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MICROBIOLOGY

Antioxidant activity of essential oils from condiment plants and their effect on lactic cultures and pathogenic bacteria

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ABSTRACT: *Studies about preservative and antioxidant activity of essential oils have been encouraged in recent years, given their importance to food industry. The aim of the current study was to evaluate the antioxidant properties and antimicrobial activity of essential oils deriving from Syzygium aromaticum, Cymbopogon citratus and Lippia alba against lactic and pathogenic bacteria responsible for food-borne diseases. Essential oil antibacterial activity was assessed through disc diffusion and macrodilution tests conducted in a mixed lactic culture of Lactobacillus delbrueckii subsp. bulgaricus and Streptococcus thermophilus (YF-L903) and of Escherichia coli (ATCC 8739), Staphylococcus aureus (ATCC 6538), Salmonella enterica (ATCC 6017) strains. Based on the chromatographic analysis results, the essential oils shown to be composed of eugenol (79.41%) which was the prevalent compound in S. aromaticum, geranial (31.89%), neral (24.52%) and β-myrcene (25.37%) in C. citratus, as well as of geranial (33.80%) and neral (25.63%) in L. alba. The observed antibacterial activity confirmed the dose-dependent effect of these three oils on all the assessed bacteria; there was halo inhibition at concentration 20μL mL^{-1}. The essential oil of S. aromaticum presented better antioxidant activity, with IC_{50} equal to 5.76μg mL^{-1} and antioxidant activity index of 6.94, and it was considered strong (AAI>2.0) in comparison to the other evaluated oils. This essential oil also presented excellent antioxidant activity at concentrations lower than the one required to inhibit lactic cultures. Based in this outcome, the essential oil from S. aromaticum can be used as preservative agent in processed food whose formulation presents lactic cultures.*
Key words: *capim cidreira, clove, lemongrass, lactic acid bacteria, probiotics.*

Atividade antioxidante do óleo essencial de plantas condimentares e efeito sobre culturas lácticas e bactérias patogênicas

RESUMO: *A atividade conservante e antioxidante de óleos essenciais são importantes ferramentas para uso na indústria de alimentos e pesquisas são estimuladas na atualidade. O objetivo deste trabalho foi avaliar as propriedades antioxidantes e a atividade antimicrobiana de óleos essenciais de Syzygium aromaticum, Cymbopogon citratuse Lippia alba (LA) contra bactérias lácticas e patogênicas com importância em doenças transmitidas por alimentos. A atividade antibacteriana dos óleos foi avaliada por testes de disco-difusão e macrodiluição em caldo, utilizando-se cultura láctica mista de Lactobacillus delbrueckii subsp. bulgaricus e Streptococcus thermophilus (YF-L903), e Escherichia coli (ATCC 8739), Staphylococcus aureus (ATCC 6538), Salmonella enterica (ATCC 6017). Os compostos dos óleos essenciais foram identificados por cromatografia, mostrando eugenol (79,41%) como composto predominante em S. aromaticum, geranial (31,89%), neral (24,52%) e β-mirceno (25,37%) em C. citratus, e o geranial (33,80%) e neral (25,63%) em L. alba. A atividade antibacteriana confirmou o efeito dose-dependente dos três óleos para todas as bactérias avaliadas, apresentando halos de inibição a partir da concentração de 20μL mL^{-1}. O óleo essencial de S. aromaticum apresentou melhor atividade antioxidante, com IC 50 IC_{50} (at concentration capable of generating 50% inhibition) igual a 5.76μg mL^{-1} e índice de atividade antioxidante (AAI) de 6,94, considerado forte (AAI>2,0) em relação aos demais óleos avaliados. O óleo essencial de S. aromaticum apresentou excelente atividade antioxidante em menor concentração que anecessário para inibir a cultura láctica, indicando que este óleo pode ser usado como conservante em alimentos processados contendo culturas lácticas.*
Palavras-chave: *capim cidreira, cravo da índia, capim limão, bactérias láticas, probioticos.*

INTRODUCTION

Many studies have been investigating the bioactive compounds reported in essential oils given their importance as food preservative agents for the food industry (KHORSHIDIAN et al., 2018). The essential oils from *S. aromaticum* (L.), *C. citratus* (DC) Stapf and *L. alba* (Mill. NE Brown) also have great potential for pharmaceutical applications (GULDIKEN et al., 2018).

