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In Vitro Antimicrobial Activities of Various Essential Oils Against Pathogenic and Spoilage Microorganisms

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HIGHLIGHTS

- Gram-positive bacteria were more sensitive than Gram-negatives against our analyzed Essential Oils (EOs).
- Yeasts were more sensitive than bacteria against our analyzed EOs.
- Oregano and thyme EOs showed the highest antimicrobial activity.

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Acronyms and abbreviations
EO=Essential Oil
MIC=Minimum Inhibitory Concentration
MBC=Minimal Bactericidal Concentration
DDA=Disk Diffusion Assay

ABSTRACT

Background: Plant-derived Essential Oils (EOs) have shown remarkable antimicrobial activity against spoilage and pathogenic microorganisms isolated from food products. The objective of the current study was to determine *in vitro* antimicrobial effects of selected EOs against these microorganisms.

Methods: Antimicrobial activity of EOs against food-borne and spoilage microorganisms was screened by disk diffusion assay; then, the Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were determined. Statistical analysis was done using SPSS 23.0 software for Windows.

Results: Oregano and thyme EOs showed the highest antimicrobial activity and the lowest MICs, while anise, fennel, garlic, and ginger showed a lower activity with significant differences (p<0.05). It was demonstrated that *Salmonella* Typhimurium, *Escherichia coli*, *Proteus mirabilis*, and *Yersinia enterocolitica* were the most sensitive bacteria to all the EOs tested (p<0.05). Among Gram-positive bacteria, *Listeria innocua* was demonstrated to be the most sensitive to most of the EOs (p<0.05). Furthermore, *Staphylococcus aureus* and *Listeria monocytogenes* were shown to be more sensitive than *Enterococcus* spp. (p<0.05). Yeasts were significantly (p<0.05) more sensitive than bacteria and were inhibited by most of the EOs.

Conclusion: The use of the analyzed EOs may be interesting to food processors because of their antimicrobial properties. However, it is necessary to test their use in food products and gauge their sensory implications.

Introduction

Plant-derived Essential Oils (EOs) are natural antimicrobials found in many plants and could be capable of decreasing growth as well as survival of some microorganisms (Calo et al., 2015). EOs in aromatic plants are among the most significant active compounds of herbs and spices (Krisch et al., 2010). Various biological characteristics, such as digestive, anti-inflammatory, sedative, antioxidant, antimicrobial, antiviral, and also cytotoxic activities have been attributed to the EOs (Bakkali et al., 2008; Burt, 2004). They are naturally occurring antimicrobials that have been shown to be

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effective against several microorganisms associated with several food products (Burt, 2004; Selim, 2010). EOs have been proposed as a viable alternative for application in food processing to avoid the use of traditional chemical additives (García-Diez et al., 2016; Ghabraie et al., 2016).

The differences in antimicrobial activity by different EOs are usually associated with their various chemical compositions that change according to seasons, geographical location of plants and/or the methodology used in EOs extraction (García-Diez et al., 2016; Kokkini et al., 1997). However, their antimicrobial activity may be attributed to their ability to penetrate through bacterial membranes and inhibit functional and lipophilic properties of the cell (Burt, 2004; Calo et al., 2015; Trombetta et al., 2005).

In recent years, consumers demand minimally processed foods. The negative perception of consumers about chemical food additives makes natural methods of preservation and natural preservatives receiving increased attention by the food industry (García-Diez et al., 2016). Non-phytotoxic oils are safe as food additives and declared as "Generally Recognized As Safe" (GRAS), which increased consumer acceptability (Jayasen and Jo, 2013). However, application of EOs is limited by taste and odor impacts, especially when used at high concentrations (Ghabraie et al., 2016). In fact, the organoleptically acceptable concentration depends on individual EO, the specific food systems, the method of application, and food product cooking methods. Indeed, it would be changed when other compounds are added to the food, too. Therefore, it is necessary to determine their lowest concentration with acceptable sensorial level in order to use them in food without any changes in smell and taste (Turgis et al., 2012).

