Article

Evaluation of chemical composition, antioxidant and anti *Listeria monocytogenes* and *Salmonella enterica* activity of the essential oil of *Mentha pulegium* and *Mentha suaveolens* collected in Morocco

Abdelaziz Ed-Dra[1], Fouzia Rhazi Filali[1], Vittorio Lo Presti[2], Badr Zekkori[3], Luca Nalbone[2], Najla Trabelsi[4], Amar Bentayeb[3], Alessandro Giuffrida[2], Filippo Giarratana[2]*

[1] Team of Microbiology and Health, Laboratory of Chemistry-Biology Applied to the Environment, Moulay Ismail University Faculty of Sciences, BP. 11201 Zitoune Meknes, Morocco
[2] Department of Veterinary Science, University of Messina, Polo Universitario dell'Annunziata, 98168 Messina, Italy
[3] Department of Chemistry, Team of Physical-Chemistry of Condensed Matter, Moulay Ismail University, Faculty of Sciences, BP. 11201 Zitoune Meknes, Morocco
[4] Center of Biotechnology of Borj Cedria, LR15CBBC05 Olive Biotechnology, University of Tunis El Manar, 2050, Tunis, Tunisia

* Correspondence: fgiarratana@unime.it; Tel.: +39 0906766889

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Abstract:
The essential oils (EOs) obtained from aromatic plants are rich in natural compounds with interesting biological effects. The aim of this study was to evaluate the chemical composition of EOs of *Mentha pulegium* (MP-EO) and *Mentha suaveolens* (MS-EO) collected from Morocco, and their antioxidant properties and antibacterial activity against *Salmonella enterica* and *Listeria monocytogenes*. The EOs were extracted by hydro-distillation, while the chemical compositions were determined by GC-MS. The antioxidant activity was evaluated by DPPH and FRAP assay. Antibacterial activity was tested with disc diffusion assay; determination of minimum inhibitory concentration, minimum bactericidal concentration and the evaluation of sub-lethally injured cell were also performed. The results of chemical composition showed the presence of compounds not still reported in EOs obtained from these plants. MS-EO was characterized by the best antioxidant and antibacterial activity *vs S. enterica* and *L. monocytogenes* respect to MP-EO. The EOs tested in this study were rich in compounds with interesting activities and they could be applied in the medical fields, as well as in food industries as natural preservatives against tested food borne pathogens.

Keywords: Essential oil; *Mentha pulegium*; *Mentha suaveolens*; *Listeria monocytogenes*; *Salmonella enterica*; antioxidant; sub-lethally injured bacteria.

1. Introduction

Several plants and herbs have been used by human for medical purpose as well as for foods preservation since ancient times [1-5]. Over the years, antibiotics in the treatment of human infections and chemical additives in the conservation of foods have taken over the lead.

The over use of antibiotics in human medicine and veterinary practice has determine the diffusion of multidrug resistant (MDR) bacteria [6-9]. This MDR has been reported in several pathogen, commensal and environmental bacteria [10-19]. The infection deriving from these MDR bacteria represent a risk for public health [16,20]. For all these reasons the World Health

Organization (WHO) required the reduction of the use of antibiotics and alternative to resolve the therapeutic failure in the treatment of MDR bacteria [20].

In this contest several studies were recently performed in order to improve the knowledge on antibacterial activity of different natural compounds and of essential oils (EOs) [21,22]. Over the years several authors have demonstrated that EOs possess not only antibacterial activity, but also antioxidant, anti-infiammatory and anti-carcinogenic properties as well as effectiveness against nematode parasites, mold and virus [23-30]. For all these properties their application has been hypothesized, not only to the treatment of human disease but also in in food as alternative to chemical preservatives for prolongation of shelf-life and safety purpose [31-33]. Several studies have demonstrated in fact their effectiveness against the two most important food borne pathogen disease such as *Listeria monocytogenes* and *Salmonella* spp. [34,35]. The bacterial infections due to the consumption of contaminated food by these two pathogens were, in fact, considered as major cause for morbidity and mortality worldwide. *L. monocytogenes* was classified among the most virulent bacteria with a high level of mortality and its frequently isolation in undercooked food and ready-to-eat food, represent a serious risk for the consumers [36-40]. *Salmonella*, instead, is considered the major foodborne pathogens worldwide and determined millions of cases of diseases each year [41]. Meat and meat products are considered as the major mean of transferring these bacteria to humans [17,42,43].

