Original Article

APPLIED FOOD BIOTECHNOLOGY, 2014, 1(1): 45-54
Journal's homepage: www.journals.sbmu.ac.ir/afb

National Nutrition & Food Technology Research Institute

Inhibitory Effects of Several Essential Oils towards *Salmonella typhimurium, Salmonella paratyphi A* and *Salmonella paratyphi B*

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Abstract

Plant essential oils are natural products extracted from plants, and because of their antimicrobial properties can be used as natural additives in foods. They are also useful for decontamination of food-born pathogens and can be a safe additive in foods. The antimicrobial activities of essential oils belonging to *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L. (spearmint)* were investigated at different concentrations (0.1, 0.3, 0.5, 1, 2, 5 and 10%v/v) against *Salmonella typhimurium, Salmonella paratyphi A* and *Salmonella paratyphi B* by using the agar well diffusion method ($P < 0.05$). Essential oils showed inhibitory effect on *Salmonella spp.* in the agar well diffusion assay. In addition, the capability of essential oils for decontamination of minced row beef, ground beef, minced raw chicken and minced raw fish inoculated with *Salmonella spp.* at 0.1 and 0.5%v/v were assessed. Reduction of the *Salmonella spp.* population was observed following the inoculation of the cultures with 0.1 and 0.5%v/v essential oils.

Article Info

Article history:
Received: 19 March 2014
Revised : 7 May 2014
Accepted: 25 May 2014

Keywords:
Antimicrobial decontamination;
Essential oil;
Salmonella spp.

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1. Introduction

From ancient times to the present, herbs and spices have been recognized as antimicrobial agents everywhere. Essential oils (volatile or ethereal) derived from plants are valuable considering their compounds having antimicrobial properties. They have antibacterial, antiviral, antimycotic, anticarcinogenic, anti-oxidant, antitoxigenic, antiparasitic, and insecticidal properties [1], and can be used as natural additives in many foods. The antibacterial properties are mainly related to phenolic components in essential oils [2]. The European Union (EU) employs essential oils largely to flavor foods, as perfumes, and in pharmaceutical industry due to their functional properties [1].

Evidence from the world health organization (WHO) [3] indicates that up to 30% of the inhabitants in the industrialized countries bear diseases derived from foods annually, which is continually threatening the public health. It is also a concern of even the well-developed countries. Considerable outbreaks of Salmonellosis initiated from food have been reported worldwide due to the poisonous effects of *Salmonella* [4]. Appropriate techniques are required to get rid of the food borne pathogens. Essential oils with their antibacterial characteristics are powerful substitutes for chemical compounds in preservation of nutrition, resulting in the enhancement of food safety. Considerable interest has been drawn towards the essential oils, which are derived from the extracts of spices, herbs and aromatic plants due to their capability to detoxify and decontaminate microorganisms [5]. Frequent usages of commercial antimicrobial drugs to treat infectious diseases have led to multiple drug resistance in pathogens [6]. The main prescription

for the suppression of *Salmonella* is antibiotics, which need to be substituted by novel efficient and safe remedies for Salmonellosis because of increasing resistance of *Salmonella* to these drugs [7]. It is the low toxicities and extensive biological activities as well as low environmental impacts of essential oils that have made them widely acceptable by the public [8]. Along with natural therapies and treatments, essential oils are also used for preservation of raw and processed foods [9].

Gram-positive bacteria are more susceptible than Gram-negative bacteria to essential oils due to their outer membrane shielding against diffusion of hydrophobic compounds out of its lipopolysaccharide layer [10]. Diffusion methods are known as a superior selection method for the antimicrobial test [11]. Capability of the test material to equally diffuse through an agar medium will influence the depth of the region of inhibition. Factors interacting with the antimicrobial action of the thyme essential oil and its active constituents have proved that detergents and solvents could reduce the essential oil's antibacterial activities [12]. Preservative features of essential oils and their constituents are affected by several factors including fats, temperature, proteins and salts [13]. Due to the impact of contamination on raw and processed foods whilst being produced, handled or sold [14], industrial use of preservatives is yet unavoidable to stop the growth of microbes disturbing foods [11].