The aim of the current study was to determine *in vitro* antimicrobial activity of various EOs against selected pathogenic and spoilage microorganisms, aiming for a future utilization in the manufacture of some food products.

Materials and methods

EOs

Twenty-three plant EOs were used in this study, including anise (*Pimpinella anisum*), basil (*Ocimum basilicum*), bay (*Laurus nobilis* L.), cardamom (*Elettaria cardamomum*), and fennel (*Foeniculum vulgare*), kindly provided by FRULACT (Gemunde Maia, Portugal); carrot (*Daucus carrot* L.), cloves (*Syzygium aromaticum*), coriander (*Coriandrum sativum*), cumin (*Cuminum cyminum*), garlic (*Allium sativum*), juniper berry (*Juniperus communis*), marjoram (*Origanum majorana*), nutmeg (*Myristica fragrans*), parsley (*Petroselinum crispum*), oregano (*Origanum vulgare* L.), rosemary (*Rosmarinus officinalis*), and sage (*Salvia officinalis*) kindly provided by Ventós Chemical (V., Barcelona, Spain); lemon (*Citrus limon*), garlic (*Allium sativum*), ginger (*Zingiber officinale*), oregano (*Origanum vulgare*) and thyme (*Thymus vulgaris*) by Casa das Essências (C.E., Oeiras, Portugal).

Microorganisms and growth conditions

All strains used in this study (Table 1) were stored at -20 ºC in Tryptic Soy Broth (TSB; Pronadisa, Madrid, Spain) with 6 g/L of Yeast Extract (YE; Lab M, Bury, UK) containing 30% (v/v) glycerol (Sigma, Steinheim, Germany), and sub-cultured twice before use in assays. Each bacterial strain was grown on Tryptic Soy Agar (TSA, Pronadisa) with 6 g/L of YE (Lab M, Bury, UK) at 37 ºC for 24 h and yeasts in Yeast Malt Agar (YMA; Lab M, Bury, UK) at 25 ºC for 48 h.

Disk Diffusion Assay (DDA)

Each inoculum was prepared by resuspending isolated colonies of each strain, previously cultured on TSA+YE or YMA, in sterile Ringer solution (Lab M, Bury, UK) in order to obtain turbidity equivalent to 0.5 in McFarland scale (bioMérieux, Marcy-l'Etoile, France).

The antimicrobial impact of EOs was evaluated by the DDA as indicated by Zaika (1987), with some modifications. Briefly, plates prepared with Mueller-Hinton Agar (MHA; Biokar, Beauvais, France), or YMA for yeasts were dried and 100 µl of standardized inoculum were uniformly spread. After that, sterilized filter paper disks (Whatman No. 5, 6 mm diameter) were applied to the surface of the seeded agar plates and 5 µl of each sterilized EO (0.22 µm syringe filter) was applied to each disk. The plates were kept at 4 ºC for 2 h to allow dispersion and incubated for 18 to 24 h at 37 ºC for all bacteria; also, the yeasts were incubated for 48 h at 25 ºC. The antimicrobial activity was visually evaluated as inhibition zone surrounding the disk and the disk diameter was measured in mm. Inhibition was only considered if the halos were greater than 10 mm, according to García-Díez et al. (2016). The DDA assay was carried out in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC)

The MIC and MBC were studied only for all EOs that resulted in inhibition halos greater than 10 mm. The assay was based on the procedures described in CLSI (2012) using 96-wells microtiter plates. The dilutions of

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the EOs were provided according to the inhibitory profile with the DDA (halos greater than 10 mm). EOs dilutions were prepared directly in the Mueller-Hinton Broth (MHB, Biokar, France) to achieve in the well each of the followings concentrations: 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, 0.39, 0.195, 0.0975, 0.0488, 0.0244, 0.0129, and 0.0060%. In each well, it was mixed 80 µl of MHB, 100 µl of each EO dilution, and 20 µl of standard suspension of each target microorganism (prepared in MHB to achieve a final cell density in each well of ca. 5 log colony forming unit/ml). Un-inoculated negative controls were included. The plates were covered, incubated for 24 h and then checked for visible growth (turbidity) in each well. The MIC was determined as the lowest concentration of EO which prevented growth. To determine the MBC, 10 µl of each well, in which no microbial growth was seen, was spread into MHA and incubated for 24 h, as stated by García-Díez et al. (2016).