Morocco for its geographical position, between two seas and a wide desert, crossed by four mountain chains is considered a favourable area for the growth of a wide range of aromatic and medicinal plants (more than 4200 different plants) [44,45]. Among aromatic plants *Mentha pulegium* and *Mentha suaveolens* and their EOs possess interesting activities, such as antibacterial, antiviral, antioxidant, anti-inflammatory and insetticidal [46-53].

The aim of this study was to improve the knowledge on the EOs obtained from *Mentha pulegium* and *Mentha suaveolens* collected from Morocco, evaluating their composition, antioxidant and antibacterial activity against *Salmonella enterica* and *Listeria monocytogenes*.

2. Results and Discussion

2.1. Chemical composion of EOs: GC-MS analysis

The chemical profile of the two tested EO was presented in Table 1. The major compounds of *M. pulegium* essential oil (MP-EO) were Limonene (35.747%), Piperitone (29.527%) and β-Thujene (8.29%); while for *M. suaveolens* essential oil (MS-EO) Cinerone (16.273%), Caryophyllene (10.85%) and Terpine-4-ol (7.278%) were the major compounds observed.

Table 1. Chemical composition of *Mentha pulegium* and *Mentha suavelons* essential oil

N°	Component	RI	Formula	% Area	
				M. pulegium	*M. suaveolens*
1	2-Hexenal	842	$C_6H_{10}O$	0.045	-
2	Tricyclene	928	$C_{10}H_{16}$	0.056	-
3	*α*-Thujene	934	$C_{10}H_{16}$	0.117	0.071
4	*α*-Pinene	941	$C_{10}H_{16}$	0.383	1.334
5	*β*-Thujene	967	$C_{10}H_{16}$	**8.29**	-
6	*β*-Pinene	982	$C_{10}H_{16}$	0.283	3.938
7	3-Octanone	987	$C_8H_{16}O$	0.805	-
8	*β*-Myrcene	991	$C_{10}H_{16}$	0.26	-
9	3-Carene	1012	$C_{10}H_{16}$	-	0.691
10	*α*-Terpinene	1018	$C_{10}H_{16}$	0.232	2.285
11	*O*-Cymene	1024	$C_{10}H_{14}$	-	0.365

12	*p*-Cymene	1025	$C_{10}H_{14}$	0.183	1.505
13	Limonene	1029	$C_{10}H_{16}$	**35.747**	**5.282**
14	1,8-Cineole	1035	$C_{10}H_{18}O$	0.117	**6.944**
15	*γ*-Terpinene	1058	$C_{10}H_{16}$	-	2.366
16	*cis*-Sabinene Hydrate	1066	$C_{10}H_{18}$	0.071	3.618
17	*p*-Menthadien-7-ol	1071	$C_{10}H_{16}O$	0.45	-
18	Terpinolene	1088	$C_{10}H_{16}$	0.112	-
19	Chrysanthenone	1124	$C_{10}H_{14}O$	**-**	2.934
20	D-Menthone	1153	$C_{10}H_{18}O$	0.152	-
21	Terpinen-4-ol	1178	$C_{10}H_{18}O$	1.133	**7.278**
22	*p*-Cymen-8-ol	1186	$C_{10}H_{14}O$	-	0.845
23	*α*-Terpineol	1193	$C_{10}H_{18}O$	0.369	1.843
24	Cinerone	1229	$C_{10}H_{14}O$	**-**	**16.273**
25	Piperitone	1248	$C_{10}H_{16}O$	**29.527**	-
26	*β*-Caryophyllene	1426	$C_{15}H_{24}$	0.34	**10.85**
27	*α*-Caryophyllene	1465	$C_{15}H_{24}$	0.56	-
28	*β*-Selinene	1488	$C_{15}H_{24}$	-	1.489
29	BicycloGermacrene	1494	$C_{15}H_{24}$	-	1.432
30	*α*-Muurolene	1499	$C_{15}H_{24}$	-	0.56
31	*γ*-Cadinene	1515	$C_{15}H_{24}$	-	0.798
32	Calamenene	1523	$C_{15}H_{22}$	-	1.336
33	*β*-Cadinene	1531	$C_{15}H_{24}$	-	0.558
Total identified (%)				**79.232**	**74.595**
Monoterpene hydrocarbons				45.734	21.455
Sysquiterpene hydrocarbons				0.9	17.023
Oxygenated monoterpenes				31.748	36.117
Other				0.85	-

The chemical composition of MP-EO was completely different from those reported in other EOs obtained from *M. pulegium* collected in different area of Morocco. Pulegone was, in fact, identified as major compound from plants collected in the capital Rabat [46] and in the province of Ouezzane [54], Taourirt [55], Oued Laou [56] and Northern region [57]. Pulegeone was the main compounds also in EOs obtained from plants collected in Algeria [49,58], Egypt [59], Bulgaria [60] and north-western Himalayas (India) [61]; while Menthone was reported in Portugal [62]. The only composition similar to those described in this study was reported in Iran with Piperitone as major compounds [63].