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S. aromaticum (L.) is native to Indonesia but; nowadays, it is grown in several parts of the world, including Brazil. This plant is one of the richest sources of phenolic compounds such as eugenol, eugenol acetate and gallic acid, fact that explains its great potential for pharmaceutical, cosmetic, food and agricultural applications (CORTÉS-ROJAS et al., 2014). The essential oil from *S. aromaticum* (L.) is known by its great antioxidant (BAKOUR et al., 2018, CORTÉS-ROJAS et al., 2014) and antimicrobial activity (PUŠKÁROVÁ et al., 2017), mainly against multidrug-resistant bacteria (NAVEED et al., 2013; ABDULLAH et al., 2015).

C. citratus, commonly known as 'lemongrass', is a widely distributed perennial herb belonging to Family *Poaceae*. This plant has been extensively consumed given its medicinal, cosmetic and nutritional effects, and pleasant aroma and taste it gives to food. Some of its important pharmacological properties were already reported in the literature (AVOSEH et al., 2015; EKPENYONG et al., 2015).

L. alba, also knowni n Portuguese as "erva cidreira or "erva cidreira Brasileira" (JANNUZZI et al., 2011), belongs to Family *Verbenaceae*. It is a very rustic and strong Brazilian shrub distributed all over the South and Central Americas (GOMES et al., 2017). Essential oil from *L. alba* is acknowledged by its broad spectrum of activities against Gram-negative and Gram-positive bacteria(ZAMORA, et al., 2018) and by its antioxidant action (TREVISAN et al., 2016).

These essential oils are classified as substances often acknowledged as safe (GRAS) by the Food and Drug Administration (FDA) (e-CFR, 2018). Although, extensive studies have evaluated their potential as food preservative, only few studies assessed their effects on lactic cultures used in the food industry. Accordingly, the aim of the current study was to assess the antioxidant and antimicrobial activity of essential oils from condiment plants on lactic cultures.

MATERIALS AND METHODS

The leaves of *C. citratus* and *L. alba* plants cultivated in the Medicinal Plant Garden of the Institute of Agricultural Sciences of Federal University of Minas Gerais (ICA/UFMG) were used for essential oil extraction based on the steam-dragging distillation technique, which was applied in a pilot distiller (Linax®, model D20, SP, Brazil). The oil was separated from the hydrolyte through liquid-liquid partitioning and, after 3 hours of extraction, it was removed with a micropipette and stored in sterile amber glass vials at 4-8°C (ANDRADE et al., 2014). The essential oil from *S. aromaticum* was provided by Ferquima (Vargem Paulista, SP, Brazil).

Chemical composition of the assessed oils was analyzed at the Laboratory Instrumental Chemistry of ICA/UFMG/Montes Claros/Brazil. Samples were subjected to chromatographic analysis 7890 A (Agilent Technologies) coupled to a mass spectrometer (MS 5975C) with a fused silica capillary column DB5-MS (30mx0.25mmx0.25µm); helium (99.9999% of purity) was used as drag gas at 1mL min^{-1} flow. The injector was kept at 220ºC, at flow split ratio 1:5 and; subsequently, it was subjected to 60ºC-240ºC (3ºC min^{-1}) for 10min. Interface temperature was kept at 240°C. The system operated in full scan with electron impact 70 eV, in the range 45-550 (m/z). Retention index of all compounds was calculated based on the retention time of a mixture comprising n-alkanes (C7-C40, Sigma USA) 20ppm, split 1:100.

The generated data were analyzed in the MSDE Chem Station software. The relative abundance (%) of the total ions was calculated by taking into account the peak area of the chromatogram (GC-MS) and organized according to the order of elution. Compounds were identified by comparing the mass spectrum to that of the library NIST 2.0 (2009), based on the relative retention index (RI), which was calculated according to VAN DEN DOOL and KRATZ (1963), and compared to information available in the literature (ADAMS, 2012).

Antibacterial activity screening

The antibacterial action of the essential oils was analyzed through the disk diffusion method with paper disks proposed by CLSI (2015a), with adaptations. The screening analysis was applied to measure the antibacterial activity of the assessed essential oils against *Escherichia coli* (ATCC 8739), *Staphylococcus aureus* (ATCC 6538), *Salmonella enterica* (ATCC 6017) strains and against a mixed lactic culture of lyophilized granules (YoFlex® Harmony 1.0) (CHR Hansen, Hoersholm, Dinamarca) of *Lactobacillus delbrueckii* subsp. *bulgaricus,* and *Streptococcus thermophilus* (YF-L903). Test inoculates were standardized in 0.9% saline solution to generate 10^4 to 10^5UFCmL^{-1}. Based on the McFarland standard; this value represented the score 0.5 for each bacterium (CLSI, 2015b).

