the results obtained in researche that verifies the antimicrobial activity of medicinal plants may result from changes in environmental conditions at collection time, the plant part studied, the procedures and protocols followed (Auricchio and Bacchi, 2003), the physicochemical characteristics of the essential oils (Nakamura et al, 1999), reciprocity of its constituents, and in the extraction form (Moreira *et al.*, 2005; Ponce *et al.*, 2003), among others.

The data shows the sanitizing potential of essential oils of *Ocimum gratissimum* and clove. Confirmation of the antiseptic character was observed in *in vitro* testing of the efficiency as a disinfectant.

Figure 3 - Inhibition halo (mm) determined by the antimicrobial effect of essential oil of *O. gratissimum*. (A) and *S. aromaticum* (B) on *E. coli* and *S. aureus* in function of the concentrations tested (μL/mL)



BAC1- *S. aureus* of sheep carcasses, BAC2- *S. aureus* ATCC 25923, BAC3- *E. coli* of sheep carcasses, BAC4- *E. coli* ATCC 8733

The minimum bactericidal concentration (MBC) is defined as the lowest concentration of essential oil that can completely destroy the inoculum (Fu *et al.*, 2007; Pozzo *et al.*, 2011). The MBC of essential oils of *Ocimum gratissimum* and *S. aromaticum* against *S. aureus* was 50 μL/mL. As for *E. coli*, it was 200 and 100 μL/mL, respectively.

The results show a lower bactericidal concentration, 50 mL, for *Ocimum gratissimum* oil against *S. aureus*, whereas for *E. coli* the same effect was only observed at 200 μL/mL, contrary to the response obtained with previously run diffusion plate tests (Table 1). The dilution method, microdilution, macrodilution or agar dilution, is applied to define the lowest bactericidal or bacteriostatic concentration of an antimicrobial agent (Alves *et al.*, 2008). It is the most efficient to establish the potency of a substance, since in the diffusion method, for example, the possibility of failure is increased due to possible errors in the preparation of the culture medium, the suspension

density, the halo measurement, unsuitable incubation time and/or temperature etc. (Ríos e Recio, 2005).

In vitro efficacy of essential oils as disinfectants

According to the Ministry of Agriculture, Livestock and Supply (Brasil, 1993), disinfectant is a substance, or a group of them, "capable of destroying non-sporulated pathogenic microorganisms in a short period of time when applied to inanimate objects." The results herein are consistent with the aforementioned definition (Brasil, 1993). There was a reduction in the number of cells within the first 5 minutes. The antiseptic composed of *Ocimum gratissimum* at the highest concentration reduced *E. coli* and *S. aureus* by 87 and 88%, respectively, and at a concentration of 200 μL/mL 58 and 78% less colonies were observed. The product based on *Ocimum gratissimum* at 400 μL/ml completely eliminated the two species early in the process. When the test was conducted with the lowest concentration, there was only a cell count decrease in *S. aureus* and *E. coli* of 67 and 27%, respectively.

Avancini and Wiest (2008) tested the effectiveness of disinfectant composed of a medicinal plant in the presence of organic matter in this present work whereas Oliveira *et al.* (2010) and Tresoldi (2008) developed the tests in the absence of this matter. These research works strengthen the results in that in all of them, likewise, found a direct relationship between infectious dose inactivation and time, i.e., the longer the disinfectant contact time, the higher the microorganism inactivation. However, Chorianopoulos *et al.* (2008) found that, in general, significant reductions in the number of colonies occur within the first 60 minutes of contact with the natural product. For the authors, extending this period does not improve the disinfection efficiency

The growing interest in antibacterial compounds, such as antibiotics and disinfectants, derived from plants arises from the need to create new strategies (Oliveira *et al.*, 2010) to control, among other things, the pathogens present in the air (Bouaziz *et al.*, 2009), zoonotics (Palaniappan and Holley, 2010), the formation of biofilms and also to meet increasing consumer demand averse to synthetic compounds. Among the alternatives to the use of disinfectants and commercial chemicals, some researchers propose the use of medicinal plants (Avancini *et al.*, 2000).

The number of research work seeking to verify the effectiveness of natural compounds, like disinfectants, is lower compared to work with commercial chemicals. The presence of compounds with proven inhibitory activity stimulates further study to obtain safer and more natural ways to preserve food. *In vitro* study results show oils and plant extracts as potentially rich sources for medicine and the food industry, due to the broad spectrum of activity against pathogens (Fu *et al.*, 2007; Kotzekidou *et al.*, 2008).

In addition to the lower cost, these substances can circumvent the negative effects caused by the indiscriminate use of conventional antibiotics, favoring veterinary medicine, farmers, consumers and the environment (Avancini and Wiest, 2008; Bouaziz *et al.*, 2009; Tresoldi, 2008).

Conclusion

The essential oils of *O. gratissimum* and *S. aromaticum* showed satisfactory *in vitro* bactericidal inhibitory and antiseptic activity on the bacteria *S. aureus* and *E. coli* isolated from sheep carcasses and being promising in the use as an alternative to antibiotics and commercial antiseptics. However, more extensive studies are necessary to verify adverse effects.

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