Also the chemical composition of MS-EO was not in accordance to those reported in different area of Morocco or in other countries; Piperitenone oxide was, in fact, the major compound of the EOs derived from plants collected from the capital Rabat [46], or from Berkane [50] and Al Hoceima region of Morocco [64], from Italy [48,65] and Romania [51] or in association with Piperitenone from Corsica [66] and from Eastern of Iberian Peninsula [67]. In Egypt, instead, Carvone was the major compounds observed in tested EO [47].

2.2. Antioxidants acitvity results

The antioxidant activity evaluated using DPPH scavenging activity and the reducing power of Iron (III) to Iron (II), showed that MS-EO has the higher activity

(IC50=0.78±0.05mg/mL; EC50=1.53±0.07mg/mL); while MP-EO presented the lower (IC50=5.11±0.14 mg/mL; EC50=6.78±0.18mg/mL) (Table 2). This difference may depend on the chemical composition of each essential oil, which allows the free radicals scavenging. The richness of *M. suaveolens* essential oil with β-Caryophyllene improves its antioxidant activity. These findings have been confirmed by worldwide studies; an *in vitro* study carried out by Dahham and his group using FRAP and DPPH scavenging assays showed that Caryophyllene has a strong antioxidant effect [68]. Also, a study performed by Basha and Sankaranarayanan on diabetic rats showed that Caryophyllene attenuates hyperglycemia mediated oxidative and inflammatory stress [69]. Moreover, the *in vivo* assay performed by Calleja and his colleagues showed that Caryophyllene has an important antioxidant activity by protecting the rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation [70].

Table 2: The antioxidant activity of the tested Essential Oils

	Ascorbic acid	***M. suaveolens***	***M. pulegium***
IC_{50} (mg/mL)*	0.031±0.001[a]	0.78±0.05[b]	5.11±0.14[c]
EC_{50} (mg/mL)*	0.095±0.002[a]	1.53±0.07[b]	6.78±0.18[d]

The same letter was assigned to the values of the same line that does not have a significant difference ($P< 0.05$).

2.3. Antibacterial activity

2.3.1. Disc diffusion method

The results of antibacterial activity of the two essential oils against the 8 strains of *S. enterica* and *L. monocytogenes,* done using the disc diffusion, were presented in Table 3.

Table 3: Inhibition diameters (mm) of the tested Essential Oils against *S. enterica* and *L. monocytogenes* strains

Strains	**ID**	***M. pulegium***	***M. suaveolens***	**Cefotaxime**
S. enterica	S. 1	13±0.2	13±0.3	14±0.1
	S. 2	12±0.2	12±0.2	13±0.1
	S. 3	13±0.4	14±0.3	29±0.2
	S. 4	11±0.1	12±0.1	31±0.2
	S. 5	12±0.2	14±0.2	29±0.2
	S. 6	12±0.3	14±0.4	28±0.3
	S. 7	13±0.2	12±0.3	28±0.2
	S. 8	14±0.3	15±0.4	27±0.2
	Mean value	**12.5±0.9**	**13.3±1.2**	**24.9±7.1**
L. monocytogenes	L. 1	18±0.3	21±0.1	27±0.1
	L. 2	19±0.2	20±0.1	25±0.1
	L. 3	14±0.1	17±0.1	26±0.1
	L. 4	17±0.2	21±0.2	28±0.2
	L. 5	18±0.2	23±0.3	31±0.2
	L. 6	13±0.1	16±0.2	34±0.3
	L. 7	15±0.1	16±0.1	29±0.1
	L. 8	14±0.1	15±0.1	27±0.1
	Mean value	**16.7±2.7**	**18.7±3.0**	**29.4±2.9**

In this preliminary evaluation, the two tested oils showed an interesting antibacterial activity. MS-EO was more effectiveness than MP-EO, with a more evident activity *vs Listeria monocytogenes.* In particularly, the inhibition diameter of MP-EO *vs S. enterica* strains has a mean value of 12.5±0.9 mm (ranged from 11±0.1 to 14±0.3); while *vs L. monocytogenes* strains the mean value was 16.7±2.7 mm with value ranged from 13±0.1 to 19±0.2. The more effectiveness MS-EO reported, instead, an inhibition diameter *vs S. enterica* strains with a mean value of 13.3±1.2 mm with value ranged from 12±0.2 - 15±0.4; while *vs L. monocytogenes* strains the mean value was 18.7±3.0 mm (ranged from 15±0.1 to 23±0.3).