Serial dilutions were prepared at essential oil concentrations 160, 80, 40, 20, and 10µL mL^{-1} at BHI (Himedia, Mumbai, India), by using 0.1%

Tween-80 (Synth, Diadema, SP, Brazil). Sterile filter paper discs (JProLab, São Paulo, SP, Brazil) -6mm diameter-were saturated with 30µL of each assessed concentration. After the discs were dry, they were placed on petri dishes with the studied inoculate. Antibiotic disks with ciprofloxacin (5mcg) (Laborclin, São Paulo, SP, Brazil) and disks with sterile distilled water were used as control. All tests were performed in triplicate and incubated at 37ºC for 18 hours. The inhibition zone diameter was expressed in mm, including the diameter of the disc. Sensitivity classification was based on PUŠKÁROVÁ et al., (2017), samples were sensitive at inhibition zone diameter was 9–14mm.

Treatments adopted for the antimicrobial activity tests, which were conducted through disc diffusion, followed a completely randomized design based on the 4x3x5 factorial arrangement (four bacteria, three oils and five concentrations); thus totaling 60 treatments, with three replicates. Results were subjected to analysis of variance (ANOVA) and linear regression in the R Statistical software (2011). Statistically significant differences were set at $p<0.05$.

Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) evaluations

The Minimal Inhibitory Concentration (MIC) was set through the macro dilution method conducted in tubes, based on CLSI (2015b), with adaptations. The oil concentrations used in the experiments were defined based on screening-test results:10 to 320µL mL^{-1} for the essential oils from *C. citratus* and *L. alba*, and 20 to 320µLmL^{-1} for the essential oil from *S. aromaticum*. Aliquots of 30µL mL^{-1} of the standardized inoculum of each strain were placed in tubes filled with 5mL of each oil dilution.

Negative controls were prepared with the three oils for the tests, and the positive controls were prepared with the four cultures. Tests were conducted in triplicate and the solutions were incubated at 35-37ºC for 24 hours. Triphenyltetrazolium chloride (TTC) (Dinâmica Química Contemporânea Ltda., Diadema, SP, Brazil) was added to the tubes to confirm absence of bacterial growth. Colors of the positive and negative controls were compared based on the methodology by DUARTE et al. (2005).

Minimal Bactericidal Concentration (MBC) was used to calculate the lowest essential oil concentration capable of stopping bacterial growth in tryptone soy agar (TSA, Himedia) (CLSI 2015b). The same procedure was applied to bacterial culture controls, oils and to culture media with Tween-80. Fisher-Bonferroni test, at 0.05 significance level, was adopted to calculate the diameter of the growth inhibition halos, which were generated at different oil concentrations.

Descriptive statistics was the instrument of choice to assess the antimicrobial activity of MIC and MBC. Results of the three tubes with each oil concentration were used in each analysis.

Antioxidant activity

The Free Radical Scavenging Capacity (RSC) was found by measuring the scavenging activity of essential oils assessed in 2.2-diphenyl-1-picrylhydrazil (DPPH) and OH radicals, as described by BOZIN et al. (2006), with adaptations. The chosen essential oil concentrations were based on previous results of antimicrobial activity and used to standardize the tests. The defined concentrations were: 25 to 400µg mL^{-1} of *C. citratus* oil, 12.5 to 200µg mL^{-1} of *L. alba* oil and 5 to 25µg mL^{-1} of *S. aromaticum* oil. Oils were mixed with 1mL of 40µg mL^{-1} solution (Sigma, St. Louis, MO) and with 95% MeOH (Synth, Diadema, Brazil) until they reached the final volume of 4mL. Both absorbance of resulting solutions and blank were recorded after1h, at room temperature-tert-butylated hydroxytoluene (BHT) (Sigma, St. Louis, MO) was the positive control. Each sample was recorded in triplicate. Readings were performed in spectrophotometer (Agilent Cary 60UV/Visible, Australia) adjusted to wave length 515nm. The RSC was calculated as follows:

$$RSC(\%)=100x(Ablank-Asample/Ablank)$$

The IC_{50} (at concentration capable of generating 50% inhibition) was graphically calculated through the calibration curve in linear range. The extract concentration vs. the corresponding scavenging effect parameter was plotted through linear regression analysis. The t test was adopted to find the significance of the regression coefficients. The experimental design was completely randomized.

