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The Effects of *Satureja hortensis* L. Essential Oil on the Growth and Survival of *Salmonella typhimorium* in Minced Poultry Meat During Refrigerated Storage

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Abstract

Background: Food products and in particular meat are regarded as one of the main sources of Salmonella genus bacteria; accordingly, researchers' attention has recently been drawn to the use of natural preservatives to increase shelf-life and eliminate food-related diseases. The present study was conducted to investigate the effect of *Satureja hortensis* L. (Summer savory plant) essential oil on the growth of *Salmonella typhimorium* in poultry meat.
Methods: In the present study, the effects of different concentrations of Summer savory essential oil (0, 100 ppm, 200 ppm, 400 ppm, and 800 ppm) alone and with 1% sodium chloride on the growth and survival of *Salmonella typhimorium* in refrigerated minced poultry meat were assessed on days zero, three, five and seven.
Results: Summer savory essential oil significantly reduced Salmonella count compared to control group ($P < 0.05$). Savory significantly reduced Salmonella count as the storage days of minced meat increased ($P < 0.05$). However, this effect on Salmonella count in the presence of 1% sodium chloride did not significantly intensify ($P > 0.05$).
Conclusions: The results showed that Summer savory essential oil has antimicrobial properties, and it can be used as a natural preservative for increasing shelf-life of meat and meat products.

Keywords: Summer Savory Essential Oil, Minced Poultry Meat, Refrigerated Conditions, *Salmonella typhimorium*

1. Background

Despite considerable advances in food preparation and storage, consumer health and safety is particularly important for public health. On average, 30% of people in industrial countries are affected by food-related diseases. Hence, the need to reduce or eradicate pathogenic microorganisms in food is still felt (1, 2).

The medication resistance in microorganisms on the one hand, and harmful effects of chemical preservatives on the other have turned into a major challenge in human and livestock health. Therefore, identifying new and natural antimicrobial compounds is highly essential to the eradication of pathogenic microorganisms and as an alternative to chemical preservatives (3).

With a scientific name of *Satureja hortensis*, Savory is from the mint family; it is an herbaceous annual plant with branched stems and dark green color, which differentiates it from other species. Savory essential oil is obtained through hot steam distillation of the leaves and leafy shoots. This essential oil is a colorless or yellowish liquid with a peppery aroma (4). Summer Savory contains 1% essential oil consisting of such compounds as carvacrol, thymol, betapenin, paracymon, limonin, and camphon. These compounds may vary depending on the climate of the growing region (5).

One of the most common causes of foodborne infectious diseases is Salmonella bacterium (6). Its main source is intestine of animals, and dense breeding of animals makes conditions conducive to the growth of this bacterium. *Salmonella typhimorium* affects both human and animal hosts. Poultry products are one of the main carriers in transmitting Salmonella, which causes such diseases as

gastroenteritis, typhoid fever, and occasionally septicemia in humans (7-9).

Their essential oils and compounds permeate into the mitochondrial and bacterial cell membrane lipids, and cause further permeability by impairing their structures. This causes leakage of ions and other cell contents, and ultimately cell death (1). Generally, the higher the amount of phenolic compounds is in the essential oil, the greater their antimicrobial property against pathogenic bacteria will be. These compounds include Carvacrol, Thymol, and Eugenol, and impaired cytoplasmic membrane, disruption in proton movement force and electrical current, and coagulation of cell contents have been proposed as their mechanism of action (10).

Sefidkon et al. investigated the effect of Savory essential oil on gram-negative and gram-positive microbes, and argued that thymol and carvacrol phenolic compounds in the essence have antimicrobial properties (11).

The studies conducted by Souri et al. (2004) (12), Cavar et al. (2008) (13), and Gulluce et al. (2003) (14), using different tests (each with its own advantages and disadvantages), reported good antimicrobial activity for savory essential oil. Previous studies have shown that gram-positive bacteria are more vulnerable to secondary plant compounds than gram-negative bacteria because permeability of secondary compounds is more difficult in gram-negative bacteria due to the presence of lipopolysaccharide cell wall (2, 15-17). Since there is much evidence confirming antimicrobial properties of the above plant, the present study was conducted to use natural preservatives to control the growth of key pathogenic bacteria such as: *Salmonella typhimorium* in the presence of sodium chloride, and also to prevent food wastage and related damage in the meat product industry, and protect consumer health.

