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Antibacterial activity of *Lippia alba*, *Myrcia lundiana* and *Ocimum basilicum* essential oils against six food-spoiling pathogenic microorganisms

[Actividad antibacteriana de los aceites esenciales de *Lippia alba*, *Myrcia lundiana* y *Ocimum basilicum* contra seis microorganismos patógenos que estropean los alimentos]

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Abstract: The aim of this study was to undertake a screening experiment on essential oils (EO) of *Myrcia lundiana*, *Ocimum basilicum* and *Lippia alba* against six food-spoiling pathogenic bacteria. Seventy-two (72) samples were initially analyzed for antimicrobial activity based on the agar diffusion test. The minimum inhibitory (MIC) and bactericidal (MBC) concentrations were determined for the 12 samples which showed greatest antimicrobial potential in this stage. Two samples of *L. alba*, three samples *of M. lundiana* and seven samples of *O. basilicum* showed a MIC of 0.12-125 µL/mL for the six tested bacteria. Of these, the EO of *O. basilicum* cultivar Maria Bonita stood out with the lowest MIC and MBC. Thus, a mixture simulating this essential oil was prepared from commercial standards of the compounds (±)-linalool, geraniol and 1,8-cineole. Significantly higher MIC and MBC were detected in the simulation compared to the respective EO, suggesting a synergistic effect between compounds.

Keywords: Verbenaceae; Myrtaceae; Lamiaceae; Volatile oil; Antimicrobial activity.

Resumen: El objetivo de este estudio fue realizar un experimento de detección en aceites esenciales (AE) de *Myrcia lundiana*, *Ocimum basilicum* y *Lippia alba* contra seis bacterias patógenas que estropean los alimentos. Setenta y dos (72) muestras fueron analizadas inicialmente para la actividad antimicrobiana basada en la prueba de difusión en agar. Se determinaron las concentraciones mínimas inhibitoria (CMI) y bactericida (CMB) para las 12 muestras que mostraron el mayor potencial antimicrobiano en esta etapa. Dos muestras de *L. alba*, tres muestras de *M. lundiana* y siete muestras de *O. basilicum* mostraron un CMI de 0.12-125 µL/mL para las seis bacterias analizadas. De estos, el AE de *O. basilicum* cultivar Maria Bonita se destacó con el CMI y CMB más bajos. Por lo tanto, se preparó una mezcla que simula este aceite esencial a partir de los estándares comerciales de los compuestos de (±)-linalol, geraniol y 1,8-cineol. Se detectaron CMI y CMB significativamente más altos en la simulación en comparación con el AE respectivo, lo que sugiere un efecto sinérgico entre los compuestos.

Palabras clave: Verbenaceae; Myrtaceae; Lamiaceae; Aceite volatil; Actividad antimicrobiana.

INTRODUCTION

Despite the great advance achieved in preservation technologies, the occurrence of foodborne disease outbreaks has increased in recent years, which has generated great concern with the quality and microbiological safety of foods (Hu *et al*., 2016). Data from the annual report of the CDC (Centers for Disease Control and Prevention) show a total of 839 outbreaks of foodborne disease reported in 2016, which resulted in 14,259 cases of disease, 875 hospitalizations and 17 deaths in the United States (CDC, 2018). In Brazil, according to the latest data from the Department of Health Surveillance, 538 outbreaks of foodborne diseases were recorded in 2016, with 9,935 people affected and seven death cases (SVS, 2018).

At the same time, in the last years, the population has shown increased interest in natural foods with decreased addition of synthetic preservatives, many of which are often harmful to health (Zhang *et al*., 2016). To meet this demand without putting the microbiological safety of foods at risk, alternative preservation technologies such as the use of natural antimicrobial additives have been sought (Melo *et al*., 2005). In this respect, one of the natural substances group currently investigated for use in food preservation are essential oils (Zhang *et al*., 2016). In addition to acting as antibacterial and antioxidant agents (Siroli *et al*., 2015; Chen *et al*., 2016; Luís *et al*., 2016; Couto *et al*., 2019), essential oils add nutritional value to foods and are generally considered safe, mainly when compared to synthetic chemical additives. As such, they constitute potentially useful substances in the food industry (Calo *et al*., 2015; Gutiérrez-del-Río *et al*., 2018).

Myrcia lundiana Kiaersk is a species of the family Myrtaceae found mainly in tropical and subtropical areas of the Southern Hemisphere (Govaerts *et al*., 2008; Alves *et al*., 2016). Essential oils of this species have shown biological properties such as larvicidal (Fontes *et al*., 2011) and fungicidal (Alves *et al*., 2016) activity.

The species *Ocimum basilicum* L., popularly known as basil, is a member of the family Lamiaceae originating from Southeast Asia and Central Africa. Basil occurs naturally all across Brazil and has multiple uses, e.g. in gastronomy, medicine, ornamentation, among others (Santos *et al*., 2012; Al Abbasy *et al*., 2015).

Lippia alba (Mill.) N. E. Br. (Brazilian lemongrass) is an aromatic plant widely used in popular medicine. The plant is native to the American continent and belongs to the family Verbenaceae. Lemongrass can be found from the northeast to the south region of Brazil (Gomes *et al*., 2011). Due to the chemical diversity presented by essential oils of *L. alba*, different chemotypes exist and, consequently, diverse biological activities are observed (Sahpaz *et al*., 2006; Jannuzzi *et al*., 2010; Tavares *et al*., 2011).

