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In vitro antibacterial activity of some essential oils against *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from meat

In vitro antibakterielle Aktivität einiger ätherischer Öle gegenüber aus Fleischprodukten isolierten Salmonella Enteritidis und Salmonella Typhimurium

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Summary

Fourteen essential oils, including basil, black pepper, cassumunar ginger, cinnamon, lemon, clove, fennel, lavender, myrtle, oregano, rosemary, curry plant, thyme and sage, were screened for their antibacterial activity against important food-borne pathogens, *Salmonella* Enteritidis and *Salmonella* Typhimurium. Essential oils have been examined by gas chromatograph coupled to mass spectrometer (GC-MS). The disc diffusion method was used as a screening test for antibacterial activity. Oregano and thyme essential oils showed the greatest inhibition zones against both *Salmonella* Enteritidis and *Salmonella* Typhimurium, while black pepper, lemon, curry plant and sage EOs expressed no antibacterial activity against tested *Salmonella* serotypes. Subsequently, minimal inhibitory concentration and minimal bactericidal concentration were determined by broth microdilution method for all essential oils that showed any inhibition zones (disc diffusion method). The essential oil that showed the highest antibacterial activity against all *Salmonella* serotypes was oregano, expressing minimal inhibitory concentration values between 0.04 and 0.23 µL/mL, and minimal bactericidal concentration values between 0.09 and 0.45 µL/mL, followed by cinnamon, clove, rosemary and thyme essential oils. The results of this study confirm the antibacterial activity of some essential oils, as well as their potential application as food preservatives.

Keywords: food preservatives, essential oils, foodborne pathogens

Introduction

Foodborne illness due to meat and meat products contamination with pathogenic bacteria has been of vital concern to public health for years. Meat and meat products can be contaminated at primary production, during processing, distribution, and preparation. Among the reported foodborne bacterial infections salmonellosis represents about 28% (da Silva Dannenberg et al., 2019). According to EFSA and ECDC (2019) *Salmonella* was identified in 1,580 notified foodborne outbreaks affecting 11,579 people in EU, while in Serbia 54 foodborne outbreaks affected 515 people in 2018. Although different serotypes have been associated with salmonellosis, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (SE) and *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ST) are responsible for most foodborne outbreaks.

To avoid food contamination and to reduce, both public health hazards and socio-economic losses, as well as to extend the shelf life of fresh and processed food synthetic additives are used for years (Oussalah et al., 2007). Lately, consumers' concerns over the safety of foods containing synthetic chemicals have led to seeking for alternatives to synthetic additives, such as the natural compounds present in plants (Radünz et al., 2019). However, spices and herbs have been used for preventing food spoilage and for extending shelf life of food since ancient times (Burt, 2004).

Essential oils (EOs), also known as volatile or ethereal oils, can be synthesized by all plant organs, that is, buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark and are stored in secretory cells, cavities, canals, epidermic cells, or glandular trichomes (Bilia et al., 2014). EOs from spices and herbs are highly complex mixtures of often hundreds of individual aroma compounds with potentially antibacterial activity against food pathogens (Hammer et al., 1999; Calo et al., 2015; Chouhan et al., 2017; Kocić-Tanackov et al., 2017). Mainly, EOs are characterized by two or three major components present in high concentrations, while others are present in traces (Chouhan et al., 2017).

The aim of this study was to chemically characterize 14 EOs and to study their antibacterial activity against 8 strains of *Salmonella* from food, using in vitro methods, disc diffusion method and broth microdilution method.

Materials and methods

Essential oils

Fourteen EOs (TerraCo d.o.o., Novi Sad, Serbia), including basil *(Ocimum basilicum),* black pepper *(Piper nigrum),* cassumunar ginger *(Zingiber cassumunar),* cinnamon (*Cinnamomum zeylanicum* Nees), lemon *(Citrus limonum),* clove (*Syzygium aromaticum* L.), fennel *(Foeniculum vulgare),* lavender *(Lavandula angustifolia),* myrtle *(Myrtus communis),* oregano *(Origanum vulgare),* rosemary *(Rosmarinus officinalis),* curry plant *(Helichrysum italicum),* thyme *(Thymus vulgaris)* and sage *(Salvia officinalis)* were used in this study. All EOs were stored in dark glass bottles and kept at 4°C per manufacturer's recommendation before use and utilized before the expiration date.

Determination of chemical composition of essential oils

The EOs were analyzed with a gas chromatograph (GC 7890B, Agilent Technologies) coupled to mass spectrometer (MS 5977A, Agilent Technologies) using a HP-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm; Agilent Technologies). High purity helium at a flow rate of 1 mL/min was used as the carrier gas. The injector was maintained at 250°C. The GC oven temperature was programmed as follows: 70°C for 2 min, followed by a gradual increase to 220°C at the rate of 4°C/min and hold at 220°C for 10 min, as previously described (Kocić-Tanackov et al., 2017). The mass spectrometer was operated in the electron ionization mode (70 eV). Data acquisition was carried out in the scan mode (range 50–450 amu), solvent delay time was 2 min. Samples were diluted in hexane with a ratio of 1 : 10 and 1 µl aliquots were injected in the split mode with split ratio of 1 : 80. The identity of the components of the essential oils was assigned by comparison of their retention indices and mass spectra with literature data (Davies, 1990; Adams, 2007) and the mass spectra databases (Wiley 10th & NIST 2011 MS Library). Retention indices (RI) were determined relative to the retention times of a series of n-alkanes with linear interpolation. The relative amounts of the components were calculated by the area normalization method, without considering response factors. The component percentages were calculated as mean values from duplicate GC-MS analyses.