The antioxidant activity was expressed by the antioxidant activity index (AAI), which was calculated as follows: final DPPH concentration (µg mL^{-1})/IC_{50}(µg mL^{-1}). Antioxidant activity was classified as low ($AAI<0.5$), moderate ($0.5<AAI<1.0$), strong ($1.0<AAI<2.0$) and very strong ($AAI>2.0$) in this test (SCHERER & GODOY (2009).

RESULTS AND DISCUSSION

Chemical composition of the essential oils

Based on information available in the literature, the major compounds of the three oils (Table 1) have antioxidant and antimicrobial activity.

Table 1 - Chemical composition of the essential oils from *Cymbopogon citratus* (CC), *Syzygium aromaticum* (SA) and *Lippia alba* (LA).

Compound	RI_{cal}	RI_{Lit}	----------% TIC----------		
			SA	CC	LA
α-pinene	932	932	-	-	1.70
Sabinene	971	969	-	-	1.00
1-Octen-3-ol	976	974	-	-	1.47
6-Methyl-5-hepten-2-one	982	985	-	0.69	1.65
β-myrcene	989	988	-	25.37	4.64
α-phellandrene	1007	1002	-	-	0.79
Cymene	1023	1022	-	-	0.63
Limonene	1028	1024	-	-	0.70
(*Z*)-ocimene	1033	1032	-	0.90	1.91
(*E*)-ocimene	1043	1044	-	0.55	2.04
β-Linalool	1099	1095	-	1.07	0.73
Unknown	1141	-	-	1.52	1.58
Unknown	1143	-	-	-	0.57
Unknown	1159	-	-	3.89	3.45
4.5-epoxy-carene	1178	1179	-	6.00	5.64
Myrtenol	1193	1194	-	-	0.86
Nerol	1222	1227	-	-	0.78
β-Citronellol	1225	1228	-	-	0.42
Neral	1238	1235	-	24.62	25.63
Geraniol	1248	1249	-	1.26	1.51
Geranial	1269	1264	-	31.89	33.80
2-Undecanona	1291	1293	-	0.51	-
Eugenol	1353	1356	79.4	-	-
Geranyl Acetate	1376	1379	-	-	0.44
(*E*)-Caryophyllene	1415	1417	7.29	0.51	3.39
Humulene	1451	1453	1.14	-	-
Germacrene D	1476	1484	-	-	2.68
α-Bisabolene	1505	1506	-	1.165	-
Unknown	1512	-	11.16	-	-
δ-Cadinene	1514	1513	0.33	-	-
Caryophyllene Oxide	1575	1582	0.65	-	1.16
Cedr-8(15)-en-10-ol	1642	1650	-	-	0.72

RI_{cal}=retention index calculated; RI_{Lit}=retention index in the literature; (-) non-detected compounds; TIC=Total Ion Chromatogram (obtained by gas chromatography/mass spectrometry).

Eugenol (79.4%) was the main compound in the essential oil from *S. aromaticum*. Approximately, 72% to 90% of the essential oil extracted from clove plants had eugenol. BHOWMIK et al. (2012) reported similar chemical compositions and KAPADIYA et al. (2018) pointed out that eugenol was the main component in essential oils from *S. aromaticum*. This compound has been associated with high antioxidant (CORTÉS-ROJAS et al., 2014; GÜLÇIN et al., 2018) and antimicrobial activity (NAVEED et al., 2013; HUSSEIN et al., 2014; ABDULLAH et al., 2015; PUŠKÁROVÁ et al., 2017).

The major compounds in the essential oil from *C. citratus* were geranial (31.89%), β-myrcene (25.37%) and neral (24.62%) (Table 1). Such result confirmed the good quality of this oil, which also presented high citral content. The quality of lemongrass plants is often determined by their citral content. Citral (3.7- dimethyl-2.6-octadienal) comprised cis-isomer neraland trans-isomer geranial compounds (ALI et al., 2017), which were responsible for the antioxidant (GUIMARÃES et al., 2011; GARCIA et al., 2015; COSTA et al., 2016; JAMUNA et al., 2017) and antibacterial activity of the assessed

oils (OLIVEIRA et al., 2013; EKPENYONG et al., 2015; AVOSEH et al., 2015; ALI et al., 2017).

Different chemotypes of *C. citratus* had different major compounds, besides showing remarkably higher percentage of volatile geranial and neral terpenoids in species distributed in Brazil (GUIMARÃES et al., 2011) and in other countries (AVOSEH et al., 2015). Myrcene was the major compound in African *C. citratus* species (AVOSEH et al., 2015; ALI et al., 2017). GUIMARÃES et al. (2011) observed this major compound in Brazil; although, at lower concentrations than the neral ones. Many studies in the literature attributed such difference in chemical compositions to a wide range of factors such as geographic location, climate conditions, harvest period, plant age and distillation method (AKINKUNMI et al., 2016; ALI et al., 2017).