On these bases, the present study proposes to undertake a screening experiment on the antimicrobial activity of essential oils of *Myrcia lundiana*, *Ocimum basilicum* and *Lippia alba* against six species of food-spoiling pathogenic bacteria.

MATERIAL AND METHODS

Plant material

Essential oils of three different species were initially tested, consisting of 23 samples of *Myrcia lundiana*, 25 samples of *Lippia alba* and 24 samples of *Ocimum basilicum*. The *M. lundiana* essential oils were obtained from leaves collected from native plants at the National Park of Serra de Itabaiana, in the state of Sergipe, Brazil (10°41′06″S, 37°25′31″W, 659 m altitude), as described by Alves *et al*. (2016). The *L. alba* essential oils were obtained from leaves of accessions kept at the Active Germplasm Bank of Medicinal and Aromatic Plants at the Federal University of Sergipe (UFS). Lastly, the essential oils of basil were obtained from commercial cultivars cultivated in the Experimental Field of the same institution, both located in São Cristóvão - SE, Brazil (11º00'S and 37º12'W).

The total 72 samples of leaves of the three species were dried in a forced-air oven at 40 ± 1ºC for five days. The essential oils were extracted by hydrodistillation in a modified Clevenger apparatus (Ehlert *et al*., 2006) using 75 g of dry leaves. Subsequently, the chemical composition of the essential oils was analyzed using a GC-MS/FID gas chromatograph system (QP2010 Ultra, Shimadzu Corporation, Kyoto, Japan) equipped with an automated sampler (AOC-20i, Shimadzu) as previously described (Alves *et al*., 2016; Silva *et al*., 2018; Pinto *et al*., 2019). The MS and FID data were simultaneously acquired using a Detector Splitting System; the split flow ratio was 4:1 (MS:FID). Quantification of each constituent was estimated by FID peak-area normalization (%). Compound concentrations were calculated from the GC peak areas, and they were arranged in order of GC elution. The retention index (van den Dool & Kratz, 1963) was obtained by injecting a C7-C30 linear hydrocarbon mixture under these same conditions, and identification of constituents was made on the basis of comparison of the retention index and MS

with those in the literature (Adams, 2017), as well as by computerized matching of the acquired mass spectra with those stored in the NIST21, NIST107, and WILEY8 mass spectral libraries of the GC-MS data system.

Microorganisms and growing and stocking condition

The following food-spoiling pathogenic microorganisms were selected: *Staphylococcus aureus* ATCC 8095, *Bacillus cereus* ATCC 4504, *Escherichia coli* ATCC 23226, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028 and *Enterobacter sakazakii* ATCC 29004.

The bacteria were cultivated in BHI medium (Brain Heart Infusion/HIMDEDIA, Mumbai, India) and incubated at 37ºC. All cultures were subjected to Gram-staining and microscopic analysis for an evaluation of degree of purity. Pure cultures of isolates were kept frozen at -20ºC in the growth medium with 20% glycerol. At the start of each experiment, each culture was transplanted to the same growth medium with addition of 1.5% agar and subsequently activated in liquid medium for 12 h.

Screening for essential oil antimicrobial activity

The activity spectrum of the essential oils against the selected bacterial strains was evaluated by the agar diffusion method (Tagg *et al*., 1976; Gahruie *et al*., 2017), wherein approximately 10^6 CFU of the microorganisms were inoculated in solid BHI medium. After inoculation, 4-mm holes were made in the medium, wherein a mixture of 25 µL of the solution of essential oil and dimethyl sulfoxide (DMSO at the concentration of 10% in relation to the oil volume) were poured. In the negative control treatment, 25 µL distilled water + DMSO (10%) were added. The plates were incubated at 37±1°C and, after 24 h the diameters of zones of inhibition (DZI) were measured in millimeters using a digital caliper. According to the size of the inhibition halo, the bacteria were classified as non-sensitive (≤8 mm); sensitive (9-14 mm); highly sensitive (15-19 mm) and extremely sensitive (≥20 mm) (Ponce *et al.*, 2003).

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

For this stage, we selected the essential oils evaluated in the agar diffusion method that provided larger inhibition halos, for the highest number of evaluated bacteria. Accordingly, essential oils ML2, ML13 and ML14 of *M. lundiana*; LA-10 and LA-22 of *L. alba* and the essential oils of basil (cultivars Dark Opal, Genovese, Mrs. Burns, Napoletano, Italian Large Leaf, Maria Bonita and Genovese × Maria Bonita) were chosen, totaling 12 samples.

The MIC of the essential oils were determined by the microdilution method (Haddouchi *et al*., 2013) standardized by CLSI M100 (CLSI, 2017). The essential oils (with 10% DMSO) were diluted (2x increments) in liquid Müller Hinton medium distributed into 96-well plates so as to obtain concentrations ranging from 125 to 0.12 µL/mL. The microorganisms were inoculated at an approximate concentration of 10^6 CFU/mL. The MIC was defined as the lowest concentration of the oil at which no visible growth was observed after 24 h of incubation at 37ºC. Microorganism growth was indicated by the turgidity of the medium and by monitoring optical density at 600 nm in a microplate reader (Synergy H1 multi-mode microplate reader). Control treatments without addition of essential oils or microorganisms were also used.

To determine the MBC, the content of the wells where no microbial growth occurred in the presence of the essential oil was transferred to a plate containing only solid BHI medium. Next, the plates were incubated at 37 ± 2°C for 48 h and the number of viable cells was checked. MBC was considered the lowest concentration at which no viable cell was detected after 48 h of incubation.