Cultures and media

Antibacterial activity of selected EOs was evaluated on eight strains of *Salmonella,* including four *Salmonella enterica* subsp. *enterica* serovar Enteritidis (SE53, SE56, SE132, SE144) isolated from poultry meat and four *Salmonella enterica* subsp. *enterica* serovar Typhimurium (ST28, ST35, ST48, ST49) isolated from minced pork and beef meat. The isolation was performed by SRPS EN ISO 6579-1:2017.

All bacterial strains were stored as frozen cultures at –80°C in Tryptic Soy Broth (TSB) (Biokar Diagnostics, Beauvais, France) medium containing 20% glycerol until examination, when these cultures were maintained on nutrient agar (Biokar Diagnostics, Beauvais, France) slants at 4°C and subcultured weekly onto fresh slants. Twenty-four hours old bacterial colonies are touched with a loop and the growth transferred to 0.85% sterile saline (HiMedia Laboratories Pvt. Ltd., Mumbai, India). The suspension is adjusted to give a turbidity equivalent to that of a 0.5 McFarland standard yielding the concentration of $1.0x10^8$ CFU/mL.

Disk diffusion method

Inhibitory effects of EOs on the eight bacterial strains were investigated by the disc diffusion method (CLSI guidelines, Institute, C.a.L.S., Document M02-A11, Wayne). First Mueller-Hinton agar (Biokar Diagnostics, Beauvais, France) plates were prepared pouring 15 mL into 90-mm Petri dishes. Then, bacteria suspension was spread with sterile swabs onto the surface of Mueller-Hinton agar carefully in three directions to achieve even growth on the surface. Sterile filter paper disc (HiMedia Laboratories Pvt. Ltd., Mumbai, India) of 6 mm diameter was placed on the center of Mueller-Hinton agar surface with sterile forceps. Then the disk was impregnated with 5 and 10 µL of undiluted EOs. As negative control discs with no EOs were used. After incubation at 37°C for 24 h, the diameters of growth-free zones around the discs were measured in mm, including the diameter of discs, and recorded. The tests were performed in triplicate for each EO.

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TABLE 1: *Chemical components (%) of the essential oils of basil (EO1), black pepper (EO2), cassumunar ginger (EO3), cinnamon (EO4), lemon (EO5), clove (EO6) and fennel (EO7).*

No.	Compounds	EO 1	EO 2	EO 3	EO 4	EO 5	EO 6	EO 7
1.	alfa-Thujene	–	1.82	–	–	–	–	–
2.	alfa-Pinene	–	10.75	1.13	0.89	1.41	–	0.24
3.	Camphene	–	0.18	–	0.40	0.17	–	–
4.	Benzaldehyde	–	–	–	4.34	–	–	–
5.	Sabinene	–	13.55	38.17	–	0.31	–	–
6.	Dihydrocamphene	–	–	–	–	–	–	0.04
7.	beta-Pinene	–	19.31	8.23	0.40	1.11	–	–
8.	Sulcatone	0.04	–	–	–	–	–	–
9.	beta-Myrcene	–	0.48	–	–	1.54	–	–
10.	Octanal	–	–	–	–	0.11	–	–
11.	alpha-Phellandrene	–	0.36	0.11	–	–	–	–
12.	3-Carene	–	7.49	–	–	4.11	–	–
13.	alpha-Terpinene	–	8.08	1.04	–	–	–	–
14.	p-Cymene	–	2.52	2.75	1.15	0.28	–	0.18
15.	Limonene	–	13.93	0.28	0.61	79.72	–	2.13
16.	1,8-Cineole	0.24	0.61	0.80	0.23	–	–	–
17.	beta-Ocimene	0.08	–	–	–	–	–	–
18.	gamma-Terpinene	–	0.85	10.06	0.07	0.12	–	0.07
19.	alpha- Terpinolen	–	0.59	0.47	–	0.45	–	0.05
20.	Fenchone	–	–	–	–	–	–	1.24
21.	Linalool	24.77	1.01	–	1.83	0.82	–	–
22.	Dihydro linalool	0.13	–	–	–	–	–	–
23.	cis-Limonene oxide	–	–	–	–	0.32	–	–
24.	trans-Limonene oxide	–	–	–	–	0.11	–	–
25.	Camphor	–	0.12	0.09	–	–	–	0.06
26.	Menthone	–	0.29	–	–	–	–	–
27.	Isoborneol	–	–	–	0.25	0.09	–	–
28.	Acetic acid, phenylmethyl ester	–	–	–	0.96	–	–	–
29.	DL-Menthol	0.23	0.32	–	–	–	–	–
30.	Terpinen-4-ol	–	1.03	35.90	0.10	–	–	–
31.	alpha-Terpineol	1.30	0.08	0.39	–	1.47	–	–
32.	Estragole	69.52	–	–	–	–	–	2.97
33.	Decanal	–	–	–	–	0.20	–	–
34.	Carveol	–	–	–	–	0.10	–	–
35.	Citronellol	–	0.23	–	–	0.07	–	–
36.	Fenchyl acetate	–	–	–	–	–	–	0.12
37.	alfa-Citral	0.22	–	–	–	1.49	–	–
38.	Carvone	–	0.30	–	–	0.20	–	–
39.	Chavicol	–	–	–	–	–	0.06	–
40.	Geraniol	–	0.19	–	–	0.10	–	–
41.	Anisaldehyde	–	–	–	–	–	–	0.72
42.	beta-Citral	0.30	–	–	–	1.75	–	–
43.	Cinnamaldehyde	–	–	–	74.93	–	–	–
44.	Safrole	–	–	–	0.06	–	–	–
45.	Anethole	–	–	–	–	–	–	88.42
46.	alpha-Cubebene	–	0.35	–	–	–	–	–
47.	Citronellol acetate	–	–	–	–	0.40	–	–
48.	Eugenol	–	0.96	–	3.10	–	85.14	–
49.	Copaene	–	3.74	–	–	–	0.11	–
50.	Geranyl acetate	–	–	–	–	0.10	–	–
51.	Anisic ketone	–	–	–	–	–	–	0.10
52.	Germacrene D	–	0.23	–	–	–	–	–
53.	Vanillin	–	–	–	0.24	–	–	–
54.	Longifolene	–	0.98	–	–	–	–	–
55.	alpha-Gurjunene	–	–	–	0.13	–	–	–
56.	Caryophyllene	0.32	7.15	–	0.15	1.98	10.20	–
57.	alpha-Bergamotene	0.52	–	–	–	–	–	–
58.	Cinnamyl acetate	–	–	–	0.05	–	–	–
59.	alpha-Humulene	0.17	0.23	0.07	–	–	2.64	–
60.	beta-Farnesene	0.18	–	–	–	–	–	–
61.	Germacrene	0.26	–	–	–	–	–	–
62.	beta-Bisabolene	0.06	–	–	–	–	–	–
63.	Calamenene	–	–	0.16	–	–	–	–
64.	delta-Cadinene	–	0.59	–	–	–	0.23	–
65.	Acetyleugenol	–	–	–	0.05	–	0.21	–
66.	alpha-Bisabolene	1.43	–	–	–	–	–	–
67.	p-Methoxycinnam-aldehyde	0.22	–	–	–	–	–	–
68.	Caryophylene oxide	–	0.11	–	–	0.33	0.82	–
69.	Humulene epoxide	–	–	–	–	–	0.10	–
70.	Ascabin	–	0.07	–	9.01	–	–	–
Total (%)		**100.00**	**98.52**	**99.64**	**98.96**	**98.85**	**99.50**	**96.35**
Not identified (%)		**–**	**1.48**	**0.36**	**1.04**	**1.15**	**0.50**	**3.65**