Thymus vulgaris and *Saturiea hortensis* have been used for centuries as spice, home remedy, drug, perfume and insecticide. In medicine, they are used as antispasmolytic, antibacterial, antifungal, secrotolytic, expect-rant, antiseptic, anthelmintic and antitussive [8, 15]. *Cuminum cyminum* (*Cumin seed*) is known with effects such as antiepileptic, perspiration, appetizer, antibronchitis and mal-digestion treatment. A kind of aldehyde, called cuminul for having the odor of *Cuminum cyminum*, with no associated harm, has been reported. *Mentha polegium* is derived from the *Labiatae* family, and *Pulegone* is the most effective constituent of its essential oil. This oil is used as an anti-cough with anti-spasmodic and antimicrobial effects. Excessive usage of *Mentha polegium* is hepatotoxic and nephrotoxic, and will cause abortion. It also functions as a disinfectant and anti-insecticidal, and is specifically prescribed for bronchitis, asthma, hysteria, flatulency wind, and arthritis. *Mentha polegium* facilitates digestion, and can be used for curing cold. *Mentha viridis L*. is also of the *Labiatae* family, otherwise known as *Spearmint* or *Common mint*. It is invigorative, anti-cough, anti-epileptic, and a sedative. It is used to treat diarrhea, mal-digestion, flu, sinusitis, and neural diseases. *Lavandula officinalis* is also an antimicrobial agent, which is employed for treatment of colitis, migrant headache, rheumatism, flu and wound treatment as well as elimination of stomach worms [16].

In this research, first, the effects of *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L. (spearmint)* essential oils on the food pathogens *S. typhimurium, S. paratyphi A* and *S. paratyphi B* were investigated. Secondly, their potentials for decontaminating raw and ground beef, raw chicken and fish artificially inoculated with *Salmonella spp.* were evaluated.

2. Materials and Methods

2.1 *Bacterial strains*

Salmonella typhimurium (PTCC 1186), *Salmonella paratyphi A* (PTCC 1100) and *Salmonella paratyphi B* (PTCC 1101) were obtained from the Iranian Research Organization for Science and Technology.

2.2 *Preparations of inocula*

Salmonella spp. was kept on the slants of Nutrient Agar at 4°C. Bacteria were sub-cultured on Brain–Heart Infusion (BHI) broth and incubated for 24 h at 37°C. The inoculum was an overnight culture in BHI broth at 37°C. The cells were harvested by centrifugation (3000×*g*, 15 min), washed twice, and resuspended with saline (NaCl, 0.85%, $w.v^{-1}$). For inoculation of the individual meats, 1 ml of the dense suspension (cfu> 10^7 $m1^{-1}$) was employed [29].

2.3 *Preparation of meats*

A piece of each of fresh raw beef, raw chicken and raw fish (1×1×1cm) was cut with a sterile scalpel, and put under the UV light in the cabinet for 20min in order to reduce the number of the microorganisms attached to the surface of the meats. In the case of ground beef, 20 grams of ground beef was weighted and then put under the UV light in the cabinet for 20min.

2.4 *Essential oils*

All essential oils were purchased from Barij Essence Co., producer of herbal medicines, Kashan, Iran.

2.5 *Antibacterial screening*

The minimum inhibitory concentration (MIC) of the essential oils belonging to *Saturiea*

hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis and *Mentha viridis L. (spearmint)* was determined for *S. typhimurium, S. paratyphi A* and *S. paratyphi B* by the agar well diffusion method. Wells were created on the agar plates with the end of a sterile Pasteur pipette. The prepared inocula (ca. 10^7cfu/plate) were spread onto the Mueller Hinton Agar media plates. The diluted essential oils dissolved in ethanol with the test concentrations (0.1%, 0.3%, 0.5%, 1%, 2%, 5%, and 10% (v/v)) were poured into the wells (20µl), and a control consisting of the same volume (20µl) of ethanol was used. The plates were incubated at 37°C for 24h and the diameter of the inhibition zones was measured in millimeters. Disregarding the radius of the wells, the results were recorded as the percentage of inhibition of bacterial growth, and compared with normal bacterial growth in the control plates (growth on the control dish was maintained as 100%). All tests were performed in triplicate.

2.6 *Decontamination of all individual meats inoculated with S. typhimurium, S. paratyphi A* and *S. paratyphi B*

For the decontamination assay, watery solutions (0.1% and 0.5% (v/v)) of *Saturiea hortensis, Thymusvulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L.* were investigated for their ability to decontaminate raw beef, chicken and fish. For this purpose, 1 ml of the prepared inocula (cfu> 10^7 $m1^{-1}$) of *S. typhimurium, S. paratyphi A* and *S. paratyphi B* was used for inoculation of the minced raw beef, ground beef, minced raw chicken and fish. After inoculation, all of these meats were left to arid for 1 h in air. Once dried, the inoculated meats were decontaminated by immersion for 5min (well shacked the meats) in clean tap water supplemented with each of the following herbs of different concentrations, 0.1% and 0.5% of *Saturiea hortensis*, 0.1% and 0.5% of *Thymus vulgaris*, 0.1% and 0.5% of *Mentha polegium*, 0.1% and 0.5% of *Cuminum cyminum*, 0.1% and 0.5% of *Lavandula officinalis*, and 0.1% and 0.5% of *Mentha viridis.* Immersion in clean tap water was used as control. In the case of ground beef, after inoculation, it was cultured in BHI broth containing 50µl of different concentrations, 0.1% and 0.5%, of each of the studied essential oils, including *Thymus vulgaris, Saturiea hortensis, Mentha polegium, Cuminum cyminum, Lavandula officinalis,* and *Mentha viridis L.* Also the BHI broth without essential oils was used as a control. The cultures were incubated at 37°C for 24h.