Statistical analysis

The comparison of the antimicrobial activity of EOs against each microorganism was carried out by one-way Analysis of Variance (ANOVA). The Tukey-Kramer test was used to determine the significant differences (p<0.05) among group means. Statistical analysis was done using SPSS 23.0 software for Windows.

Results

The data of the antimicrobial activity assessed by DDA showed that in general the antimicrobial activities of the different tested EOs were varied and dependent on the type of oil and type of microorganism (data not shown). In general, oregano from "Casa das Essências": C.E. and oregano from "Ventós" Chemical: V. had the EOs that showed significantly (p<0.05) higher antimicrobial activity than the others. On the other hand, EOs of lemon, ginger, and anise were the ones that showed meaningfully (p<0.05) lower antimicrobial activity. It was demonstrated that *S.* Typhimurium, *Escherichia coli*, *Proteus mirabilis*, and *Yersinia enterocolitica* were the most sensitive bacteria to all the tested EOs (p<0.05). Among Gram-positive bacteria, *L. innocua* was demonstrated to be the most sensitive to most of the EOs (p<0.05). Furthermore, *Staphylococcus aureus* and also *L. monocytogenes* were shown to be more sensitive than *Enterococcus* spp. (p<0.05). Yeasts were significantly (p<0.05) more sensitive than bacteria and were inhibited by most of the EOs.

Results of MIC and MBC of the tested EOs are presented in Tables 2 to 6. Through the results obtained in the DDA, all EOs with halos lower than 10 mm were excluded and not tested for determination of MICs. Values of MIC and MBC were, on average, higher for Gram-negative microorganisms than for Gram-positives.

EOs of bay, cloves, oregano (C.E.), oregano (V.), and thyme presented MICs between 0.0488% and 1.56% for Gram-negative bacteria (Tables 2 and 3). However, the EO that demonstrated the lowest inhibitory concentration against all the analyzed microorganisms was the oregano (V.) (0.0975-0.0488%). Hence, the most sensitive microorganisms were *S.* Typhimurium, *E. coli*, as well as *Y. enterocolitica*.

Regarding Gram-positive bacteria, there was a higher number of EOs demonstrating low inhibitory concentrations. For the non-spore forming bacteria, EOs of bay, cloves, coriander, cumin, marjoram, oregano (C.E.), oregano (V:), rosemary, and thyme presented MICs between 0.0244 and 3.125% (Tables 4 to 6). Strains of *St. aureus*, *L. monocytogenes*, and *L. innocua* were the most sensitive. *Enterococcus* spp. were the most resistant.

Bay, basil, cloves, coriander, oregano (C.E)., oregano (V.), rosemary, and thyme had the EOs with the lowest MICs (between 0.0488% and 3.125%) for the spore forming bacteria; *B. cereus* was the most sensitive (data not shown).

Regarding yeasts, these were extremely sensitive, demonstrating low MICs for most of the EOs, with the exception of anise, basil, fennel, juniper berries, nutmeg, and parsley that showed higher MICs (between 3.125% and 100%; data not shown).

Discussion

Through the analysis of the results obtained for DDA and MIC, it was possible to verify that Gram-positive bacteria are more sensitive than Gram-negative ones, which is in accordance with previous reports (Hyldgaard et al., 2012; Nazzaro et al., 2013). The cell wall of Gram-positive bacteria allows hydrophobic molecules to readily penetrate into cells and act on both cell wall and cytoplasm; but Gram-negative bacteria have an outer membrane that contains lipopolysaccharides that could act as a barrier against macromolecules and hydrophobic compounds, making them more resistant to these same compounds (Nikaido, 1994, 2003). The lowest inhibitions observed in the DDA for EOs were in accordance with the highest MIC and MBC values obtained. Conversely, the lowest MIC and MBC values of EOs of thyme and oregano (V.) were similar with their previously observed high antimicrobial activity observed in the DDA. The values of MIC and MBC were similar, with small differences, being considered bactericidal in their mode of action.