Citral was the major component in the essential oil from *L. alba* (59.43%=geranial 33.8%+neral25.63%). This result was consistent with the one recorded for citral chemotype by JEZLER et al. (2011) in Bahia, Brazil, and by PANDELÓ et al. (2012) in Rio de Janeiro, Brazil. Many authors relied on the similarity between major compounds found in *L. alba* specimens, given the lack of established standardization to differentiate chemotypes in this species (JANUZZI et al., 2011). PIERRE et al. (2011) observed genetic diversity in *L. alba.* MARQUES et al. (2018) and TELLES et al. (2012) recorded great variations in the composition of the essential oil from *L. alba*, which suggested the existence of a large number of chemo types. Several factors, such as the plant part used for distillation, plant development stage and plant geographical origin, were associated with the observed variance in the composition (MACHADO et al., 2014; MARQUES et al., 2018).

Antibacterial activity

The growth inhibition response of all oils was dose-dependent ($p<0.05$) and changed according to the used bacterium (Figure 1). Higher *S. aromaticum* oil concentrations (40µL mL^{-1} a 320µL mL^{-1}) were required to inhibit the growth of all bacteria. *S. aureus* growth inhibition required lower *L. alba* oil concentration (40µL mL^{-1}) than other oil types and bacteria. The essential oil from *C. citratus* inhibited the growth of all bacteria at concentration 40µL mL^{-1} (Table 2). There was no standard halo size to indicate the antimicrobial activity of essential oils, such as the ones used for conventional antimicrobials. Essential oils presenting antimicrobial activity were the ones that generated 9-14mm growth-inhibition halos (PUŠKÁROVÁ et al., 2017).

The lowest concentration of essential oils from *L. alba* and *C. citratus* presenting inhibitory activity (MIC) was 10µL mL^{-1}, whereas that of *S. aromaticum* was 20µL mL^{-1} (Table 2), regardless of the inhibition-halo size. All bacteria recorded the same MIC value. The lactic culture recorded lower MBC value in all oils than the ones recorded for the other bacteria,whereas *S. aureus* recorded higher MBC value in all oils than the ones recorded for the other bacteria.

The effect of essential oils from *S. aromaticum*, *C. citratus* and *L. alba* on the lactic culture used in our study had not been described in literature, so far. Studies evaluating the effects of other essential oils have also reported lower MIC values for lactic cultures than the ones recorded for pathogenic bacteria. Thyme oil recorded lower MIC values for starter co-cultures such as *Lactococcus lactis* subsp. *lactis* and *L. lactis* subsp. c c*remoris* than for *Staphylococcus aureus* and *Listeria monocytogenes* (CARVALHO et al., 2015). SOUZA et al. (2016), also observed similar results when they evaluated the MIC value of oregano essential oil on these very same bacteria.

The literature has already described the use of spices in dairy products with beneficial effects on lactic cultures. GRANATO et al. (2018) conducted a review study on the latest discoveries in the dairy sector about new food added with herbal extracts and their beneficial impact on food quality. Few studies assessed these essential oils but, based on their results, these oils had no negative effects on different lactic cultures.

MAHMOUDI et al. (2017) conducted a review study on the feasibility of using essential oils in probiotic dairy products. They concluded that the treatment applied at mid concentrations enabled the largest number of viable probiotic bacteria. MARCIAL et al. (2016) evaluated the effect of the essential oil from *Origanum vulgare* on lactic starter cultures and did not find negative effects on the growth, or on the metabolic activity, of lactic acid bacteria such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus.* Although, some researchers have already suggested that, as starter cultures, lactic acid bacteria were relatively resistant to the toxic effects of some essential oils, others advocate that the application of EO doses enough to control pathogenic bacteria could negatively affect the growth and survival of starter cultures comprising lactic acid bacteria; they also speculate about a potential decrease in acid production, which could affect the proper sensory characteristics and safety of the products (CARVALHO et al., 2015).