Broth microdilution method

The antibacterial effectiveness of each EO was further studied by determining their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) using the guideline (Institute, C.a.L.S., Document M07-A9, Wayne) and a protocol previously reported (Kocić-Tanackov et al., 2017). Sterile plastic, disposable, microtiter plates with 96 U shaped bottom wells were used (Nuova Aptaca SRL, Canelli, Italy). Into each well aliquot of 100 µL Muller-Hinton broth (Oxoid, Basingstoke, UK) were added. Then, tested EO (100 µL) was added to the first well and 1 : 1 serial dilution was made down to the column number 12 in order to get dilution ranging from 454.54 to 0.22 µL/mL. From the last well 100 µL of the mixture was throwaway. At the end, suspension of the tested bacteria (10^8 CFU/mL) was added in an amount of 10 µL to each well. Depending on preliminary results, cinnamon, clove, oregano and thyme EOs were dissolved in 10% dimethylsulfoxide (DMSO) (Lach-Ner sro, Czech Republic). Positive control was obtained using Mueller-Hinton broth and bacterial suspension, while the negative control was obtained using Mueller-Hinton broth and EOs. Controls were set up with DMSO in amounts corresponding to the quantity present in the test solution. DMSO did not show any inhibitory activity. Microtiter plates were incubated at 37°C for 24 h. Next, from each well the content were taken with a sterile wire loop and spread onto the Mueller-Hinton agar and incubated at 37°C for 24 h. The lowest concentrations that inhibited the growth in the well (clear broth suspension), but still showed slightly visible growth on the plate were defined as Minimal Inhibitory Concentrations (MICs). To detect the Minimum Bactericidal Concentration (MBC), 10 µl aliquots from each well that did not grow in the MIC test were inoculated onto Mueller-Hinton agar plates. The lowest concentration with no visible growth was defined as the MBC. All tests were performed in duplicates for each EO.

Statistical analysis

The mean values ± standard deviations were calculated. Analyses of variance (ANOVA), followed by Duncan's test, was performed to determine the significant difference between essential oils at $P \leq 0.05$. Statistical analysis was undertaken using the statistical software R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

TABLE 2: *Chemical components (%) of the essential oils of lavender (EO8), myrtle (EO9), oregano (EO10), rosemary (EO11), curry plant (EO12), thyme (EO13) and sage (EO14).*