2.7 *Enumeration of microorganisms*

Enumeration of *Salmonella spp*. was derived from pour plating proper dilutions of the bacteria grown in BHI broth on Plate Count Agar (PCA, Oxoid). In order to enumerate *Salmonella spp.*, 100µl of a suitable dilution in Ringer's solution with quarter strength of all individual meats were surface plated on PCA. Enumerations were carried out after incubating the plates at 37°C for 24h.

2.8 *Statistical analysis*

All experiments were carried out in triplicate. The data obtained were analyzed for significant differences between the control and experimental groups, using the SPSS software (Ver. 16.0), involving the independent student's t-test. P value <0.05 was considered statistically significant.

3. Results and Discussion

3.1 *Well diffusion method to determine the inhibition of Salmonella spp. growth*

The antimicrobial effects of essential oils from *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L.* against *S. typhimurium, S. paratyphi A* and *S. paratyphi B,* as determined by the agar well diffusion assay, are demonstrated in Figure 1. The entire essential oils tested here showed antibacterial activities toward *S. typhimurium, S. paratyphi A* and *S. paratyphi B.* As shown in the figure, there is a linear correlation between the concentration of the essential oils and the radius of the inhibition zones and therefore there is an agreeable reproducibility of the achieved inhibition zones. It can be observed that *S. typhimurium* is more susceptible to *Saturiea hortensis, Thymus vulgaris, Cuminum cyminum* and *Mentha viridis L.* essential oils than *S. paratyphi A* and *S. paratyphi B* (Figure 1a, b, d and f). Additionally, in the case of *Saturiea hortensis* and *Thymus vulgaris* essential oils, *S. paratyphi A* is more vulnerable than *S. paratyphi B.* In fact, *S. paratyphi A* was susceptible to these two test essential oils (Figure 1a and b). The inhibition zones of *Mentha polegium* essential oil toward *S. typhimurium, S. paratyphi A* and *S. paratyphi B* demonstrated that *S. typhimurium* is more sensitive to this essential oil than *S. paratyphi A* and *S. paratyphi B,* except concentrations of 10% for *S. paratyphi A* and 5% and 10% for *S. paratyphi B* (Figure 1c). *Cuminum cyminum* showed higher antibacterial activities at

low and high concentrations toward *S. paratyphi A* and *S. paratyphi B*. In the case of *S. paratyphi A*, the 0.3% and 0.5% concentrations of *Cuminum cyminum* were better than its 1% and 2% concentrations in inhibiting the growth of microorganism. In the case of *S. paratyphi B* essential oils, its 0.1% and 0.3% concentrations were better than the 0.5% and 5% concentrations in inhibiting the bacterial growth (Figure 1d).

There were no linear relationships between *S. paratyphi A* and *S. paratyphi B* for *Cuminum cyminum*, and between the all tested microorganisms in case of *Lavandula officinalis* (Figure 1d and e). In addition, there were no linear relationships between *S. paratyphi A* and *S. paratyphi B* in the cases of *Mentha polegium* and *Mentha viridis L.* (Figure 1c and f). Figure 1f shows that *Mentha viridis L.* was more or the same inhibitor towards *S. paratyphi B* at lower concentrations (0.1%, 0.3%, 0.5%, 1% and 2%) than *S. paratyphi A*; however, it had a less inhibitory effect against *S. paratyphi B* than *S. paratyphi A* at higher concentrations (5%, 10%). Figure 1c demonstrates that *S. paratyphi A* was more susceptible to *Mentha polegium* at lower concentrations (0.1%, 0.3%, 0.5%, 1% and 2%) than *S. paratyphi B*, whereas it was less sensitive at higher concentrations (5% and 10%).