Antibacterial activity of the major compounds of basil cultivar Maria Bonita

In this stage, mixtures were made with the major compounds of cultivar Maria Bonita, simulating the chemical composition of the essential oil. Of all evaluated samples, this cultivar showed the greatest antimicrobial potential against the six bacteria evaluated in the previous stages. The mixtures were obtained from commercially acquired compounds (standards) (Sigma Chemical Co., USA), by mixing them in the same proportion in which they are found in the selected essential oil. This mixture was termed 'essential oil simulation'. As shown in Table 1, 77.23% linalool, 13.28% geraniol and 5.11% 1,8-cineole were mixed. After the preparation, the antimicrobial activity in the mixture was evaluated by the same previously mentioned methods (MIC and MBC) and compared to the antimicrobial activity presented by the respective essential oil.

Table No. 1
Chemical composition of the essential oils of the three species used in this study (*Myrcia lundiana*, *Lippia alba* and *Ocimum basilicum*).

Plants		Compounds								
		C01	C02	C03	C04	C05	C06	C07	C08	C09
	RRIo	933	974	1015	1080	1183	1186	1234	1224	1253
	RRIl	932	974	1026	1096	1195	1186	1249	1235	1264
ML 002		2.26	5.51	14.81	7.63	-	4.40	-	0.48	0.73
ML 13		3.97	7.67	11.77	1.13	-	4.68	-	1.83	2.66
ML 14		3.20	8.23	10.53	3.12	-	3.29	-	0.94	1.37
LA 10		0.36	-	-	1.30	-	-	-	34.30	45.57
LA 22		-	-	6.60	86.00	-	-	-	-	-
Dark Opal		-	-	15.42	53.96	-	-	-	-	-
Genovese		-	-	8.19	49.57	2.13	-	-	-	-
Mrs. Burns		-	-	0.79	38.52	-	-	0.27	19.89	25.43
Napoletano		-	-	11.1	50.10	6.05	-	-	-	-
Italian Large Leaf (Isla)		-	-	10.04	57.96	-	-	-	-	-
Maria Bonita		-	-	5.11	77.23	-	-	13.28	-	-
Genovese x Maria Bonita		-	-	4.00	67.07	-	-	2.05	6.22	6.58

Compounds: C01: α-pinene, C02: β-pinene, C03: 1,8 cineole, C04: linalool, C05: methyl chavicol, C06: α-terpineol, C07: geraniol, C08: neral, C09: geranial. RRIo: Relative Retention Index - observed; RRIl: Relative Retention Index- literature (Adams, 2007). (Data extracted from Pinto *et al*., 2019; Pinto, 2017 and Alves *et al*., 2016).

Table No. 1
Continuation ...

Plants		Compounds								
		C10	C11	C12	C13	C14	C15	C16	C17	C18
	RRIo	1280	1338	1346	1415	1422	1490	1498	1585	1626
	RRIl	1280	1356	1347	1432	1417	1489	1498	1582	1638
ML 002		2.52	-	42.59	-	5.59	3.71	3.43	1.34	-
ML 13		1.27	-	32.39	-	3.76	5.06	3.77	6.71	-
ML 14		0.70	-	38.68	-	3.12	2.16	1.43	7.77	-
LA 10		-	-	-	-	1.99	-	-	2.42	-
LA 22		-	-	-	-	1.48	-	-	1.08	-
Dark Opal		-	6.58	-	8.82	-	-	-	-	2.15
Genovese		-	29.69	-	2.20	-	-	-	-	2.14
Mrs. Burns		-	-	-	2.68	-	-	-	0.46	-
Napoletano		-	4.86	-	7.51	-	-	-	-	4.43
Italian Large Leaf (Isla)		-	12.59	-	5.72	-	-	-	-	3.44
Maria Bonita		-	-	-	1.32	-	-	-	-	0.83
Genovese x Maria Bonita		-	-	-	0.96	-	-	-	0.65	1.69

Compounds: C10: methyl nerolate, C11: eugenol, C12: nerolic acid, C13: α-(E)-bergamotene, C14: (*E*)-caryophyllene, C15: β-selinene, C16: α-selinene, C17: caryophyllene oxide, C18: epi-α-cadinol. RRIo: Relative Retention Index - observed; RRIl: Relative Retention Index- literature (Adams, 2007). (Data extracted from Pinto *et al*., 2019; Pinto, 2017 and Alves *et al*., 2016).

Statistical analysis

All the antimicrobial activity assays were performed in triplicate. The data were expressed as means, which were compared by the Scott-Knott test at the 5% significance level ($p<0.05$) using Sisvar® software version 5.6 (Ferreira, 2011).

RESULTS

Of the 25 *L. alba* essential oils tested, two (LA-10 and LA-22) showed larger inhibition zones against the bacterial strains tested (not shown results).

In the MIC and MBC assays, the LA10 and LA22 essential oils showed MIC between 3.9-104.16 µL/mL against the six evaluated bacteria. Of the two samples of essential oils used, greater bactericidal potential was found in LA10, whose MBC ranged from 7.82 µL/mL (*B. cereus*) to 62.60 µL/mL (*S. aureus*). For LA22, it was not possible to determine

the MBC within the evaluated concentration range for most of the evaluated bacteria (Table No. 2).

Table No. 2
Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of the essential oils of *Lippia alba* (LA) against six foodborne pathogenic bacteria. Results are expressed in µL/mL.