No.	Compounds	EO 8	EO 9	EO 10	EO 11	EO 12	EO 13	EO 14
1.	alfa-Thujene	–	0.31	–	0.12	0.04	0.06	–
2.	alfa-Pinene	1.36	35.47	0.29	28.23	3.77	0.40	–
3.	Camphene	0.07	0.44	0.05	3.44	0.06	0.07	–
4.	beta-Pinene	0.63	1.02	0.19	3.16	0.09	1.28	–
5.	3-Octanone	0.65	–	–	–	–	–	–
6.	beta-Myrcene	0.30	0.24	0.09	0.16	0.19	0.64	0.96
7.	3-Octanol	0.15	–	–	–	–	–	–
8.	alpha-Phellandrene	–	–	–	0.06	0.30	0.18	0.35
9.	3-Carene	0.43	8.80	–	0.22	–	–	–
10.	alpha-Terpinene	–	0.22	–	–	–	–	–
11.	p-Cymene	1.65	2.41	1.61	2.04	0.43	40.91	0.16
12.	Limonene	2.72	0.56	0.14	3.43	1.48	0.39	0.94
13.	1,8-Cineole	1.13	19.58	0.23	11.54	–	0.06	–
14.	alpha-Ocimene	–	–	–	–	–	–	0.29
15.	o-Cymene	–	–	–	–	–	0.07	–
16.	beta-Ocimene	0.04	–	–	–	–	–	–
17.	gamma-Terpinene	–	1.13	0.13	0.34	0.27	10.37	–
18.	Linalool oxide	0.59	–	–	–	–	–	–
19.	alpha-Terpinolen	–	0.79	–	0.36	0.45	–	0.49
20.	Linalool	23.88	6.28	0.18	1.03	1.21	2.18	23.95
21.	Isofenchol	–	–	–	0.46	–	–	–
22.	Limona ketone	–	–	–	–	0.23	–	–
23.	Dihydrolinalool	–	–	–	–	–	–	0.32
24.	Camphor	2.82	0.32	0.10	1.23	–	–	–
25.	Menthone	–	0.15	–	–	–	–	–
26.	Isoborneol	1.73	–	–	–	–	–	–
27.	Borneol	–	–	–	24.87	–	–	–
28.	endo-Borneol	0.12	–	–	0.31	–	–	0.10
29.	DL-Menthol	–	0.27	–	–	–	–	–
30.	Isononyl acetate	10.05	–	–	–	–	–	–
31.	Terpinen-4-ol	–	0.85	–	0.65	–	0.76	–
32.	alpha-Terpineol	1.71	5.02	0.24	11.86	–	–	4.77
33.	gamma-Terpineol	0.27	0.88	–	1.57	–	–	0.66
34.	Nerol	–	–	–	–	3.66	–	0.08
35.	Carvone	–	0.42	–	–	–	–	–
36.	Geraniol	–	0.40	–	0.45	0.08	–	–
37.	Linalyl acetate	25.33	0.87	–	0.21	–	–	56.41
38.	Borneol acetate	1.84	–	–	1.30	–	–	–
39.	Thymol	–	–	7.74	–	–	40.36	–
40.	Carvacrol	–	–	81.00	–	–	1.44	–
41.	Limonene dioxide	–	1.52	–	–	–	–	–
42.	alpha-Cubebene	–	–	–	–	0.27	–	–
43.	alpha-Terpinenyl acetate	12.58	–	–	1.23	–	–	–
44.	Eugenol	–	0.61	–	–	0.09	–	–
45.	Neryl acetate	0.29	–	–	–	18.15	–	1.51
46.	Copaene	0.11	0.76	0.13	–	0.65	–	–
47.	Geranyl acetate	0.05	0.60	–	–	0.11	–	2.29
48.	Longifolene	0.06	0.88	–	0.25	0.34	–	0.10
49.	Caryophyllene	5.21	3.79	6.69	0.63	21.48	–	2.13
50.	beta-Cubebene	–	–	–	–	0.17	–	–
51.	Cumarin	1.43	–	–	–	–	–	–
52.	alpha-Bergamotene	–	–	–	–	1.33	–	–
53.	alpha-Himachalene	–	–	–	–	5.34	–	–
54.	alpha-Humulene	0.57	0.49	0.66	0.07	3.08	–	0.35
55.	gamma-Himachalene	–	–	–	–	3.34	–	–
56.	alpha-Cedrene	–	–	–	–	0.83	–	–
57.	beta-Himachalene	–	–	–	–	13.34	–	–
58.	beta-Bisabolene	–	–	–	–	0.19	–	–
59.	Calamenene	–	–	–	–	–	–	0.72
60.	delta-Cadinene	0.07	–	0.09	–	0.30	–	–
61.	Cadina-1,4-diene	–	–	–	–	–	–	0.07
62.	alpha-Bisabolene	–	–	–	–	0.70	–	–
63.	Elemol	–	–	–	–	0.16	–	–
64.	Nerolidol	–	–	–	–	0.30	–	–
65.	Caryophylene oxide	0.21	–	0.31	–	0.74	–	0.41
66.	Longiborneol	–	–	–	–	0.16	–	–
67.	gama-Eudesmol	–	–	–	–	0.43	–	–
68.	Thujopsene	–	–	–	–	0.19	–	–
69.	alpha-Cedrene	–	–	–	–	0.56	–	–
70.	beta-Eudesmol	–	–	–	–	0.34	–	–
71.	alpha-Atlantone	–	–	–	–	4.76	–	–
Total (%)		**98.05**	**95.11**	**99.87**	**99.19**	**89.62**	**99.17**	**97.06**
Not identified (%)		**1.95**	**4.89**	**0.13**	**0.81**	**10.38**	**0.83**	**2.94**

Results and discussion

Chemical composition of essential oils

GC-MS analysis detected several compounds for each tested EO. The components identified in the EOs are listed in Tables 1 and 2. Estragole (69.52%) was the main constituent of basil *(Ocimum basilicum)* EO followed with linalool (24.77%). Constituents such as β-pinene (19.31%), limonene (13.93%), sabinene (13.55%), α-pinene (10.75%), α-terpinene (8.08%), 3-carene (7.49%) and caryophyllene (7.15%) were found to be the prevalent in black pepper *(Piper nigrum)* EO. Sabinene (38.17%) and terpinen-4-OL (35.90%) were compounds determined at highest percentage in cassumunar ginger *(Zingiber cassumunar)* EO. Main component of cinnamon (*Cinnamomum zeylanicum* nees) EO was cinnamaldehyde (74.93%). In the EO of lemon *(Citrus limonum)* limonene (79.72%) was the major component. Eugenol (85.14%) was the main constituent of clove (*Syzygium aromaticum* L.) EO, followed with caryophyllene (10.20%). Anethole (88.42%) dominated in fennel *(Foeniculum vulgare)* EO. Linalyl acetate (25.33%), linalool (23.88%), α-terpinenyl acetate (12.58%) and isononyl acetate (10.05%) were principal constituents of lavender *(Lavandula angustifolia)* EO. Two main constituents of myrtle *(Myrtus communis)* EO were α-pinene (35.47%) and 1,8-cineol (19.58%). Similar compositions were quantified for rosemary *(Rosmarinus officinalis)* EO, since α-pinene (28.23%) was also the most abundant compound, followed by borneol (24.87%), α-terpineol (11.86%) and 1,8-cineol (11.54%). Carvacrol (81.00%) was the major component of oregano *(Origanum vulgare)* EO, followed by thymol (7.74%). Three major components of curry plant *(Helichrysum italicum)* EO were caryophyllene (21.48%), neryl acetate (18.15%) and β-himachalene (13.34%). The EO of thyme *(Thymus vulgaris)* was characterized by high contents of p-cymene (40.91%) and thymol (40.36%). Linalyl acetate (56.41%) was the main constituent of sage *(Salvia officinalis)* EO, followed by linalool (23.95%).