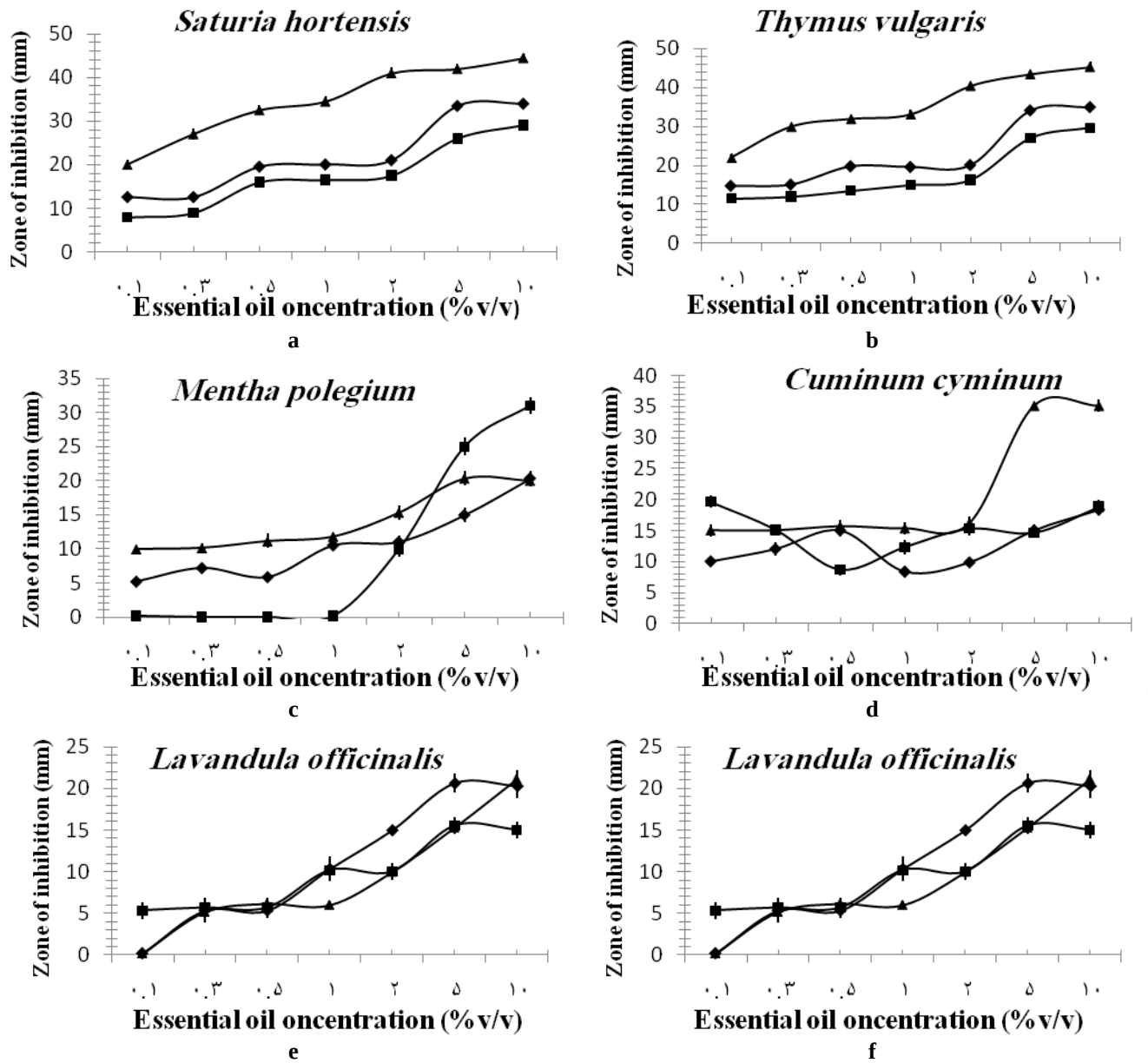

Figure 1. Antimicrobial effect of essential oils toward *Salmonella* spp. (-♦- *S. paratyphi A*, -■- *S. paratyphi B*, -▲- *S. typhimurium*)

The MIC of 0.1% concentration of essential oils inhibited the growth of all microorganisms tested in the case of *Saturiea hortensis, Thymus vulgaris,* and *Cuminum cyminum* (Figure 1a, b and d).

The concentration of 0.1% was the MIC for *Mentha polegium* toward *S. paratyphi A* and *S. typhimurium,* and this essential oil had the MIC of 2% concentration against *S. paratyphi B* (Figure 1c). The concentration of 0.3% was the MIC for *Lavandula officinalis* and *Mentha viridis L.* against *S. typhimurium* (Figure 1e and f). *Lavandula officinalis* had the MIC of 0.3% concentration toward *S. paratyphi A* and *S. paratyphi B* and *Mentha viridis L.*, had the MIC of 5% and 2% concentrations against *S. paratyphi A* and *S. paratyphi B,* respectively (Figure 1e and f).