	MIC		MBC	
Bacteria	LA10	LA22	LA10	LA22
S. aureus	5.17a	104.16b	62.60a	>125 b
B. cereus	7.81a	52.08b	7.82a	62.50b
L. monocytogenes	31.25a	52.08b	41.66a	>125 b
E. sakazakii	41.66a	31.25a	62.50a	104.16a
E. coli	3.90a	104.16b	52.08a	>125 b
S. typhimurium	3.90a	104.16b	41.66a	>125b

***>125: minimum bactericidal concentration not detected in the tests performed up to the concentration limits tested. Means followed by the same letter in the lines, for MIC and MBC, do not differ according to the Scott-Knott test at the 5% probability level.**

Of the 23 essential oils of *M. lundiana*, three (ML2, ML13 and ML14) showed antimicrobial activity against the tested bacteria, producing inhibition zones of 7 to 18.5 mm (not shown results). The bacteria were considered sensitive and highly sensitive to the essential oils of this species, except *E. sakazakii*, which did not show sensitivity to any of the samples of *M. lundiana* essential oil (inhibition halo ≤8.0 cm) and *E. coli*, which showed no sensitivity to the ML14 essential oil.

These three essential oils (EO) of *M. lundiana* showed MIC between 3.25-125 µL/mL against the six evaluated bacteria. The ML2 essential oil stood out with the highest inhibitory potential against the bacteria *S. aureus*, *L. monocytogenes*, *E. coli* and *S. typhimurium* and bactericidal action against *L. monocytogenes*, *E. coli* and *S. typhimurium*. Samples ML13 and ML14 showed no bactericidal activity within the tested concentration range (0.12-125 µL/mL) (Table No. 3).

Table No. 3
Inhibition halos (mm) and minimum inhibitory (MIC) and bactericidal (MBC) concentrations of essential oils of *Myrcia lundiana* (ML) against six foodborne pathogenic bacteria. Results are expressed in µL/mL.

	MIC			MBC		
Bacteria	ML2	ML13	ML14	ML2	ML13	ML14
S. aureus	3.25a	62.5b	83.33b	>125a	>125a	>125a
B. cereus	13.02a	15.62a	31.25b	>125a	>125a	>125a
L. monocytogenes	13.02a	31.25b	26.04b	62.5a	>125b	>125b
E. sakazakii	104.16a	125a	125a	>125a	>125a	>125a
E. coli	7.81a	83.33b	62.5b	52.08a	>125b	>125b
S. typhimurium	31.25a	52.08b	125c	62.5a	>125b	>125b

***>125: minimum bactericidal concentration not detected in the tests performed up to the concentration limits tested. Means followed by the same letter in the lines, for MIC and MBC, do not differ according to the Scott-Knott test at the 5% probability level.**

Of the 24 essential oils of *O. basilicum* evaluated in this study, seven exhibited antimicrobial activity against all tested bacteria, which were also classified as highly sensitive and extremely sensitive, forming inhibition halos of 15 to 20 mm (not shown results). The mean MIC and MBC values obtained in the experiment involving the selected EO of the species *O. basilicum* presented MIC within the evaluated concentration range (0.12-125 µL/mL) for the six tested bacteria. However, in terms of bactericidal effect, it was not possible to establish the minimum bactericidal concentrations within the evaluated range (0.12-125 µL/mL) for some EO; thus, they were classified as >125 µL/mL.

By contrast, some EO such as those of cultivars Mrs. Burns and Genovese presented an MBC of 1.95 and 2.60 µL/mL, respectively, against *E. sakazakii*, while Maria Bonita showed the same MBC value against *B. cereus* (Table No. 4). Cultivar Maria Bonita showed the most promising results

among all EO of *O. basilicum*. This EO inhibited all evaluated bacteria, presenting inhibitory activity at concentrations up to 1.95 µL/mL for Gram-positive and Gram-negative bacteria (Table No. 4). In terms of bactericidal activity, the EO of this cultivar was efficient against all evaluated bacteria at concentrations between 2.6-62.6 µL/mL. This EO showed bactericidal activity against Gram-negative bacterium lines such as *E. sakazakii* and *E. coli* at concentrations up to 10.41 µL/mL and 3.9, respectively (Table No. 4).

Table No. 4
Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of essential oils of *Ocimum basilicum* against six foodborne pathogenic bacteria. Results are expressed in µL/mL.

	MIC						
Bacteria	Dark Opal	Genovese	Mrs. Burns	Napoletana	Italian Large Leaf	Gen. x M.Bonita	Maria Bonita
S. aureus	125.0c	125.0c	26.00b	12.98a	31.25b	15.62a	15.6a
B. cereus	13.01a	20.8b	6.50a	26.04b	26.04b	31.25b	1.95a
L. monocytogenes	62.50d	62.50d	31.25c	62.5d	62.50d	13.01b	3.90a
E. sakazakii	10.41b	2.60a	1.95a	5.20a	7.8b	15.6c	10.4b
E. coli	3.25a	13.01b	15.62c	31.25d	15.62c	7.81b	1.95a
S. typhimurium	52.08c	62.50c	62.50c	52.08c	52.08c	0.97a	31.25b

Means followed by the same letter in the lines do not differ according to the Scott-Knott test at the 5% probability level.

Table No. 4
Continuation ...