Earlier studies found similar chemical compositions but in different proportions (Menon et al., 2003; Tomaino et al., 2005; Soković et al., 2010; Teixeira et al., 2013; Mazzarrino et al., 2015; Verma et al., 2018). The proportion differences in chemical compositions of EOs from a particular species of plant could be attributed to harvesting seasons (Hussain et al., 2008), geographical origin (Falei-

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TABLE 3: *The inhibition zones (expressed in mm) obtained testing the Salmonella Enteritidis strains against 14 EOs (mean ± SD).*

Essential oils	SE53		SE56		SE132		SE144	
	5µL	10µL	5µL	10µL	5µL	10µL	5µL	10µL
Basil	10.00±0.00^{f}	11.00±0.00^{e}	8.33±0.58^{e}	10.00±0.00^{e}	7.00±0.00^{f}	9.00±1.00^{h}	7.00±0.00^{d}	8.67±1.15^{e}
Black pepper	–	–	–	–	–	–	–	–
Cassumunar ginger	16.33±0.58^{d}	25.00±1.73^{c}	12.33±0.58^{d}	22.33±2.08^{c}	12.67±0.58^{d}	20.00±0.00^{d}	13.67±0.58^{c}	23.33±1.15^{c}
Cinnamon	23.33±0.58^{c}	28.67±1.15^{b}	20.33±1.15^{b}	23.33±1.15^{bc}	18.33±1.15^{c}	25.33±1.15^{c}	17.67±1.15^{b}	21.33±1.15^{c}
Lemon	–	–	–	–	–	–	–	–
Clove	12.67±2.08^{e}	14.67±0.58^{d}	14.67±0.58^{c}	15.00±0.00^{d}	11.33±0.58^{d}	15.67±0.58^{e}	15.67±0.58^{bc}	16.67±0.58^{d}
Fennel	–	–	–	8.00±0.00^{f}	–	–	–	7.00±0.00^{e}
Lavender	–	–	–	–	–	7.00±0.00^{j}	–	7.17±0.29^{e}
Myrtle	7.00±0.00^{g}	12.67±0.58^{e}	–	–	7.00±0.00^{f}	13.33±0.58^{f}	–	8.67±1.15^{e}
Oregano	25.00±1.00^{b}	26.50±1.32^{c}	23.67±2.08^{a}	24.67±1.53^{b}	24.67±0.58^{b}	29.67±1.53^{b}	34.33±1.53^{a}	36.33±2.08^{b}
Rosemary	7.83±0.76^{g}	8.33±1.15^{f}	7.67±1.15^{e}	10.67±1.53^{e}	7.00±0.00^{f}	11.67±1.15^{g}	–	7.17±0.29^{e}
Curry plant	–	–	–	–	–	–	–	–
Thyme	27.33±0.58^{a}	38.33±1.53^{a}	25.00±0.00^{a}	30.67±0.58^{a}	29.00±1.00^{a}	44.00±1.00^{a}	36.67±3.06^{a}	50.67±2.08^{a}
Sage	–	–	–	–	–	–	–	–

(–) Diameter of inhibitory zone < 6 mm considered as no antimicrobial activity. Values are mean diameter of inhibitory zone (mm) ± SD of three replicates. Different letters in the column indicate statistically significant differences ($P < 0.05$).

ro et al., 2003), climate effects on the plants (Gachkar et al., 2007) as well as different parts of the same plant (Burt, 2004), and storage, processing conditions, the EO extraction and analysis method (Melo et al., 2015).

Antibacterial activity of essential oils

Tables 3 and 4 show inhibition zones for EOs against all examined *Salmonella* strains. Observing inhibition zones broad variations were noticed. The strain sensitivity to each EOs was classified by the diameter of the inhibition zones, including diameter of paper disc (6 mm), as follows: not sensitive (–) for diameter smaller than 8 mm, sensitive (+) for diameter 9–14 mm; very sensitive (++) for diameter 15–19 mm, and extremely sensitive (+++) for diameter larger than 20 mm (Ponce et al., 2003).

Black pepper, lemon, curry plant and sage EOs expressed no antibacterial activity and no inhibition zone was observed for any of the tested bacteria. Contrary, Al-Turki (2007) reported inhibition effects of black pepper and sage EOs against *Salmonella* Enteritidis. The lowest potential was observed in lavender and fennel EOs, while basil, myrtle and rosemary EOs were more effective, with visible inhibition zones matching (+) sensitive range. Good inhibition zones were obtained for EOs of clove and cassumunar ginger, as previously reported (Thanissery et al., 2014; Verma et al., 2018), with inhibition zones larger than 11.0 mm (5 µL) and 15.00 mm (10 µL). The essential oils with much larger inhibition zones (> 20 mm) than other oils in the disc diffusion method were those of cinnamon (16.5 mm applying 5 µL; > 20 mm applying 10 µL), oregano and thyme.