2.3.2. Evaluation of MIC and MBC

The determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), reported in Table 4 confirmed MS-EO has the more effectiveness (4 dilution more) respect to MP-EO, and *vs L. monocytogenes* strains. MP-EO presented, in fact, value for MIC and MBC of 2% respect to 0.5% of MS-EO vs *S. enterica*; while *vs L. monocytogenes* these values were 1% and 0.25% for MP-EO and MS-EO respectively. MIC and MBC were correspondent for both the tested EOs and *vs* all the tested strains.

Table 4: MIC and MBC in percentage (%) of the tested essential oils against *S. enterica* and *L. monocytogenes*

Strains	**ID**	***M. pulegium***		***M. suaveolens***	
		MIC	MBC	MIC	MBC
S. enterica	S. 1	2	2	0.5	0.5
	S. 2	2	2	0.5	0.5
	S. 3	2	2	0.5	0.5
	S. 4	2	2	0.5	0.5
	S. 5	2	2	0.5	0.5
	S. 6	2	2	0.5	0.5
	S. 7	2	2	0.5	0.5
	S. 8	2	2	0.5	0.5
L. monocytogenes	L. 1	1	1	0.25	0.25
	L. 2	1	1	0.25	0.25
	L. 3	1	1	0.25	0.25
	L. 4	1	1	0.25	0.25
	L. 5	1	1	0.25	0.25
	L. 6	1	1	0.25	0.25
	L. 7	1	1	0.25	0.25
	L. 8	1	1	0.25	0.25

Regarding the great activity against *L. monocytogenes* compared to *S. enterica*, it is known that Gram + bacteria are generally more sensitive to EOs than Gram - bacteria. The greater resistance in Gram negative bacteria is due to the complexity of its membrane, which limits the diffusion of hydrophobic compounds through lipopolysaccharide coverage [71]. Thus, less quantity of EOs are necessary to diffuse through the external membrane and the lipid bilayer and to reach the bacterial membrane [72].

2.4. Determination of the percentage of injured cells

The determination of injured cells percentage showed that the two tested EOs create damage in the cells with different degrees (Table 5). Also, with this test, MS-EO confirmed its capacity to

induce more damage in the tested bacteria respect to MP-EO. MS-EO showed, in fact, the highest results against *S. enterica* (5.00%±0.08) and *L. monocytogenes* (5.23%±0.07). Moreover, the percentage of injured cell of *L. monocytogenes* was higher than that of *S. enterica* for all the tested essential oils (Table 5).

Table 5: Percentage of sublethally injured cell of *S. enterica* and *L. monocytogenes* after their exposition to different essential oils during 24 hours

Bacteria	Blank	*M. pulegium*	*M. suaveolens*
S. enterica	3.72±0.04[a]	4.03±0.12[b]	5.00±0.08[c]
L. monocytogenes	2.53±0.05[a]	3.77±0.19[b]	5.23±0.07[c]

The same letter was assigned to the values of the same line that does not have a significant difference ($P< 0.05$).

Different mechanisms have been proposed to explain the mechanisms of action of the essential oils on bacteria [73-75]. The EOs create damages in the cell by increasing the cell membrane permeability, changing cell morphology and decreasing ATP synthesis, because the membrane potential is the driving force of ATP synthesis [74]. Several studies by microscopic visualization have showed that essential oils can affected the membrane integrity and destroyed the phospholipid bilayer [75,76].

The uses of EOs with a concentration lower than MIC is not able to inhibit the bacterial growth but can create damages in the bacterial cell [77]. These damages allow the weakness of bacterial membrane, which became sensitive to osmotic pressure exerted by different compounds. These findings were for a major interest to control the survival of pathogenic bacteria in food products and to reduce the used quantity of EOs.