3.2 *Decontamination of minced raw beef, ground beef, minced raw chicken and minced raw fish by essential oils*

Decontaminations of *S. typhimurium, S. paratyphi A* and *S. paratyphi B,* which had been used to artificially inoculate minced raw beef, ground beef, minced raw chicken and minced raw fish by *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L.* essential oils are indicated in Tables 1-4. Watery solutions with 0.1% and 0.5%v/v of individual essential oils were employed to decontaminate the tested microorganisms. Decrease in the microorganisms' growth was observed after decontaminating the meats (except in some cases for ground beef) with the mentioned essential oils. As shown in Table 1, all *Salmonella* strains were decontaminated significantly with the entire set of essential oils. Minced raw beef was considerably decontaminated of *S. typhimurium* by *Saturiea hortensis* and *Mentha polegium* (Table 1).

In the case of *S. paratyphi A, Cuminum cyminum, Lavandula officinalis* and *Mentha polegium* at the concentration of 0.1% were the greatest essential oils for decontamination of minced raw beef (Table 1). *Saturiea hortensis, Thymus vulgaris* and *Lavandula officinalis* were the best essential oils tested to decontaminate *S. paratyphi B* in minced raw beef; the concentration of 0.1% of *Saturiea hortensis* was the best whilst *Thymus vulgaris* and *Lavandula officinalis* were the most excellent at the concentration of 0.5% (Table 1).

Table 1. Decontamination of minced raw beef inoculated with *Salmonella spp.* at 0.1% and 0.5% concentrations of essential oils ($P < 0.05$)

Bacteria	Concentration of essential oils	*Saturiea hortensis* (Log cfu.g^{-1})	*Thymus vulgaris* (Log cfu.g^{-1})	*Mentha pulegium* (Log cfu.g^{-1})	*Cuminum-cyminum* (Log cfu.g^{-1})	*Lavandula officinalis* (Log cfu.g-1)	*Menthaviri-dis L.* (Log cfu.g^{-1})	*Control* (Log cfu.g^{-1})
S. typhi-murium	0.10%	0.30±0.05	1.20±0.05	0.14±0.02	1.50±0.08	1.40±0.1	1.01±0.06	3.36±0.13
	0.50%	0.40±0.05	0.82±0.08	0.18±0.03	1.44±0.06	1.43±0.12	1.20±0.1	3.36±0.13
S. paratyph i A	0.10%	1.85±0.05	1.45±0.1	0.73±0.1	0.37±0.08	0.163±0.11	1.33±0.06	4.57±0.5
	0.50%	1.33±0.05	1.75±0.08	1.65±0.03	1.29±0.05	1.66±0.02	1.23±0.08	4.57±0.5
S. paratyph i B	0.10%	0.72±0.05	1.26±0.09	1.10±0.04	1.65±0.12	1.02±0.1	1.56±0.06	4.55±0.2
	0.50%	1.42±0.05	0.14±0.01	1.20±0.02	1.74±0.06	0.76±0.11	1.46±0.08	4.55±0.2

As observed in the case of ground beef (Table 2), due to the existence of fat inside the meat, no desirable decontamination effects were found by the essential oils. Furthermore, in the case of raw chicken, the results were not so fair for some of the essential oils (Table 2). Only *Mentha viridis L.* and *Mentha polegium* at a concentration of 0.5% were found to decontaminate *S. paratyphi A* from ground beef. In fact, all essential oils (except *Lavandula officinalis*) at concentration of 0.5% decontaminated *S. paratyphi A* but they were mediocre (Table 2).

For minced raw chicken, *Thymus vulgaris* at 0.1% for decontamination *S. typhimurium* was considerable (Table 3). Decontamination of *S. paratyphi A* of minced raw chicken by *Lavandula officinalis, Thymus vulgaris* and *Cuminum cyminum* was satisfactory at the concentrations of 0.1%, 0.5% and 0.5%, respectively (Table 3). *Thymus vulgaris* was also a good decontaminating agent for *S. paratyphi B* (Table 3).

Table 2. Decontamination of ground beef inoculated with *Salmonella spp.* at 0.1% and 0.5% concentrations of essential oils (P <0.05)