TABLE 4: *The inhibition zones (expressed in mm) obtained testing the Salmonella Typhimurium strains against 14 EOs (mean ± SD).*

Essential oils	ST28		ST35		ST48		ST49	
	5µL	10µL	5µL	10µL	5µL	10µL	5µL	10µL
Basil	–	8.00±0.00^{e}	8.67±0.58^{e}	9.00±0.00^{e}	8.50±1.32^{f}	12.17±1.76^{e}	7.17±0.29^{e}	13.00±1.00^{e}
Black pepper	–	–	–	–	–	–	–	–
Cassumunar ginger	11.67±1.15^{c}	19.33±1.53^{d}	12.33±0.58^{d}	18.33±0.58^{c}	14.33±0.58^{e}	25.67±0.58^{c}	13.00±0.00^{d}	20.67±0.58^{c}
Cinnamon	17.00±1.00^{b}	25.67±3.21^{c}	24.67±0.58^{a}	26.00±1.00^{b}	25.67±1.15^{b}	28.67±0.58^{b}	16.33±1.53^{c}	21.33±0.58^{c}
Lemon	–	–	–	–	–	–	–	–
Clove	16.67±0.58^{b}	18.00±0.00^{d}	15.67±0.58^{c}	17.00±0.00^{c}	16.00±1.00^{d}	16.67±1.15^{d}	15.67±0.58^{c}	18.33±0.58^{d}
Fennel	–	–	–	–	–	–	–	8.00±0.00^{fg}
Lavender	–	–	–	–	–	8.83±0.29^{f}	–	7.67±058^{g}
Myrtle	7.67±0.58^{d}	10.67±1.53^{e}	8.33±0.58^{e}	11.33±0.58^{d}	8.50±1.32^{f}	13.33±0.58^{e}	7.67±0.58^{e}	9.33±0.58^{f}
Oregano	26.67±1.15^{a}	29.67±1.53^{b}	22.67±1.53^{b}	26.67±2.52^{b}	23.17±0.29^{c}	25.00±1.00^{c}	22.33±1.53^{b}	24.00±0.00^{b}
Rosemary	–	–	–	8.00±0.00^{e}	7.83±0.29^{f}	8.83±0.29^{f}	–	–
Curry plant	–	–	–	–	–	–	–	–
Thyme	26.00±1.73^{a}	34.33±2.08^{a}	25.33±0.58^{a}	34.67±1.53^{a}	34.00±0.00^{a}	35.33±0.58^{a}	25.67±0.58^{a}	35.00±1.73^{a}
Sage	–	–	–	–	–	–	–	–

(–) Diameter of inhibitory zone < 6 mm considered as no antimicrobial activity. Values are mean diameter of inhibitory zone (mm) ± SD of three replicates. Different letters in the column indicate statistically significant differences ($P < 0.05$).

Ten out of 14 tested EOs showed antibacterial activity against both SE and ST. Similar results were obtained by Teixeira et al. (2013) for basil, clove and lemon EOs against ST. However, applying double the amount of EOs we used they observed antibacterial activity for sage and rosemary, showing inhibition zones of 10 mm and 9 mm, respectively. In our study, sage did not show antibacterial activity, while rosemary did for SE with small inhibition zones (7–10.5 mm) and only one ST strain. Yet, sage has been reported both to show and not antibacterial activity (Soković et al., 2010; Kocić-Tanackov et al., 2017). Examining nine EOs, including, as we did, basil, cinnamon, clove, oregano and thyme, basil EO was the most effective in inhibiting SE (Rattanachaikunsopon and Phumkhachorn, 2010). Generally different inhibition zones among used EOs obtained in the disc diffusion method could be explained by the solubility of the EOs which limits diffusion of EOs through the agar medium (Soković et al., 2010) and the volume of applied EO. Overall, in our study cinnamon, oregano and thyme EOs proved to be the most active. Similar results have been reported previously (Elgayyar et al., 2001; Soković et al., 2010; Teixeira et al., 2013; Melo et al., 2015; Ebani et al., 2019).

TABLE 5: *Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of different essential oils against Salmonella Enteritidis.*

Essential oils	Concentration (µL/mL)							
	SE53		SE56		SE132		SE144	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Basil	28.41	56.82	28.41	56.82	14.21	28.41	14.21	28.41
Cassumunar ginger	14.21	28.41	7.11	14.21	7.11	14.21	1.78	3.56
Cinnamon	0.45	0.89	0.23	0.45	0.23	0.45	0.23	0.45
Clove	0.45	0.89	0.23	0.45	0.45	0.89	0.45	0.89
Fennel	TNP	TNP	28.41	56.82	TNP	TNP	TNP	TNP
Myrtle	0.89	1.78	7.11	14.21	7.11	14.21	7.11	14.21
Oregano	0.04	0.09	0.09	0.18	0.23	0.45	0.23	0.45
Rosemary	0.89	1.78	0.89	1.78	0.23	0.45	1.78	3.56
Thyme	0.23	0.45	0.89	1.78	0.45	0.89	0.45	0.89

TNP – Test Not Performed. Values are mean diameter of inhibitory zone (mm) ± SD of two replicates.

TABLE 6: *Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of different essential oils against Salmonella Typhimurium.*

Essential oils	Concentration (µL/mL)							
	ST28		ST35		ST48		ST49	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Basil	7.11	14.21	3.56	7.11	14.21	28.41	7.11	14.21
Cassumunar ginger	7.11	14.21	7.11	14.21	7.11	14.21	14.21	28.41
Cinnamon	0.89	1.78	0.45	0.89	0.45	0.89	0.23	0.45
Clove	0.45	0.89	0.23	0.45	0.45	0.89	0.45	0.89
Lavender	TNP	TNP	TNP	TNP	14.21	28.41	TNP	TNP
Myrtle	1.78	3.56	7.11	14.21	14.21	28.41	14.21	28.41
Oregano	0.18	0.36	0.09	0.18	0.09	0.18	0.18	0.36
Rosemary	TNP	TNP	TNP	TNP	0.89	1.78	TNP	TNP
Thyme	0.23	0.45	0.23	0.45	0.23	0.45	0.89	1.78

TNP – Test Not Performed. Values are mean diameter of inhibitory zone (mm) ± SD of two replicates.