	MBC						
Bacteria	Dark Opal	Genovese	Mrs. Burns	Napoletana	Italian Large Leaf	Gen. x M.Bonita	Maria Bonita
S. aureus	125.0c	125c	26.04a	26.04a	62.5b	31.25a	31.25a
B. cereus	>125b	>125b	>125b	>125b	>125b	>125b	2.6 a
L. monocytogenes	125.0c	>125c	>125c	>125c	125.0c	13.01a	31.25b
E. sakazakii	10.41b	2.60a	5.20a	5.20a	13.01c	15.62c	10.41b
E. coli	13.01a	52.08b	62.50b	62.50b	15.62a	125.0c	3.95a
S. typhimurium	52.08b	62.50b	52.08b	52.08b	52.08b	3.95a	62.5b

***>125: minimum bactericidal concentration not detected in the tests performed up to the concentration limits tested.**
Means followed by the same letter in the lines do not differ according to the Scott-Knott test at the 5% probability level.

Due to the greater antimicrobial potential of the EO of cultivar Maria Bonita, the antimicrobial activity of its major compounds was evaluated. In this respect, the major compounds (linalool, 1,8-cineole and geraniol), which were acquired commercially, were mixed to generate the simulation of this EO (Table No. 1).

A comparison between the EO of cultivar Maria Bonita with the mixture of its major compounds revealed that the EO showed significantly lower MIC values than its simulation (Table No. 5) against the bacteria *B. cereus* (1.95 vs. 31.25 µL/m), *L. monocytogenes* (3.9 vs. 31.26 µL/m), *E. coli* (1.95 vs. 15.62 µL/m) and *S. typhimurium* (31.25 vs. 62.50 µL/m). Similarly, the EO also showed greater bactericidal effect compared to its simulation, showing significantly lower MBC values for all evaluated bacteria, except *S. typhimurium*, for which MBC was 62.5 µL/mL in both treatments (Table No. 5).

DISCUSSION

Of the 25 samples of *L. alba* essential oils tested in this experiment, LA10 stood out for its antimicrobial ability. The major compounds in this EO are neral (34.30%) and geranial (45.57%), which, together, form citral. Found mainly in herbs and citric fruits, citral (3, 7-dimethyl-2, 6-octadienal) is a monoterpene that has already been highlighted for its antimicrobial activity (Weerawatanakorn *et al*., 2015). This compound acts by rupturing the lipid structure of the bacterial cell wall, causing protein denaturation and cell membrane destruction, which lead to leakage of cytoplasmic content and, eventually, cell death (Saddiq & Khayyat, 2010). Antimicrobial activity of other EO of *L. alba* against foodborne pathogenic bacteria such as *E. coli* has already been demonstrated, though these oils have limonene and carvone as their major compounds (Aquino *et al*., 2010).

Table No. 5
Minimum inhibitory (MIC) and bactericidal (MBC) concentrations of essential oil of *Ocimum basilicum* cultivar Maria Bonita and its simulation (containing the major components present its essential oil) against six foodborne pathogenic bacteria. Results are expressed in µL/mL.

Bacteria	MIC		MBC	
	Essential oil	Simulation	Essential oil	Simulation
S. aureus	15.62a	15.40a	31.26a	62.51b
B. cereus	1.95a	31.25b	2.60a	>125.00b
L. monocytogenes	3.9a	31.26b	31.25a	125.00b
E. sakazakii	10.41a	15.62a	7.81a	62.51b
E. coli	1.95a	15.62b	3.9a	31.25b
S. typhimurium	31.25a	62.5b	62.5b	62.5b

***Means followed by the same letter in the lines, for MIC and MBC, do not differ from each other according to the Scott-Knott test at the 5% probability level**

The three EO of *M. lundiana* which showed the best antimicrobial performance (ML2, ML13 and ML14) belong to the same chemical group and present the following major compounds in their composition: nerolic acid (32.39-42.59%), 1,8-cineole (10.53-14.81%) and β-pinene (5.51-8.23%) (Alves *et al*., 2016). The EO of the species *M. lunadiana* (ML2), which exhibited the lowest MIC and MBC, contained high concentrations of nerolic acid, but also showed the 1,8-cineole, linalool alcohols as major compounds. The other oils (ML13 and ML14), in turn, showed 1,8-cineole, nerolic acid and caryophyllene oxide as their major compounds. Essential oils of *M. lundiana* have already been mentioned for their potential antifungal activity against *Fusarium solani* (Alves *et al*., 2016).

The greater antibacterial potential shown by the EO of cultivar Maria Bonita in relation to the others is related to its chemical composition. Various studies conducted with *Ocimum* essential oils reinforce the activity of these compounds. Essential oils of basil found in Oman with similar chemical composition to that of the EO of cultivar Maria Bonita used in the present study have been reported as having antibacterial activity against Gram-positive bacteria such as *S. aureus* and *B. cereus* and moderate activity against Gram-negative bacteria such as *E. coli* and *Salmonella typhimurium* (Al Abbasy *et al*., 2015). In another study, EO of basil were shown to have antimicrobial effects against strains of the bacterium *Salmonella enteritidis* in *in vitro* assays. Moreover, at the concentration of 50 ppm, the oil reduced the count of bacteria inoculated in pork sausage from 5 to 2 log cfu/g, after storage for three days at 4ºC. The EO used in the afore-mentioned study had linalool (64%), 1,8-cineole (12%), eugenol (3%) (Rattanachaikunsopon & Phumkhachorn, 2010) as their major compounds.