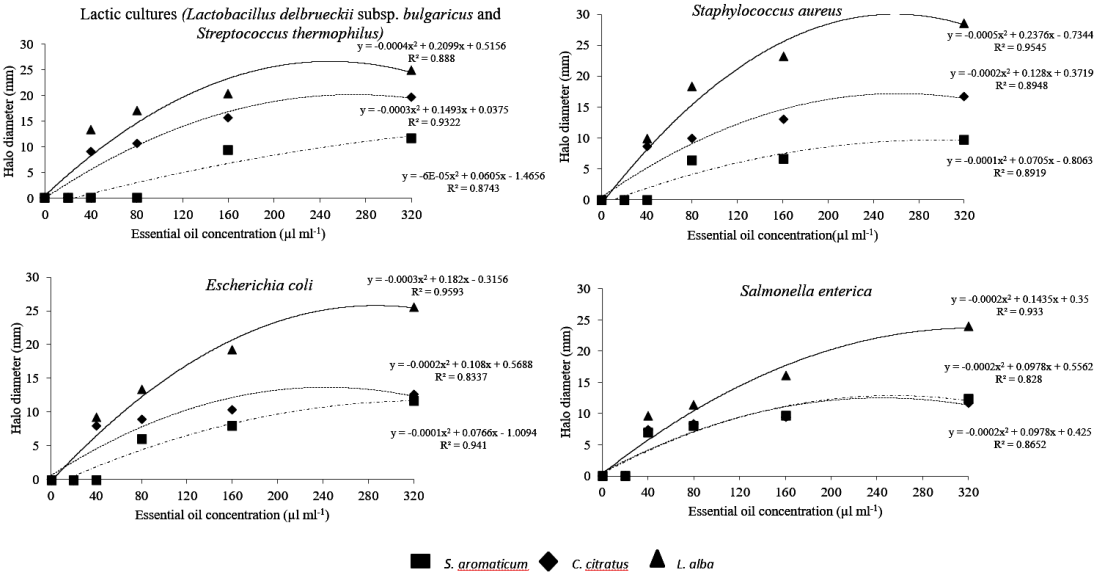


Figure 1 - Effect of the *Syzygium aromaticum*, *Cymbopogon citratus* and *Lippia alba* essential oils against lactic cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophiles*), *Staphylococcus aureus*, *Salmonella enterica* and *Escherichia coli*.

The essential oil from *S. aromaticum* presented antimicrobial activity against *S. aureus* (NAVEED et al., 2013; HUSSEIN et al., 2014; ABDULLAH et al., 2015; PUŠKÁROVÁ et al., (2017), *E. coli* (NAVEED et al., 2013; PUŠKÁROVÁ et al., 2017) and *Salmonella* spp (NAVEED et al., 2013; PUŠKÁROVÁ et al; 2017). NAVEED et al. (2013) and ABDULLAH et al. (2015) reported clinical isolates identified as multidrug resistant in a study based on the agar diffusion method. HUSSEIN et al., (2014) and PUŠKÁROVÁ et al. (2017) used a broth microdilution method.

C. citratus results changed depending on the adopted method. MILLEZI et al. (2014) used the disk diffusion technique in agar and reported the ability of the oil to inhibit *S. aureus*, *E. coli* and *Salmonella enterica* halo formation at higher concentrations. ALI et al. (2017) addressed the

Table 2 - Screening analysis of the antimicrobial activity, minimum Inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oils against Lactic culture and the pathogenic bacteria.

	--------*Syzygium aromaticum*-------			----------*Cymbopogon citrates*----------			----------*Lippia alba*----------		
Bacterium	Concentration with antimicrobial activity*	MIC	MBC	Concentration with antimicrobial activity*	MIC	MBC	Concentration with antimicrobial activity*	MIC	MBC
----------Concentration oil µL mL^{-1}----------									
Lacticculture	160	20	30	80	10	12.5	40	10	12.5
S. aureus	320	20	40	160	10	20	40	10	17.5
S. enterica	160	20	35	160	10	20	40	10	15
E. coli	320	20	35	160	10	20	40	10	17.5

*Diameter of 9–14mm.

antibacterial activity of *C. citratus* oil and evidenced a clear growth-inhibition zone (>18mm) formed by *E. coli*, *Staphylococcus aureus* and *Salmonella tiphi*. AZEVEDO et al. (2016) adopted this methodology to address the antimicrobial activity of the essential oil from *Cymbopogon flexuosus* against enteric *S. aureus* and *E. coli* isolated from laying hens. Based on their results, the concentration 160µL mL^{-1} recorded the largest inhibition zone diameters for all bacteria during disk diffusion. There was inhibitory effect (MIC) at concentration 80µL mL^{-1}, but no bactericidal effect was recorded. Citral was the major compound in the essential oils from *C. citratus* and *C. flexuosus*.