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Table 1: Microbial strains and their sources used in this study

Microorganisms	Species	Sources
Gram-positives	*Bacillus cereus* *Bacillus subtilis* *Bacillus*(*Geobacillus*) *stearothermophilus* *Listeria monocytogenes* SCOTT A *Listeria innocua* 2030c *Staphylococcus aureus* 18N (Methicillin-resistant *St. aureus* - MRSA) *Staphylococcus aureus* 2037 M1 (Methicillin- sensitive *St. aureus* - MSSA)	ESB culture collection
	Enterococcus faecalis ATCC 29212 *Staphylococcus aureus* ATCC 29213	ATCC
	Enterococcus faecalis DSMZ 12956 *Enterococcus faecium* DSMZ 13590 *Enterococcus flavescens* DSMZ 7370 *Enterococcus casseliflavus* DSMZ 20680 *Enterococcus gallinarum* DSMZ 20628	DSMZ
	Listeria monocytogenes L7946 *Listeria monocytogenes* L7947	McLauchlin et al. (1997)
Gram-negatives	*Acinetobacter baumannii* R *Acinetobacter baumannii* S-1 *Acinetobacter baumannii* S-2 *Acinetobacter calcoaceticus* R *Acinetobacter calcoaceticus* S *Klebsiella pneumoniae* *Proteus mirabilis* *Proteus vulgaris* *Pseudomonas aeruginosa* *Salmonella* Braenderup *Salmonella* Enteritidis *Salmonella* Enteritidis 417536 *Salmonella* Enteritidis 545047 *Salmonella* Typhimurium *Yersinia enterocolitica*	ESB culture collection
	Escherichia coli ATCC 25922	ATCC
	Yersinia enterocolitica NCTC 10406	NCTC
Yeasts	*Candida albicans* *Saccharomyces cerevisiae*	ESB culture collection

ESB: culture collection of Escola Superior de Biotecnologia; DSMZ: German Collection of Microorganisms and Cell Cultures; ATCC: American Type Culture Collection; NCTC: National Collection of Types Cultures - Culture Collection of Public Health England. S - Sensitive to several tested antibiotics; R - Resistant to several antibiotics.

Regarding the used EOs, it was possible to state that oregano and thyme were the EOs with the greatest inhibitory capacity for all the bacteria tested in DDA. The MIC and MBC values that were observed in this study were similar to those previously reported in the literature (García-Diez et al., 2016; Sokovic et al., 2010). Oregano (V.) was the EO that presented the lowest MIC, with values between 0.195 and 0.0244%. The lowest MIC (0.0244%) was observed against *L. innocua*, nevertheless a MIC of 0.0975% was observed for the majority of the microorganisms under study. According to these concentrations, this EO could be considered with great inhibitory potential. These results are in agreement with previous studies, which also reported a high antimicrobial activity of oregano and thyme (Dobre et al., 2011; Semeniuc et al., 2017). Although two different oregano oils were used in our study, oregano (V.) has a greater inhibitory capacity, which may be due to their different origins. According to Kokkini et al. (1997), the type of extraction of EOs and the different seasons of the year could produce different amounts of compounds related to each EO. This antimicrobial activity is probably due to its main components, including carvacrol for oregano and thymol for thyme. Thymol as well as carvacrol are hydrophobic compounds, which induce structural and functional damages to cytoplasmic membrane (Sikkema et al., 1995). Hyldgaard et al. (2012) described that thymol could be involved in the rupture of the inner and outer membrane and the interaction with membrane proteins and intracellular targets, while carvacrol owes its mechanism of action to its ability to position into the membrane, which increases their permeability.