A study published in 2017 examined the antioxidant and antimicrobial activity of 10 cultivars of basil (*O. basilicum* L.) and two other *Ocimum* species: *O. sanctum* and *O.* × *citriodorum*. All EO evaluated in the experiment inhibited the growth of both tested bacteria (*E. coli* and *S. aureus*). According to the authors, this finding reinforces the fact that EO of this species are active against Gram-positive and Gram-negative bacteria (Koroch *et al*., 2017).

The fact that the EO of cultivar Maria Bonita presents only *R*-(-)-linalool (Silva, 2011) and showed greater antimicrobial potential than its simulation [(±)-linalool] suggests that (-)- isomer potentiating the antimicrobial activity. The antibacterial activity of the linalool isomers are distinct according literature report (Silva *et al*., 2018). Besides, previous studies have demonstrated the greater effect of EO when compared to their major compounds separately. Essential oils of cinnamon, geranium and thyme showed greater growth-inhibition effect on bacteria such as *E. coli* and *S. aureus* than their major compound, linalool (Dorman & Deans, 2000; Chang *et al*., 2001), alone. The EO used in this stage (Maria Bonita) contains a high concentration of linalool, which is a monoterpene alcohol of high value in the cosmetics market, in addition to being used as a flavoring agent by the food and perfumery industries. Linalool has been mentioned for its biological activities; e.g. acaricidal (Prates *et al*., 1998), bactericidal and fungicidal effects (Belaiche *et al*., 1995).

Considering the many groups of different chemical compounds present in the EO, it is very likely that their antimicrobial activity is not attributed to only one specific mechanism, but that there are

several targets in the cell. In the bacterial cell, many targets can be hit causing inhibition of growth or even death of the microorganism; e.g., cell wall degradation, cytoplasmic membrane damage, membrane protein damage, cell content leakage, cytoplasmic coagulation and interruption of the proton-motive force (Burt, 2004).

Although other cultivars in this study also showed these compounds as major elements of their composition, though at different concentrations and in different combinations, the presence of other minor compounds in this oil may potentiate its activity. Because we cannot affirm that the major component is the only element responsible for biological activity, there may often be an interaction between the different components of an EO (Weerawatanakorn *et al*., 2015). However, these combinations between major compounds may generate several types of interactions, which may have synergetic, additive, or antagonistic effects (Katiki *et al*., 2017).

CONCLUSION

Among the essential oils of the three evaluated species, the samples of *O. basilicum* and *L. alba* exhibited the greatest antimicrobial potential, inhibiting the growth of Gram-positive and Gram-negative bacteria. Linalool, citral and 1,8-cineole are the major components of these species. The essential oil of basil—more specifically, that of cultivar Maria Bonita—stood out between the two species. A comparative analysis between the essential oil of this cultivar and its simulation obtained from a mixture of commercial standards revealed that the simulation exhibited less antimicrobial activity, suggesting the existence of a synergistic interaction between the major and minor compounds. The essential oils tested in this study –especially that of basil cultivar Maria Bonita– presented great antimicrobial potential. Therefore, they may be used in the future as natural additives by the food industry, improving preservation and adding nutritional value to the products.

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REFERENCES

Adams RP. 2017. **Identification of essential oil components by gas chromatography/mass spectroscopy**. Publisher Allured Publishing Corporation, Carol Stream, USA. **https://doi.org/10.1016/s1044-0305(97)00026-3**

Al Abbasy DW, Pathare N, Al-Sabahi JN, Khan SA. 2015. Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimum basilicum* Linn.). **Asian Pac J Trop Med** 5: 645 - 649. **https://doi.org/10.1016/s2222-1808(15)60905-7**

Alves MF, Nizio DAC, Sampaio TS, Nascimento AF, Brito FA, Melo JO, Arrigoni-Blank MF, Gagliardi PR, Machado SMF, Blank AF. 2016. *Myrcia lundiana* Kiaersk native populations have different essential oil composition and antifungal activity against *Lasiodiplodia theobromae*. **Ind Crops Prod** 85: 266 - 273. **https://doi.org/10.1016/j.indcrop.2016.03.039**

Aquino LCL, Santos GG, Trindade RC, Alves JAB, Santos PO, Alves PB, Blank AF, Carvalho LM. 2010. Atividade antimicrobiana dos óleos essenciais de erva-cidreira e manjericão frente a bactérias de carnes bovinas. **Alim Nutr** 21: 529 - 535.

Belaiche T, Tantaoui-Elaraki A, Ibrahimy A. 1995. Application of a two levels factorial design to the study of the antimicrobial activity of three terpenes. **Sci Aliment** 15: 571 - 578.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods. **Int J Food Microbiol** 94: 223 - 253. **https://doi.org/10.1016/j.ijfoodmicro.2004.03.022**

Calo JR, Crandall PG, O'Bryan CA, Ricke SC. 2015. Essential oils as antimicrobials in food systems- A review. **Food Control** 54: 111 - 119. **https://doi.org/10.1016/j.foodcont.2014.12.040**

CDC [Centers for Disease Control and Prevention]. 2018. **Surveillance for foodborne disease outbreaks, United States, 2016, Annual Report.** Department of Health and Human Services. Atlanta, Georgia, USA.