MACHADO et al. (2014) adopted a methodology similar to the one used in this study to test the antimicrobial activity of *L. alba* oil. Their chemotypes came from other sources and their results were different from ours. *S. aureus* showed larger inhibition halos (1.5±0.7mm) at lower oil concentrations (1.5%) than *E. coli* and *Salmonella choleraesuis*. MIC and MBC results recorded for *S. aureus* were lower (0.29mg mL^{-1}) than the ones recorded for *E. coli* and *Salmonella choleraesuis*.

ISLAM et al. (2018) investigated the growth-inhibition effect of *L. alba* oil on *E. coli* and recorded halo size 15mm and MIC value 31.25mg mL^{-1}. The antimicrobial activity of essential oils extracted from three *L. alba* specimens against *S. aureus in vitro* was investigated and determined through the microdilution method (PORFIRIO et al., 2017). The MIC and MBC results changed from specimen to specimen. The lowest MIC and MBC value was 0.5mg mL^{-1}. However, the aforementioned authors adopted a different methodology and chemotypes from other sources; therefore, it was not possible comparing their results to ours.

The action mechanism of essential oils against microorganisms has not yet been entirely elucidated, thus, the effects on these organisms cannot be attributed to a single mechanism (KHORSHIDIAN et al., 2018; NAZZARO et al., 2013). Several regions in microorganisms can be the sites subjected to the action of essential oils. These actions comprise cell permeability increase, membrane change in fatty acids and effects on membrane proteins (KHORSHIDIAN et al., 2018). There were cases in which these actions completely changed the cell morphology (NAZZARO et al., 2013).

The different results recorded for the assessed oils and bacteria can be associated with several factors such as plant origin, the adopted methodology and the extraction of active compounds (RADULOVIĆ et al., 2013; ZAMORA et al., 2018). Oil antibacterial activity depends on the existing action sites in the target cells (NEGI, 2012) and on the inherent features of each microorganism. Consequently, differences between studies could not possibly reflect their differences between essential oil features (ZAMORA et al., 2018).

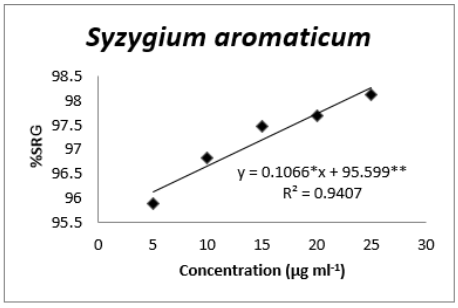
Effect of the essential oils from *S. aromaticum*, *C. citratus* and *L. alba* on the lactic culture used in our study had not been described in the literature, so far. RAMOS et al. (2017) studied the addition of lyophilized extract with herbal extracts composed of *S. aromaticum*, *Ilex paraguariensis* and *Cymbopogon citratus* to fermented milk. They observed increased total phenolic content and antioxidant activity (DPPH assays) and recorded sensory acceptance index higher than 70%. The effects of the essential oils on the lactic culture were not described in their study.

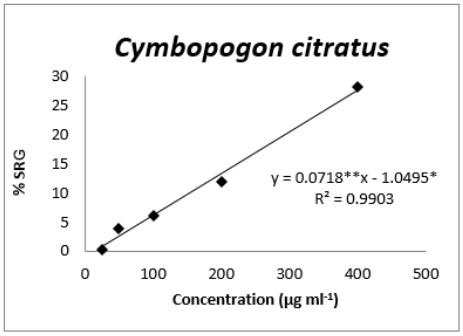
Antioxidant activity

The scavenging activity of the assessed oils was concentration-dependent (Figure 2), except for *Lippia alba*. Their ability to donate hydrogen atoms or electrons in order to turn DPPH into its reduced form (DPPH-H) was investigated through the DPPH assay (BOZIN et al., 2006).

The essential oil from *S. aromaticum* presented the best scavenging potential at all essential oil concentrations (95.89% to 98.5%). The IC_{50} (5.76µg mL^{-1}) and AAI (6.94) recorded for this oil evidenced its strong antioxidant activity (AAI>2.0) (SCHERER & GODOY, 2009). The essential oils from *C. citratus* and *L. alba* recorded low antioxidant activity (AAI<0.5) (SCHERER & GODOY, 2009). Both oils showed lower scavenging potential, even when they were tested at higher oil concentrations (Figure 2). The IC_{50} results were higher (710.99µg $mL^{-1}$and 1.168µg mL^{-1}, respectively), and the AAI ones were lower (0.056 and 0.034, respectively), than the ones recorded for *S. aromaticum* and for tert-butylated hydroxytoluene (BHT) (IC_{50}=161.44µg mL^{1}; AAI= 0.247). The lower IC_{50} indicated higher DPPH free radical scavenging activity. Free radical-scavenging is one of the mechanisms used by antioxidants to inhibit lipid oxidation (GÜLÇIN et al., 2012).