Determination of MIC and MBC

EOs that showed any antibacterial activity in the disc diffusion method were further investigated by the microdilution method whose results are presented in Tables 5 and 6. The determination of MIC and MBC concentrations by broth microdilution method is more sensitive than the disc diffusion method. This method revealed that the oregano EO had remarkable antibacterial effects, inhibiting all the bacteria in very small concentrations (from 0.04 µL/mL). The antibacterial activity of oregano is associated with the presence of carvacrol and thymol. In particular, carvacrol has been reported to be a component of oregano EO present in very high quantity (75–95%) (Gounaris et al., 2002). Oregano EO in present study had a relevant amount (81.00%) of this component, which explains the strong antibacterial effects and particularly low MIC values. Oregano EO has been reported earlier to have strong antibacterial activity not only against *Salmonella* strains, but on a number of bacteria, such as *Acinetobacter baumanii, Aeromonas veronii* biogroup *sobria, Enterococcus faecalis, Escherichia coli, Listeria monocytogenes, Lactobacillus plantarum, Klebsiella pneumoniae, Pseudomonas aeruginosa, Serratia marcescens, Staphylococcus aureus* and *Yersinia enterocolitica* (Hammer at al., 1999; Elgayyar et al., 2001).

MIC and MBC values for cinnamon, clove, rosemary and thyme EOs were very similar, 0.23–1.78 µL/mL and 0.45–3.56 µL/mL, respectively. The bacteriostatic and bactericidal properties of these EOs are supposed to be associated with the high levels of their main components, cinnamaldehyde, eugenol, α-pinene, 1,8-cineole and thymol, which has been reported to have significant antibacterial activity (Burt, 2004; Klein et al., 2013). Although, the mechanism of action of EOs has not been fully understood, the low MIC values could be explained by the chemical compositions of the EOs. Phenolic compounds, such as carvacrol, thymol and eugenol, are capable to partition the cytoplasmic membrane of gram-negative bacteria, such as *Salmonella* spp., cause leakage of intracellular materials, and finally to cell lysis (Burt, 2004). Thymol and carvacrol were found to manifest bacteriostatic effects from 40 ppm and 50 ppm, respectively, and bactericidal effect from 100 ppm, followed by linalool (180 ppm/720 ppm), α-pinene (400 ppm/no bactericidal effect), 1,8-cineol (1400 ppm/2800 ppm), and α-terpineol (600 ppm/no bactericidal effect) (Klein et al., 2013). Carvacrol changes the fatty acid composition of the membrane forming channels through it, and causing loss of ions from the cytoplasm. Thymol cause upregulation of genes involved in synthesis of outer membrane proteins and accumulation of outer membrane proteins in misfolded pattern. Intracellularly thymol affects energy-generating processes and lowers the ability of cell to recover (Chouhan et al., 2017). Cinnamaldehyde can covalently cross-link DNA, proteins and amine groups, causing inhibition of enzymes, while p-cymene causes swelling of the cytoplasmic membrane (Burt, 2004; Mazzarrino et al., 2015). The presence of cinnamaldehyde (74.93%) in cinnamon EO may have enhanced the antibacterial effect against both *S.* Enteritidis and *S.* Typhimurium, as previous study demonstrated (Ebani et al.,

2019). Despite the powerful action of major components, there is some evidence than components present only as a trace have a critical part in antibacterial activity (Burt, 2004). Synergistic effects of some components are crucial for their antibacterial activity.

After reviewing many published articles on the antibacterial effects of EOs we noticed that the comparison between results of MIC is often difficult. This difficulty comes from applying different test methods, bacterial strains and EOs with different compositions (Hammer et al., 1999). However, it has to be underlined that; beside chemical composition of EOs the strain biodiversity of *Salmonella* species has also a crucial importance in antibacterial activity of EOs (Mazzarrino et al., 2015). The powerful antibacterial effect of oregano EO against tested food-borne pathogens (SE and ST) may be useful in food industry. However, strong aroma could limit the utilization (Elgayyar et al., 2001).

Conclusion

In conclusion, this study confirms that many EOs have potential use as antibacterials against important food-borne pathogens, such as *Salmonella* Enteritidis and *Salmonella* Typhimurium. Our first screening showed that oregano and thyme EOs were the most effective essential oils followed by clove, cassumunar ginger and cinnamon. When these five selected EOs were tested using microdilution method, it was found that oil of oregano showed the highest antibacterial activity. Present study, together with previous studies, provides support to the antibacterial properties of some EOs against food-borne pathogens. The results demonstrate the promising possibility in using these EOs to improve the microbial safety of foods and for increasing the shelf life. Considering antibacterial effects of several EOs they can be effectively used in meat industry as natural alternatives to synthetic additives. Future research will focus on the effectiveness of tested EOs in meat matrices.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

Adams RP (2007): Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing, Coral Stream, IL.

Al-Turki AI (2007): Antibacterial effect of thyme, peppermint, sage, black pepper and garlic hydrosols against *Bacillus subtilis* and *Salmonella enteritidis*. J Food Agric Environ, 5(2): 92–94.

Bilia AR, Guccione C, Isacchi B, Righeschi C, Firenzuoli F, Bergonzi MC (2014): Essential oils loaded in nanosystems: a developing strategy for a successful therapeutic approach. Evid Based Complement Alternat Med, 1–14.

Burt S (2004): Essential oils: their antibacterial properties and potential applications in foods – a review. Int J Food Microbiol, 94(3): 223–253.

Calo JR, Crandall PG, O'Bryan CA, Ricke SC (2015): Essential oils as antimicrobials in food systems – A review. Food Control, 54: 111–119.

Chouhan S, Sharma K, Guleria S (2017): Antimicrobial activity of some essential oils – present status and future perspectives. Medicines, 4(3): 58.