Chang ST, Chen PF, Chang SC. 2001. Antibacterial activity of leaf essential oils and their constituents from *Cinnamomum osmophloeum*. **J Ethnopharmacol** 77: 123 - 127. **https://doi.org/10.1016/s0378-8741(01)00273-2**

Chen Z, He B, Zhou J, He D, Deng J, Zeng R. 2016. Chemical compositions and antibacterial activities of essential oils extracted from *Alpinia guilinensis* against selected foodborne pathogens. **Ind Crops Prod** 83: 607 - 613. **https://doi.org/10.1016/j.indcrop.2015.12.063**

CLSI [Clinical and Laboratory Standards Institute]. 2017. **M100 – Performance standards for antimicrobial susceptibility testing.** CLSI, Wayne, USA.

Couto HGSA, Blank AF, Oliveira e Silva AM, Nogueira PCL, Arrigoni-Blank MF, Nizio DAC, Pinto JAO. 2019. Essential oils basil chemotypes: Major compounds, binary mixtures, and antioxidant activity. **Food Chem** 293: 446 - 454. **https://doi.org/10.1016/j.foodchem.2019.04.078**

Dorman HJD, Deans SG. 2000. Antimicrobial agents from plants: antibacterial activity of plant volatile oils. **J Appl Microbiol** 88: 308 - 316. **https://doi.org/10.1046/j.1365-2672.2000.00969.x**

Ehlert PAD, Blank AF, Arrigoni-Blank MF, Paula JWA, Campos DA, Alviano CS. 2006. Tempo de hidrodestilação na extração de óleo essencial de sete espécies de plantas medicinais. **Rev Bras Plant Med** 8: 79 - 80. **https://doi.org/10.1590/s1516-05722013000300002**

Ferreira DF. 2011. Sisvar: a computer statistical analysis system. **Ciênc Agrotec** 35: 1039 - 1042. **https://doi.org/10.1590/s1413-70542011000600001**

Fontes JEN, Matos IL, Machado SMF, Ribeiro AS, Sócrates CHC. 2011. **Atividade larvicida do óleo essencial das folhas frescas de *Myrcia lundiana* e *Myrcia guianensis* (Myrtaceae) frente ao *Aedes aegypti*.** Resumo da 34° Reunião Anual da Sociedade Brasileira de Química, Sociedade Brasileira de Química, Florianópolis, Brasil.

Gahruie HH, Ziaee E, Eskandari MH, Hossini SMH. 2017. Characterization of basil seed gum-based edible films incorporated with *Zataria multiflora* essential oil nanoemulsion. **Carbohyd Polym** 166: 93 - 103. **https://doi.org/10.1016/j.carbpol.2017.02.103**

Gomes SVF, Nogueira PCL, Moraes VRS. 2011. Aspectos químicos e biológicos do gênero *Lippia* enfatizando *Lippia gracilis* Schauer. **Eclét Quím J** 36: 64 - 77. **https://doi.org/10.1590/s0100-46702011000100005**

Govaerts R, Sobral M, Ashton P, Barrie F, Holst BK, Landrum LR, Mastsumoto K, Mazine FF, Niclughadha E, Proença CEB, Silva LHS, Wilson PG, Lucas EJ. 2008. **World Checklist of Myrtaceae**. Royal Botanic Gardens, London, England.

Gutiérrez-del-Río I, Fernández J, Lombó F. 2018. Plant nutraceuticals as antimicrobial agents in food preservation: terpenoids, polyphenols and thiols. **Int J Antimicrob Agents** 52: 309 - 315. **https://doi.org/10.1016/j.ijantimicag.2018.04.024**

Haddouchi F, Chaouche TM, Zaouali Y, Ksouri R, Attou A, Benmansour A. 2013. Chemical composotion and antimicrobial activity of the essential oils from four *Ruta* species growing in Algeria. **Food Chem** 141: 253 - 258. **https://doi.org/10.1016/j.foodchem.2013.03.007**

Hu K, Renly S, Edlund S, Davis M, Kaufman J. 2016. A modeling framework to accelerate food-borne outbreak investigations. **Food Control** 59: 53 - 58. **https://doi.org/10.1016/j.foodcont.2015.05.017**

Jannuzzi H, Mattos JKA, Vieira RF, Silva DB, Bizzo HR, Gracindo LA. 2010. Avaliação agronômica e identificação de quimiotipos de erva cidreira no Distrito Federal. **Hortic Bras** 28: 412 - 417. **https://doi.org/10.1590/s0102-05362010000400006**

Katiki LM, Barbieri AME, Araujo RC, Veríssimo CJ, Louvandini H, Ferreira JFS. 2017. Synergistic interaction of ten essential oils against *Haemonchus contortus in vitro*. **Vet Parasitol** 243: 47 - 51. **https://doi.org/10.1016/j.vetpar.2017.06.008**

Koroch AR, Simon JE, Juliani HR. 2017. Essential oil composition of purple basils, their reverted green varieties (*Ocimum basilicum*) and their associated biological activity. **Ind Crops Prod** 107: 526 - 530. **https://doi.org/10.1016/j.indcrop.2017.04.066**

Luís Â, Duarte A, Gominho J, Domingues F, Duarte AP. 2016. Chemical composition, antioxidant, antibacterial and anti-quorum sensing activities of *Eucalyptus globulus* and *Eucalyptus radiata* essential oils. **Ind Crops Prod** 79: 274 - 282. **https://doi.org/10.1016/j.indcrop.2015.10.055**

Melo NR, Soares NDFF, Gonçalves MPJC. 2005. Nisina: um conservante natural para alimentos. **Rev Ceres** 52: 921 - 938.