Bacteria	Concentration of essential oils	*Saturiea hortensis* (Log cfu.g^{-1})	*Thymus vulgaris* (Log cfu.g^{-1})	*Mentha pulegium* (Log cfu.g^{-1})	*Cuminum cyminum* (Log cfu.g^{-1})	*Lavandula officinalis* (Log cfu.g^{-1})	*Mentha viridis L.* (Log cfu.g^{-1})	*Control* (Log cfu.g^{-1})
S. typhimurium	0.10%	1.25±0.05	1.08±0.09	1.44±0.02	1.24±0.15	0.79±0.1	1.49±0.06	1.25±0.09
	0.50%	1.02±0.05	0.37±0.04	0.82±0.02	1.21±0.06	0.80±0.1	1.42±0.05	1.25±0.09
S. paratyphi A	0.10%	1.02±0.08	1.37±0.05	0.99±0.03	1.06±0.15	1.54±0.12	0.84±0.09	1.95±0.06
	0.50%	0.66±0.02	0.75±0.05	0.43±0.08	0.63±0.04	1.79±0.1	0.36±0.12	1.95±0.06
S. paratyphi B	0.10%	0.86±0.05	1.55±0.09	1.22±0.08	1.70±0.08	1.70±0.05	0.56±0.01	1.73±0.09
	0.50%	1.36±0.09	0.75±0.16	1.30±0.03	1.88±0.15	0.75±0.1	1.16±0.08	1.73±0.09

Cuminum cyminum, *Mentha viridis L.* and *Thymus vulgaris* at concentrations of 0.1%, 0.1% and 0.5%, respectively, were significant agents in decontaminating *S. typhimurium* from minced raw fish (Table 4). *Saturiea hortensis* showed to be relatively suitable for decontamination of *S. paratyphi B* from minced raw fish (Table 4). None of the remaining results seemed to be remarkable. The results of decontamination tests indicated no linear relationship between the concentrations of essential oils and their decontamination quantities.

The objective of the present study was to assess the antibacterial effects of essential oils from *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L. (spearmint)* towards *Salmonella spp*. using the agar well diffusion assay. In its second phase, the present work was conducted to evaluate the effect of decontamination of a piece of minced raw beef, ground beef, minced raw chicken and fish by all studied essential oils' suspensions on the inoculated *S. typhimurium, S. paratyphi A* and *S. paratyphi B.* Many researches have revealed that phenolic compounds (thymol and carvacrol) are the main compounds of *Saturiea hortensis* and *Thymus vulgaris* [17], but in *Mentha polegium*, oxygen containing monoterpens like pulegone and cineol were the main constituents of the oil [18]. The major compounds in *Cuminum cyminum* were the monoterpenes beta-pinene, *p*-cymene and gamma-terpinene [19]. Linalool, 1,8-cineole, borneol and camphor the oxygenated monoterpenes were the predominant class of components in *Lavandula officinalis* [20]. Mkaddem et al. reported that *Mentha viridis* was rich in carvone, cineole, and limonene [21]. The main components of these plants are different but they have good inhibitory effects on bacteria.

Watery solutions of *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L.* essential oils were investigated for their capability to decontaminate the test meats. A reduction in the number of the *Salmonella* was noticed subsequent to washing the individual meats with 0.1% and 0.5% (v/v) of the test essential oils.

Diffusion methods are recognized as a worthwhile selection method for antimicrobial tests [5]. Ability of the test material to uniformly diffuse through an agar culture will affect the diameter of the region of inhibition. High volatility and inferior solubility of most essential oils and their active compounds in the aqueous phase are considered as disadvantages in obtaining results [22, 23]. Furthermore, quantitative results are available through dilution methods [5]. However, it is significant to standardize the test methods.

This study demonstrated that essential oils and their compounds are potent for decontamination of foods. The concentrations tested here were the same or lower compared to those of the clove essential oil (0.5% and 1%) tested for anti-listeric activity in meat and cheese, and the concentrations of *Oregano* essential oil (0.5%, 1.0% and 2.0%) tested for inhibition activity against *Salmonella* in Tarama salad, but higher compared to the concentrations of thyme essential oil, carvacrol and thymol (0.1% and 0.05%) for decontamination of fresh lettuce inoculated by *Shigella sonnei* [24], and the same or lower to the concentrations of basil methyl chavicol (0.1% and 1.0%) tested for decontamination of fresh lettuce [25].

Because of interactions between the food mixture and the phenolic compounds of essential oils, higher levels of such antibacterial materials are required to halt microbial growth in foods than in culture media [13]. In the fat content of the food, hydrophobic antibacterial essential oil

components may be better absorbed, and thus preventing access to bacterial cells, which grow better in the hydrophilic regions of the food [26]. Further, it is recommended to combine proteins in the food with the phenolic compounds in the oil [27]. It seems that high fat content in fish and meat products reduces essential oils' antibacterial activities. e.g. effectiveness of *Oregano* oil at 0.5 $\mu g.ml^{-1}$ against the spoilage organism *Photobacterium phosphoreum* on cod fillets is more than on salmon (a kind of fatty fish) [28]. Food structure has a significant task in compare with laboratory media regarding inhibitions for the decline of antimicrobial activity. The inhibitory activities of certain phenolic composites are definitely affected by intense proteins in model food systems or in broths [13, 29].