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Table 2: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of tested Essential Oils (EOs) in *Salmonella* spp. and *Escherichia coli* (results are expressed in % of EO)

	***S.* Braenderup**		***S.* Enteritidis** *		***S.* Typhimurium**		***E. coli* ATCC 25922**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Anise	100	100	100	100	100	100	100	100
Basil	100	100	100	100	100	100	100	100
Bay	0.39	0.39	0.195	0.195	0.195	0.195	0.39	0.78
Carrot	100	100	100	100	100	100	100	100
Cloves	0.195	0.195	0.195	0.195	0.195	0.195	0.195	0.195
Coriander	100	100	1.56	1.56	0.78	0.78	0.78	0.78
Cumin	100	100	100	100	1.56	1.56	1.56	3.125
Fennel	100	100	100	100	100	100	100	100
Juniper berries	100	100	100	100	100	100	100	100
Lemon	100	100	100	100	100	100	100	100
Marjoram	100	100	100	100	0.78	0.78	0.78	0.78
Nutmeg	100	100	100	100	12.5	12.5	12.5	12.5
Parsley	100	100	50	50	100	100	50	50
Pepper mint	100	100	0.78	0.78	0.39	0.39	0.39	0.39
Oregano (C.E)	0.78	1.56	1.56	1.56	0.78	1.56	0.78	0.78
Oregano (V.)	0.0975	0.0975	0.0975	0.0975	0.0488	0.0488	0.0488	0.0488
Rosemary	100	100	100	100	0,78	0.78	3.125	3.125
Sage	100	100	100	100	0.78	0.78	0.78	0.78
Thyme	0.78	0.78	1.56	1.56	0.78	0.78	0.78	0.78

*Three strains: *S.* Enteritidis; *S.* Enteritidis 417536; *S.* Enteritidis 545047.

Table 3: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of tested Essential Oils (EOs) in *Klebsiella pneumoniae*, *Proteus* spp., and *Yersinia* spp. (results are expressed in % of EO)

	K. pneumoniae		***P. vulgaris***		***P. mirabilis***		***Y. enterocolitica* NCTC 10406**		***Y. enterocolitica***	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Anise	100	100	100	100	100	100	100	100	12.5	25
Basil	25	25	100	100	25	25	50	50	100	100
Bay	0.39	0.39	0.78	0.78	0.78	0.78	0.78	0.78	0.195	0.39
Carrot	100	100	100	100	100	100	100	100	100	100
Cloves	0.195	0.195	0.195	0.195	0.0975	0.0975	0.195	0.195	0.39	0.39
Coriander	0.78	0.78	100	100	0.78	0.78	1.56	1.56	0.39	0.39
Cumin	3.125	3.125	100	100	1.56	3.125	3.125	3.125	0.78	1.56
Fennel	100	100	100	100	100	100	100	100	50	50
Juniper berries	100	100	100	100	100	100	100	100	100	100
Lemon	100	100	100	100	100	100	100	100	100	100
Marjoram	1.56	1.56	100	100	3.125	3.125	1.56	1.56	1.56	1.56
Nutmeg	100	100	100	100	100	100	100	100	6.25	6.25
Parsley	100	100	100	100	50	50	25	25	50	50
Pepper mint	100	100	0.78	0.78	0.78	0.78	0.39	0.78	0.195	0.39
Oregano (C.E)	1.56	1.56	0.78	0.78	0.78	0.78	0.78	1.56	0.78	0.78
Oregano (V.)	0.0488	0.0488	0.0975	0.0975	0.0975	0.0975	0.0488	0.0488	0.0488	0.0975
Rosemary	50	50	100	100	12.5	12.5	6.25	6.25	0.78	0.78
Sage	100	100	100	100	1.56	1.56	1.56	1.56	0.78	0.78
Thyme	1.56	1.56	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78

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Table 4: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of tested Essential Oils (EOs) in *Enterococcus* spp. (results are expressed in % of EO)