The different oil concentrations set for the antioxidant activity assays were based on the antimicrobial activity in order to find the concentrations that best meet the aim of this study. The concentration 30µL mL^{-1} was compatible with the MIC (20µL mL^{-1}) of the oil in the pathogen culture, and with the MBC in the lactic culture (25µL mL^{-1}) (Table 2). The essential oils from *C. citratus* and *L.*





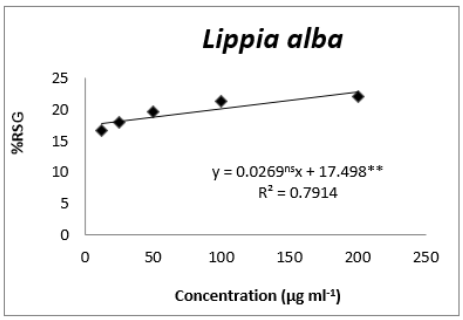


Figure 2 - Free Radical Scavenging Capacity (RSC) the essential oils from *Syzygium aromaticum*, *Cymbopogon citratus*, and *Lippia alba*, using DPPH methodology.

alba at the concentration defined based on MIC (10µL mL^{-1}) and on MBC (12.5µL mL^{-1}) did not show antioxidant activity.

The different antioxidant activity of the essential oils from *S. aromaticum, C. citratus* and *L. alba* was already described in the literature. This activity depends on the oil origin, on the adopted oil obtainment technique and on the methodology used for oil evaluations. The essential oil from *S. aromaticum* showed better antioxidant activity in the present study than in other studies. According to GÜLÇIN et al. (2018), clove oil applied at concentration 45µg mL^{-1} recorded 83.6 scavenging effect on DPPH radical standards. The DPPH free radical scavenging activity of clove oil increased under increased concentration (r^2=0.8786). The IC_{50} value recorded for clove oil was 21.50µg mL^{-1}.

The antioxidant behavior of the citral reported in the essential oils from *C. citratus* and *L. alba* resulted from the co-oxidation with the substrate, due to very fast self-termination and cross-termination of the oxidative chain (BASCHIERI et al., 2017). JAMUNA et al. (2017) assessed the scavenging activity of the essential oil from *C. citratus* against DPPH and observed that the highest essential oil concentration (100g ml^{-1}) led to better DPPH

scavenging activity than other concentration levels. BAYALA et al. (2018) observed the antioxidant activity of *C. citratus* scavenging DPPH and recorded 67.58% inhibition at 8mg mL^{-1}. REYES-SOLANO (2017) analyzed the essential oil from *L. alba* and reported IC_{50}=12.45mg mL^{-1} in the DPPH assay.

The antioxidant activity of essential oils, based on their radical scavenging activity and lipid oxidation inhibition (KHAYYAT & ROSELIN, 2018), is an important tool for food industry. Lipid oxidation has been described as the major cause of low-quality food and as a challenge to manufacturers and food scientists. Thus, the use of antioxidants is the most effective, convenient and economical way to prevent lipid oxidation. However, it is necessary conducting further studies, since a number of intrinsic and extrinsic factors (SHAHIDI & ZHONG, 2015) can influence the effectiveness of antioxidants.

Our results are relevant, given the interest in essential oils and in their application for food preservation. Such interest has grown in recent years because of consumers negative perception about synthetic food preservatives. The survival of beneficial bacteria and the improvement the food-hygiene quality due to the use of natural preservative agents, besides the possible synergic effects of essential oils and lactic cultures, are current market requests worldwide.

CONCLUSION

Essential oils from *S. aromaticum*, *C. citratus* and *L. alba* showed major compounds compatible with antioxidant and antimicrobial activity. All oils inhibited the growth of all bacteria. Lower concentrations of the oils exerted bactericidal effect on the lactic cultures and higher concentrations on S. aureus in comparison with the other bacteria. The essential oil from *S. aromaticum* recorded excellent antioxidant activity at the lowest concentration, since it was capable of inhibiting *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus* cultures. This outcome indicated the potential of such essential oil to work as antioxidant at concentrations that do not affect lactic cultures.

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DECLARATION OF CONFLICTING OF INTERESTS

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

AUTHORS' CONTRIBUTIONS

The authors contributed equally to the manuscript.

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