It has been also reported that reduction of water content as well as increase in protein and fat contents of foods will result in the increase of resistance to sage essential oil [30].

Table 3. Decontamination of minced raw chicken inoculated with *Salmonella spp.* at 0.1% and 0.5% concentrations of essential oils ($P < 0.05$)

Bacteria	Concentra-tion of essential oils	*Saturiea hor-tensis* (Log $cfu.g^{-1}$)	*Thymus vulgaris* (Log $cfu.g^{-1}$)	*Mentha pule-gium* (Log $cfu.g^{-1}$)	*Cuminum cyminum* (Log $cfu.g^{-1}$)	*Lavandula officinalis* (Log $cfu.g^{-1}$)	*Mentha viri-dis L.* (Log $cfu.g^{-1}$)	Control (Log $cfu.g^{-1}$)
S. typhi-murium	0.10%	1.23±0.04	0.28±0.02	0.47±0.06	0.72±0.08	0.41±0.05	0.39±0.04	1.36±0.2
	0.50%	1.27±0.09	1.15±0.1	1.08±0.08	0.75±0.09	1.17±0.1	0.92±0.15	1.36±0.2
S. paratyphi A	0.10%	0.91±0.05	0.75±0.09	1.52±0.08	0.63±0.1	0.17±0.03	1.04±0.01	1.56±0.05
	0.50%	0.87±0.13	0.21±0.06	1.52±0.04	0.32±0.06	1.16±0.1	0.82±0.1	1.56±0.05
S. paratyphi B	0.10%	0.52±0.08	0.32±0.06	0.72±0.11	0.83±0.08	1.52±0.09	0.66±0.01	1.98±0.15
	0.50%	0.82±0.09	0.32±0.06	0.45±0.02	1.63±0.15	1.34±0.02	1.13±0.08	1.98±0.15

Table 4. Decontamination of minced raw fish inoculated with *Salmonella spp.* at 0.1% and 0.5% concentrations of essential oils

Bacteria	Concentra-tion of essential oils	*Saturiea hor-tensis* (Log $cfu.g^{-1}$)	*Thymus vulgaris* (Log $cfu.g^{-1}$)	Menthapule-gium (Log $cfu.g^{-1}$)	*Cuminum cyminum* (Log $cfu.g^{-1}$)	*Lavandula officinalis* (Log $cfu.g^{-1}$)	*Mentha viri-dis L.* (Log $cfu.g^{-1}$)	Control (Log $cfu.g^{-1}$)
S. typhimurium	0.10%	0.98±0.1	0.44±0.02	0.45±0.06	0.09±0.02	0.80±0.03	0.22±0.05	1.29±0.11
	0.50%	0.87±0.04	0.23±0.06	0.38±0.10	0.45±0.03	0.54±0.1	0.70±0.06	1.29±0.11
S. paratyphi A	0.10%	0.57±0.02	0.69±0.1	0.88±0.08	0.55±0.10	0.62±0.09	0.54±0.01	1.30±0.12
	0.50%	0.69±0.03	0.44±0.06	0.47±0.08	0.52±0.06	0.64±0.1	0.43±0.13	1.30±0.12
S. paratyphi B	0.10%	0.28±0.03	0.29±0.04	0.19±0.05	0.23±0.06	0.24±0.03	0.34±0.01	1.0±0.09
	0.50%	0.18±0.03	0.25±0.06	0.37±0.04	0.33±0.05	0.28±0.06	0.28±0.06	1.0±0.09

The action of essential oils increases in the presence of high levels of water or salt [29, 31]. It has been revealed that bacteria defuse more easily into strained chicken and rice than beef, with fewer amounts of water, being rich in proteins and fats while lacking carbohydrates. Essential oils have hydrophobic nature so they dissolve much more easily in the lipid constituents than in the watery part of the food. Therefore, essential oils fail to reach microbial flora [12]. Addition of protein to diluted low-fat cheese acts as an inhibiting factor with respect to the effect of clove oil against *S. enteritidis* [32]. A fat coat may surround bacterial cells resulting in the probable reduction of essential oil penetration [33]. In addition, pH of food can influence the activity of essential oils. Dissolving of essential oil in the lipids of the bacterial surface increases with decreasing of pH [12]. It has been found that in low-fat foods (cucumber and yoghurt salad), antibacterial effect of Mint oil is much higher than high-fat products (pâté and fish roe salad) against *Listeria monocytogenes* and *S. enteritidis* [29]. This improved effectiveness can also be partly related to the low pH (4.3) of cucumber and yoghurt salad and fish roe salad (pH 4.9), compared with that of pâté (pH 6.8). It is worth noting that fat percentage might have higher impact on the antibacterial effect of essential oils than pH. Required mint oil concentration against *S. enteritidis* in low-fat yoghurt and cucumber was 5–20 $\mu g.ml^{-1}$ [29]. Antibacterial activity of essential oils might be restricted with the physical shape of foods. It has been shown that gel mixture severely lessens the inhibition of *Oregano* oil towards *S. typhimurium*

in comparison with broth due to penetration obstructions of the gel matrix [34].