	***E. faecalis* ATCC 29212**		***E. faecalis* DSMZ 12956**		***E. faecium* DSMZ 13590**		***E. flavescens* DSMZ 7370**		***E. gallinarium* DSMZ 20628**		***E. casseliflavus* DSMZ 20680**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Anise	100	100	100	100	100	100	100	100	100	100	100	100
Basil	25	50	25	25	100	100	100	100	100	100	25	25
Bay	0.39	0.39	0.78	0.78	0.39	0.39	0.78	0.78	0.39	0.39	0.78	0.78
Carrot	100	100	0.0975	0.0975	0.195	0.195	0.0975	0.0975	0.39	0.39	25	25
Cloves	0.195	0.195	0.39	0.39	0.195	0.195	0.0975	0.195	0.39	0.39	0.195	0.195
Coriander	1.56	1.56	0.78	0.78	0.78	0.78	0.78	0.78	1.56	1.56	1.56	1.56
Cumin	100	100	50	100	12.5	12.5	25	25	100	100	50	50
Fennel	100	100	100	100	100	100	100	100	100	100	100	100
Juniper berries	100	100	6.25	6.25	3.125	3.125	100	100	100	100	100	100
Lemon	100	100	100	100	100	100	100	100	100	100	100	100
Marjoram	1.56	1.56	1.56	1.56	0.78	0.78	0.78	0.78	0.78	0.78	0.78	0.78
Nutmeg	100	100	100	100	100	100	100	100	3.125	3.125	100	100
Parsley	100	100	25	25	100	100	25	25	25	25	100	100
Pepper mint	100	100	6.25	12.5	12.5	25	0.78	1.56	100	100	100	100
Oregano (C.E)	0.78	0.78	0.78	0.78	0.78	0.78	0.39	0.39	0.78	0.78	0.78	0.78
Oregano (V.)	0.195	0.195	0.0975	0.0975	0.0975	0.0975	0.0488	0.0975	0.0975	0.0975	0.0975	0.0975
Rosemary	12.5	12.5	0.78	0.78	0.78	0.78	0.78	0.78	3.125	3.125	3.125	3.125
Sage	1.56	1.56	3.125	3.125	100	100	1.56	1.56	100	100	1.56	1.56
Thyme	1.56	1.56	0.78	0.78	1.56	1.56	0.78	0.78	0.78	0.78	0.78	0.78

Table 5: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of tested Essential Oils (EOs) in *Staphylococcus* spp. (results are expressed in % of EO)

	***St. aureus* ATCC 29213**		***St. aureus* 18N (MRSA)**		***St. aureus* 2037 M1 (MSSA)**	
	MIC	MBC	MIC	MBC	MIC	MBC
Anise	100	100	100	100	100	100
Basil	50	50	100	100	100	100
Bay	0.39	0.39	0.39	0.39	0.39	0.39
Carrot	12.5	12.5	0.195	0.195	0.39	0.39
Cloves	0.195	0.195	0.195	0.195	0.0975	0.0975
Coriander	1.56	1.56	0.78	0.78	0.78	0.78
Cumin	3.125	6.25	3.125	3.125	3.125	3.125
Fennel	100	100	100	100	100	100
Juniper berries	100	100	100	100	100	100
Lemon	25	25	50	50	100	100
Marjoram	1.56	1.56	0.78	0.78	0.78	0.78
Nutmeg	100	100	100	100	100	100
Parsley	100	100	25	25	25	25
Pepper mint	0.78	1.56	1.56	1.56	0.195	0.195
Oregano (C.E)	0.78	0.78	0.78	1.56	0.78	0.78
Oregano (V.)	0.0975	0.0975	0.0975	0.0975	0.0975	0.195
Rosemary	1.56	1.56	1.56	1.56	1.56	1.56
Sage	3.125	3.125	100	100	0.78	0.78
Thyme	0.78	1.56	0.78	0.78	0.78	0.78