3. Materials and Methods

3.1. Plant collection and essential oil extraction

The plant of *Mentha pulegium* (Linneo, 1753) and *Mentha suaveolens* (Ehrh, 1972) were manually collected from their natural habitat in Ifrane and El Hajeb (Morocco). Plants identification was based upon the morphology of their leaves and stems. In laboratory, they were air dried at room temperature in absence of light for 15 to 20 days.

EOs extraction was done by hydro-distillation. Two-hundred grams for each dried plant was immersed in 1 L of distilled water in a round glass flask of 2 L and boiled for 3 hours using Clevenger-type apparatus (IsoLab Laborgäte GmbH, Wetheim, Germany). The water traces which can be presented in EOs were eliminated using anhydrous sodium sulfate (Sigma-Aldrich, Buchs, Switzerland) and the resulting products were stored in sealed glass vials at 4°C until used.

3.2. Essential oil analysis

The analyses were carried out by GC-MS, an Agilent 6890N Gas Chromatograph equipped with a HP-5MS capillary column (50 m x 0.200 mm i.d., film thickness 0.33 μm). The GC oven temperature was programmed to increase from 60 to 250°C at a rate of 4°C/min and finally held for 15 min. The transfer line temperature was 250°C. Helium was used as the carrier gas at a flow rate of 1.1 mL/min with a split ratio equal to 1/100. The quadrupole mass spectrometer was scanned over the 35-465 m/z with an ionizing voltage of 70eV and an ionization current of 150mA. The percentage composition of oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of mass spectra with corresponding data in the computer mass spectra libraries (NIST MS SEARCH 2.0).

3.3. Antioxidant activity

The antioxidant activity was tested using DPPH radical scavenging method and the reducing capacity of Fe (III) to Fe (II). The IC_{50} equivalent to the concentration of EOs providing 50% of inhibition of DPPH was performed according to the method of Ed-Dra et al. [53]. EC_{50} equivalent to the concentration of EOs providing an absorbance of 0.5 for reducing power of Fe (III) to Fe (II) was, instead, determined using the method described by Elsharkawy et al. [78].

3.4. Antibacterial activity

The tetsted EOs were liposoluble compounds, so in order to facilitate their diffusion in the culture medium, they were mixed with Tween 80 (Biolife, Milan, Italy) at the final concentration of 5%. The antibacterial activity was performed against eight different strains of *Salmonella enterica* and *Listeria monocytogenes* (Table 6) by using disc diffusion test and broth dilution assay. The evaluation of the percentage of sub-lethally injured cells to EOs was also performed. All working cultures used were prepared by inoculating a loopful from the frozen stock (-80°C) in the different media.

Table 6. Description of strains tested.

Strains	ID strains	Information	Serotype	Origin
L. monocytogenes	L1	ListME222	-	Wild type-ice cream
	L2	ATCC 13932	4b	Human
	L3	ListME212	-	Wild type-meat product
	L4	ATCC 7644	1/2c	Human
	L5	ATCC 19111	1/2	Poultry
	L6	ListME1	-	Wild type-smoked salmon
	L7	ListME9	-	Wild type-fresh salmon
	L8	ListME13	-	Wild type-smoked salmon
S. enterica	S1	MG869132*	Typhimurium	Wild type-meat product
	S2	KX355308*	Typhimurium	Wild type-meat product
	S3	KX355300*	Kentucky	Wild type-meat product
	S4	KX355309*	Corvallis	Wild type-meat product
	S5	KX355310*	Kentucky	Wild type-meat product
	S6	KX355311*	Saintpul	Wild type-meat product
	S7	MG869130*	Kentucky	Wild type-meat product
	S8	KX355302*	Kentucky	Wild type-meat product

[1] ATCC: American Type Culture Collection; *Accession number

3.4.1. Disc diffusion method

The disc diffusion method was used to test the *in vitro* antibacterial activity of the tested EOs against 8 strains of *S. enterica* and *L. monocytogenes*, considering the reported high difference of response of different strains of the same bacteria to EOs [79]. According to the method described by Mazzarino et al. [80], a 0.5 McFarland (10^8 CFU/mL) bacterial suspension was prepared in physiological water (0.9% NaCl) and inoculated by swabbing on plates containing Mueller-Hinton Agar (Biolife, Milan, Italy). Ten 10 µL of each EO was dropped on 6 mm diameter sterile paper discs (Biolife, Milan, Italy), and a disc with 10 µL of Tween 80 was used as a negative control and Cefotaxime (30 µg) was used as a reference test. All the plates were incubated at 37°C for 24 hours and the inhibition diameter was measured in millimetres (disk included).