It has been reported that several essential oil components have valuable antibacterial activities, with MICs of 0.05–5 µl ml^{-1} *in vitro*. The results of various case studies on fresh meat, meat products, fish, dairy products, milk, vegetables, fruit and cooked rice showed that necessary concentration to obtain a major antibacterial effect is observed in the range of 0.5–20 µl ml^{-1} in foods and approximately 0.1–10 µl ml^{-1} in solutions for washing fruit and vegetables [1]. Effect of *Oregano* oil even in fatty fish dishes is found more compared to mint oil; which has been confirmed from couple of research on fish roe salad with two essential oils at the concentration of 5–20 µg.ml^{-1} [11, 29]. Cinnamaldehyde and thymol at 50°C inhibited the growth of *Salmonella spp.* on alfalfa seeds and sprouts at 200-600 µg.ml^{-1} but they were not efficient at 70°C [35]. It has been shown that carvacrol, citral and geraniol inhibit *S. typhimurium* in culture medium and on fish cubes at a range of 5-30 µg.ml^{-1} [36]. *Oregano* oil is reported to have good inhibition against *S. enteritidis* in Trama salad at a range of 5-20 µg.ml^{-1} [11]. Mint oil does not have any inhibitory effect toward *S. enteritidis* in Tramosalata at the range of 5-20 µg.ml^{-1}, but this essential oil has an inhibitory effect on *S. enteritidis* in Tzatziki (cucumber and yogurt salad, pH 4.3) [29]. It has been reported that the growth of *Salmonella* spp. was inhibited by the essential oils of orange, cinnamon, thyme, oregano, bay, allspice, coriander, grapefruit, lemon, and clove [7]. Bactericidal effect towards *Salmonella* has been shown with carvacrol in pieces of fish stored at 4°C [23]. The extract of *Capsicum annuum* bell pepper type has been found to have inhibitory effects against *S. typhimurium* and *Pseudomonas aeruginosa*, which were used to inoculate minced beef meat mixed with various concentrations of the extract, stored at 7ºC for 1 week. Additionally, the joint effect of *C. annuum* extract and NaCl on bacterial growth has been evaluated. The MIC of *C. annuum* to stop *S. typhimurium* growth was 1.5ml per 100 g of meat; the addition of 1%, 2%, 3% and 4% w/w of sodium chloride was no more effective on inhibition against *Salmonella* [37].

Few studies regarding the role of essential oils and their components as food preservatives are available. The effectiveness of essential oils as a preservative has been investigated up to now in Tarama salad (a traditional Greek appetizer), Tzatziki, pâté [11, 29], naturally contaminated beef meat [38], meat and cheese [10], and vacuum-packed ham [26]. In this study, it was shown that essential oils have potential to be used for decontamination of meat products. The use of essential oils as antimicrobial agents in foodstuff is frequently discouraged due to their lipophilicity and volatility. Therefore, the potential role of antimicrobial action would be lost. Some sources point the finger at the volatility and lipophilicity of essential oils, and hesitate in utilizing them for antimicrobial requirements. Considering the decontaminating feature of *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L. (spearmint)* essential oils, they persuade us to emphasize using them as decontamination agents despite the attitude about the volatility and lipophilicity of essential oils in carbohydrate-rich foods [24]. Recently, we have shown that essential oils are opulent sources of small molecules with the diverse features, and their application as well as their effective compounds would be valuable in numerous facets of pharmaceutical industry due to their compounds' diversity [39, 40]. Since, a number of essential oils have been found to cause allergic reaction in consumers, it is suggested that more safety studies be carried out prior to their eventual extensive utilization, especially at higher concentrations in foods [1].

4. Conclusion

The main purpose of the present study was to evaluate the antibacterial efficiency of essential oils as decontaminating agents of food products. The results achieved here may recommend that the essential oils of *Saturiea hortensis, Thymus vulgaris, Mentha polegium, Cuminum cyminum, Lavandula officinalis* and *Mentha viridis L. (spearmint)* possess antibacterial activities, and thus can be used in biotechnological fields as natural preservative components in foods or pharmaceutical products.

Acknowledgment

Our special thanks to Dr. Fariborz Shojaee Aliabadi from Faroogh Life Sciences Research Laboratory for supporting us and for his helpful ideas.

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