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Table 6: Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of tested Essential Oils (EOs) in *Listeria* spp. (results are expressed in % of EO)

	***L. monocytogenes* 7946**		***L. monocytogenes* 7947**		***L. monocytogenes* SCOOT A**		***L. innocua* 2030c**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Anise	100	100	100	100	100	100	100	100
Basil	100	100	100	100	100	100	100	100
Bay	0.39	0.39	0.39	0.39	0.39	0.39	0.39	0.39
Carrot	0.79	0.79	0.39	0.39	0.39	0.39	0.0975	0.0975
Cloves	0.195	0.39	0.0488	0.0488	0.195	0.195	0.39	0.78
Coriander	0.78	0.78	0.39	0.39	0.78	0.78	0.78	0.78
Cumin	1.56	3.125	1.56	3.125	1.56	1.56	3.125	3.125
Fennel	100	100	100	100	100	100	100	100
Juniper berries	100	100	12.5	12.5	25	25	3.125	6.25
Lemon	100	100	100	100	100	100	3.125	3.125
Marjoram	1.56	1.56	0.0064	0.0064	1.56	1.56	0.78	0.78
Nutmeg	12.5	12.5	3.125	3.125	6.25	6.25	12.5	12.5
Parsley	25	25	50	50	50	50	50	50
Pepper mint	3.125	6.25	0.195	0.195	1.56	1.56	6.25	12.5
Oregano (C.E)	0.78	1.56	0.39	0.39	0.78	0.78	0.78	1.56
Oregano (V.)	0.0975	0.195	0.0975	0.0975	0.0975	0.0975	0.0244	0.0244
Rosemary	1.56	1.56	0.0488	0.0488	0.0975	0.0975	0.78	0.78
Sage	1.56	1.56	0.0975	0.0975	1.56	1.56	0.78	0.78
Thyme	0.78	0.78	0.39	0.78	0.78	0.78	0.39	0.78

Sokovic et al. (2010) demonstrated that oregano EO, thyme EO, and their principal compounds were the most active against *Bacillus subtilis*, *St. epidermidis*, *St. aureus*, *S.* Enteritidis, *S.* Typhimurium, *E. coli*, *P. mirabilis*, *Pseudomonas aeruginosa*, as well as *L. monocytogenes*. In a study carried out by Silva et al. (2013), it was also demonstrated that among the evaluated EOs, the greatest effectiveness was achieved when thyme and oregano were used, which showed activity against all the tested bacterial strains. Similarly, Gutierrez et al. (2008) showed that *B. cereus*, *E. coli*, *L. monocytogenes*, and *Ps. aeruginosa* were sensitive to the oregano EO. Burt (2004) also reviewed that EOs had antimicrobial effect against some microorganisms in different food products such as boiled rice, carrots, soft cheese, and fish. Regarding yeasts, the results obtained in the present study are in agreement with other studies showing that oregano EO exhibited a broad spectrum of activity against *Candida* spp. (Khosravi et al., 2011) and *Saccharomyces cerevisiae* was the most sensitive microorganism to all EOs tested (Çoskun et al., 2016).

Conclusion

The current study demonstrated that tested EOs had *in vitro* antimicrobial effect against food-borne pathogens such as *Salmonella* spp., *L. monocytogenes*, *St. aureus*, and *E. coli* and also against some spoilage bacteria. EOs of oregano and thyme showed the greatest inhibitory effect against the different microorganisms. These differences could be associated to several factors such as chemical composition of the EOs or to the specific sensitivity of the target microorganism among others.

Taking into account the large number of oils and the number of investigated microorganisms, this study is of great importance to their potential users, namely the food industry, since it covers several food-borne pathogenic microorganisms. However, it is necessary to test their use in each food matrix and gauge their sensory implications.

Author contributions

H.A., M.C., and P.T. designed the study and wrote the manuscript; M.C. conducted the experimental work; H.A. and M.C. analyzed the data. All authors revised and approved the final manuscript.

Conflicts of interest

No conflict of interest declared.

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