Clinical and Laboratory Standards Institute (2012): Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Ninth Edition. Document M07-A9, Vol.32. No.2. Wayne, PA, USA.

Clinical and Laboratory Standards Institute. (2012): Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard-Eleventh Edition. Document M02-A11, vol. 32, No. 1, Wayne, PA, USA.

da Silva Dannenberg G, Funck GD, da Silva WP, Fiorentini ÂM (2019): Essential oil from pink pepper (*Schinus terebinthifolius* Raddi): Chemical composition, antibacterial activity and mechanism of action. Food Control, 95: 115–120.

Davies NW (1990): Gas chromatographic retention indices of monoterpenes and sesquiterpenes on methyl silicon and Carbowax 20M phases. J Chromatogr A, 503: 1–24.

Ebani VV, Nardoni S, Bertelloni F, Tosi G, Massi P, Pistelli L, Mancianti F (2019): In Vitro Antimicrobial Activity of Essential Oils against *Salmonella enterica* Serotypes Enteritidis and Typhimurium Strains Isolated from Poultry. Molecules, 24(5): 900.

Elgayyar M, Draughon FA, Golden DA, Mount JR (2001): Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J Food Prot, 64(7): 1019–1024.

European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC) (2019): The European Union One Health 2018 Zoonoses Report. EFSA Journal, 17(12): e05926.

Faleiro ML, Miguel MG, Ladeiro F, Venancio F, Tavares R, Brito JC, Figueiredo AC, Barroso JG, Pedro LG (2003): Antimicrobial activity of essential oils isolated from Portuguese endemic species of *Thymus*. Lett Appl Microbiol, 36(1): 35–40.

Gachkar L, Yadegar D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2007): Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem, 102(3): 898–904.

Gounaris Y, Skoula M, Fournaraki C, Drakakaki G, Makris A (2002): Comparison of essential oils and genetic relationship of *Origanum intercedens* to its parental taxa in the island of Crete. Biochem Syst Ecol, 30(3): 249–258.

Hammer KA, Carson CF, Riley TV (1999): Antimicrobial activity of essential oils and other plant extracts. J Appl Microbiol, 86(6): 985–990.

Hussain AI, Anwar F, Sherazi STH, Przybylski R (2008): Chemical composition, antioxidant and antimicrobial activities of basil *(Ocimum basilicum)* essential oils depends on seasonal variations. Food Chem, 108(3): 986–995.

Klein G, Rüben C, Upmann M (2013): Antimicrobial activity of essential oil components against potential food spoilage microorganisms. Curr Microbiol, 67(2): 200–208.

Kocić-Tanackov S, Blagojev N, Suturović I, Dimić G, Pejin J, Tomović V, Šojić B, Savanović J, Kravić S, Karabasil N (2017): Antibacterial activity essential oils against *Escherichia coli, Salmonella enterica* and *Listeria monocytogenes*. Arch Lebensmittelhyg, 68: 88–95.

Mazzarrino G, Paparella A, Chaves-López C, Faberi A, Sergi M, Sigismondi C, Compagnone D, Serio A (2015): *Salmonella enterica* and *Listeria monocytogenes* inactivation dynamics after treatment with selected essential oils. Food Control, 50: 794–803.

Melo ADB, Amaral AF, Schaefer G, Luciano FB, de Andrade C, Costa LB, Rostagno MH (2015): Antimicrobial effect against different bacterial strains and bacterial adaptation to essential oils used as feed additives. Can J Vet Res, 79(4): 285–289.

Menon AN, Padmakumari KP, Jayalekshmy A (2003): Essential oil composition of four major cultivars of black pepper (*Piper nigrum* L.) III. Journal of essential oil research, 15(3): 155–157.

Oussalah M, Caillet S, Saucier L, Lacroix M (2007): Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157: H7, *Salmonella* Typhimurium, *Staphylococcus aureus* and *Listeria monocytogenes.* Food control, 18(5): 414–420.

Ponce AG, Fritz R, Del Valle C, Roura SI (2003): Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensm Wiss Technol, 36(7): 679–684.

Radünz M, da Trindade MLM, Camargo TM, Radünz AL, Borges CD, Gandra EA, Helbig E (2019): Antimicrobial and antioxidant activity of unencapsulated and encapsulated clove (*Syzygium aromaticum,* L.) essential oil. Food Chem, 276: 180–186.

Rattanachaikunsopon P, Phumkhachorn P (2010): Antimicrobial activity of basil *(Ocimum basilicum)* oil against *Salmonella enteritidis* in vitro and in food. Biosci Biotechnol Biochem, 74(6): 1200–1204.

Soković M, Glamočlij, J, Marin PD, Brkić D, van Griensven LJ (2010): Antibacterial effects of the essential oils of commonly consumed medicinal herbs using an in vitro model. Molecules, 15(11): 7532–7546.

Teixeira B, Marques A, Ramos C, Neng NR, Nogueira JM, Saraiva JA, Nunes ML (2013): Chemical composition and antibacterial and antioxidant properties of commercial essential oils. Ind Crops Prod, 43: 587–595.

Thanissery R, Kathariou S, Smith DP (2014): Rosemary oil, clove oil, and a mix of thyme-orange essential oils inhibit *Salmonella* and *Campylobacter* in vitro. J Appl Poul Res, 23(2): 221–227.

Tomaino A, Cimino F, Zimbalatti V, Venuti V, Sulfaro V, De Pasquale A, Saija A (2005): Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem, 89(4): 549–554.

Verma RS, Joshi N, Padalia RC, Singh VR, Goswami P, Verma SK, Chanda D, Verma RK, Darokar MP, Chauhan A, Kandwal MK (2018): Chemical composition and antibacterial, antifungal, allelopathic and acetylcholinesterase inhibitory activities of cassumunar-ginger. J Sci Food Agric, 98(1): 321–327.

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