Pinto JAO, Blank AF, Nogueira PCL, Arrigoni-Blank MF, Andrade TM, Sampaio TS, Pereira KLG. 2019. Chemical characterization of the essential oil from leaves of basil genotypes cultivated in different seasons. **Bol Latinoam Caribe Plant Med Aromat** 18: 58 - 70. **https://doi.org/10.35588/blacpma.19.18.1.05**

Pinto VS. 2017. **Influência da idade da planta na composição química do óleo essencial de** *Lippia alba* **e de um ciclo de seleção recorrente na atividade formicida**. Thesis, Universidade Federal de Sergipe, São Cristóvão, Brasil.

Ponce AG, Fritz R, Valle C, Roura SI. 2003. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. **LWT-Food Sci Technol** 36: 679 - 684. **https://doi.org/10.1016/s0023-6438(03)00088-4**

Prates HT, Leite RC, Craveiro AA, Oliveira AB. 1998. Identification of some chemical components of the essential oil from molasses grass (*Melinis minutiflora* Beauv.) and their activity against cattle-tick (*Boophilus microplus*). **J Braz Chem Soc** 9: 193 - 197. **https://doi.org/10.1590/s0103-50531998000200013**

Rattanachaikunsopon P, Phumkhachorn P. 2010. Antimicrobial activity of basil (*Ocimum basilicum*) oil against *Salmonella Enteritidis in vitro* and in food. **Biosci Biotechnol Bioquím** 74: 1200 - 1204. **https://doi.org/10.1271/bbb.90939**

Saddiq AA, Khayyat SA. 2010. Chemical and antimicrobial studies of monoterpene: Citral. **Pestic Biochem Physiol** 98: 89 - 93. **https://doi.org/10.1016/j.pestbp.2010.05.004**

Sahpaz S, Dermont C, Joseph H. 2006. The essential oil of *Lippia alba*: analysis of samples from french overseas departments and review of previous works introduction. *Lippia alba* (Mill.) N. E. B rown is an aromatic shrub mainly found in all regions of South and Central America. **Chem Biodivers** 3: 1116 - 1125. **https://doi.org/10.1002/cbdv.200690113**

Santos FCC, Vogel FSF, Monteiro SG. 2012. Efeito do óleo essencial de manjericão (*Ocimum basilicum* L.) sobre o carrapato bovino *Rhipicephalus* (Boophilus) *microplus* em ensaios *in vitro*. **Semina Ciênc Agrár** 33: 1133 - 1140. **https://doi.org/10.5433/1679-0359.2012v33n3p1133**

SVS [Secretaria de Vigilância em Saúde]. 2018. **Análise Epidemiológica dos Surtos de Doenças Transmitidas por Alimentos no Brasil.** Coordenação de Vigilância Alimentar, Brasil.

Silva DC, Blank AF, Nizio DAC, Sampaio TS, Nogueira PCL, Arrigoni-Blank MF. 2018. Chemical diversity of essental oils from native populations of *Eplingella fruticosa*. **Crop Breed Appl Biotechnol** 18: 205 - 214. **https://doi.org/10.1590/1984-70332018v18n2n29**

Silva GB. 2011. **Isolamento, caracterização, quantificação e avaliação da pureza enantiomérica de linalol, carvona e limoneno em óleos essenciais de espécies aromáticas**. Thesis, Universidade Federal de Sergipe, São Cristóvão, Brasil.

Silva LL, Balconi LS, Gressler LT, Garlet QI, Sutili FJ, Vargas APC, Baldisserotto B, Morel, AF, Heinzmann BM. 2018. *S*-(+)- and *R*-(-)-linalool: a comparison of the *in vitro* anti-*Aeromonas hydrophila* activity and anesthetic properties in fish. **An Acad Bras Cienc** 89: 203 - 212. **https://doi.org/10.1590/0001-3765201720150643**

Siroli L, Patrignani F, Gardini F, Lanciotti R. 2015. Effects of sub-lethal concentrations of thyme and oregano essential oils, carvacrol, thymol, citral and trans-2-hexenal on membrane fatty acid composition and volatile molecule profile of *Listeria monocytogenes*, *Escherichia coli* and *Salmonella enteritidis*. **Food Chem** 182: 185 - 192. **https://doi.org/10.1016/j.foodchem.2015.02.136**

Tagg JR, Dajani AS, Wannamaker LW. 1976. Bacteriocins of gram-positive bacteria. Bacteriocins of Gram-Positive Bacteria. **Bacteriol Rev** 40: 722 - 756. **https://doi.org/10.1128/mmbr.40.3.722-756.1976**

Tavares IB, Momenté VG, Nascimento IR. 2011. *Lippia alba*: estudos químicos, etnofarmacológicos e agronômicos. **Pesquisa Aplic Agrotecnol** 4: 204 - 220. **https://doi.org/10.5777/paet.v4.n1.12**

Van Den Dool H, Kratz PDA. 1963. A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. **J Chromatogr A** 11: 463 - 471. **https://doi.org/10.1016/s0021-9673(01)80947-x**

Weerawatanakorn M, Wu JC, Pan MH, Ho CT. 2015. Reactivity and stability of selected flavor compounds. **J Food Drug Anal** 23: 176 - 190. **https://doi.org/10.1016/j.jfda.2015.02.001**

Zhang Y, Liu X, Wang, P. Jiang Y, Quek S. 2016. Antibacterial activity and mechanism of cinnamon essential oil against *Escherichia coli* and *Staphylococcus aureus*. **Food Control** 59: 282 - 289. **https://doi.org/10.1016/j.foodcont.2015.05.032**