3.4.2. Determination of MIC and MBC with broth dilution method

In a sterile microtubes containing 100 µL of Brain Heart Infusion (BHI) (Biolife, Milan, Italy) with the added of the 5% of Tween 80 for *S. enterica* and Tryptone Soya Yeast Extract Broth (TSYEB) (Biolife, Milan, Italy) plus 5% of Tween 80 for *L. monocytogenes*, a decreasing concentration of EOs from 4% to 0.0625% was prepared. Four µL of 10^8 CFU/g bacterium suspensions were then added to each tube. All the suspensions were mixed and incubated at 37°C for 24h. The tubes presenting the law concentration and without growth of bacteria were considered as the minimum inhibitory concentration (MIC). Whereas, the minimum bactericidal concentration (MBC) was determined by sub-culturing the samples from the microtubes in which a visible growth of bacteria was present, on Tryptone Soya Agar (TSA) (Biolife, Milan, Italy) for *S. enterica* and on Tryptone Soya Yeast Extract Agar (TSYEA) (Biolife, Milan, Italy) for *L. monocytogens*, then the plates were incubated at 37°C for 24 h. The lower concentration that does not present any growth on media was considered as the minimum bactericidal concentration (MBC).

3.4.3. Determination of the percentage of sub-lethally injured cells to the EOs

The number of injured cells was according to the method described by Silva-Angulo et al. [77] with few modifications. The single strains of *S. enterica* and *L. monocyt*ogenes were inoculated in BHI and TSYEB respectively with a concentration of EOs equal to the half of MIC and incubated at 37°C for 24h. Series of decimal dilution were done in sterile peptone water (1g peptone, 8.5g NaCl, 1L of water) (Biolife, Milan, Italy) and 100 µL of each dilution were pour-plated on TSA and TSA with 5% of NaCl (TSA-S) plates for *S. enterica* and on TSYEA and on TSYEA with 5% of NaCl (TSYEA-S) plates for *L. monocytogenes*. The non-selective media (TSA and TSYEA) support the growth of un-injured and EO-injured cells, whereas the selective media (TSA-S and TSYEA-S) support only the growth of un-injured bacteria.

All the plates (selective and non-selective media) were incubated at 37°C for 24 - 48 hours. Then, the number of colony forming unit was counted in each plate, and the percentage of sub-lethally injured cells was estimated using the following equations:

For *Listeria monocytogenes*: [1-(CFU on TSYEA-S/CFU on TSYEA)]x100 (1)

For *Salmonella enterica*: [1-(CFU on TSA-S/CFU on TSA)]x100 (2)

3.5. Statistical analysis

All tests were performed in triplicate. The obtained data were presented as means ± standard deviation. The significance of difference between test and control groups was statistically analysed using Student test with a probability level of $p < 0.05$. Excel Microsoft was used as software for data processing.

4. Conclusions

The results of this study showed the presence of compounds not still reported in EOs obtained from these plants. This difference in their composition can be related to different period of plant life as well as growth condition of the plants [81-83]. It is reported, in fact, that plants grown in arid regions, in mountains or in soil deficient in certain mineral produce metabolites to withstand stress condition [82-84].

Regarding the antibacterial activity, we have confirmed by the calculation of the percentage of sub-lethally injured cells, that the synergistic effect between the EOs and salt can improve their antibacterial activity *vs S. enterica* and especially *vs L. monocytogenes*. This data could be used in further studies in order to control the survival of bacteria by using the mixtures of EOs with other substances that are able to create an osmotic pressure on bacterial cells wall.

The EOs in generally and those reported in this study can be used in the therapeutic treatments of these pathogens in human and in veterinary practice, instead of antibiotics, as required by World Health Organization in order to reduce the diffusion of multidrug resistant bacteria.

Another important application is in food industries as natural additives. EOs, in fact, are just used in foods traditionally associated with these plants to improve the safety and extend their shelf life, reducing the use of chemical preservatives [77,85,86]. Combination of these EOs and the association of osmotic substance, like the salt, can be used in food to improve the effectiveness and contemporary to reduce their minimum effective dose thus minimizing the loss in sensory quality caused by higher concentration when added alone [85]. The combined use of EOs at lower concentrations can, in fact, allow the achieving of a balance between sensory acceptability, health benefit and bactericidal efficacy.

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533 **Sample Availability:** Samples of the compounds are available from the authors.

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