2. Methods

2.1. Preparation of Essential Oil and Bacterial Strain

The study bacterium (standard *Salmonella typhimorium*) (PTCC1730) was procured from the Pasteur institute. Furthermore, the standard Savory essential oil was purchased from Barij Essences Company, Kashan. All culture media used in the present study were made by Germany's Merck company.

2.2. Analysis of Constituent Compounds of the Essential Oil Using GC-MS

The essential oil obtained was decomposed by the gas chromatography device attached to a Mass-Spectrometer (GC/MS) in the food and drug laboratory of Tabriz University of Medical Sciences, and its constituent compounds were identified. The GC-MS device used in the present study was of the Agilent 6890 type, with a 30 m long capillary column, internal diameter of 0.25 mm, and internal layer thickness of 0.25 μm of HP-5MS type, with column temperature program: initial temperature of 70°C maintained for 2 minutes, then rising to 22°C at a rate of 15°C per minute, and column temperature rising to 300°C for 2 minutes.

2.3. Disc Diffusion Test

This test was used to determine the presence or absence of antimicrobial activity of Savory essential oil. To this end, first, the study bacteria were cultured in Muller Hinton Broth for 24 hours. Then, the opacity of bacterial culture was visually adjusted and compared with McFarland 0.5 standard, so that 1.5×10^8 CFU/mL of bacteria were obtained, and then cultured on Muller Hinton Agar. Next, 20 μL of Savory essential oil was added to the standard discs (6 mm), and discs were placed on Muller Hinton agar and incubated at 37°C. After 24 hours, the diameter of no-growth zone formed around the discs was measured with a ruler under light and reported in mL.

2.4. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC and MBC were determined using Broth Microdilution MIC testing method, in which a 96-well polystyrene panel is used. Using the standard half-McFarland method, the amount of bacterial inoculation was found 1.5×10^8 CFU/mL. Then, it was diluted by one-twentieth to reach bacterial inoculation of 5×10^6 CFU/mL. Next, up to seven double dilutions of Savory essential oil solutions were prepared in tubes containing nutrient broth. To determine MIC and MBC, first, 160 μL of nutrient broth, and then 20 μL of the prepared concentrations of Savory essential oil, and finally 20 μL of bacterial inoculation were added to the microplate wells. For positive control, 20 μL of bacterial inoculation, 180 μL of nutrient broth, and 20 μL of 96% ethanol and 10% dimethyl-sulfoxide were added to the wells, and for negative control, 180 μL of nutrient broth with 20 μL of bacterial inoculation was added. Then, microplates were incubated at 37°C for 24 hours. Afterward, wells were visually examined in terms of opacity, and the first transparent well was taken as MIC and the second as MBC. Then, for confirmation, 10 μL was taken from the first transparent well together with one transparent and an adjacent opaque well, and cultured in the nutrient agar.

2.5. Preparation of Bacterial Inoculum

For bacterial inoculation, *Salmonella typhimorium* was prepared in BHI broth culture medium, and incubated for

24 hours, which was re-cultured in BHI medium incubated for 24 hours. Then, it received an 18-hour culture in nutrient culture medium. The standard concentration was prepared by adding nutrient broth culture medium to 0.85% normal saline and matching its opacity to that of the standard half-McFarland solution. Based on the standard half-McFarland, this stage contained 1.5×10^8 CFU/mL bacteria. Next, 0.1 mL of this solution was added to 99.9 mL (100 mL) of normal saline, and thus the initial concentration of bacteria was diluted by 1000 times. In the next stage, 1 mL was added to 100 grams of the meat, and thus, *Salmonella typhimorium* count in every gram of meat reached 10^3 CFU (18).

2.6. Meat Preparation

After preparation, the meat was transferred to the laboratory and minced in a sterile mincing machine after aseptic removal of the skin and bones. Then, the minced meat was divided into five 100 g samples as control, 100 ppm, 200 ppm, 400 ppm, and 800 ppm groups. Next, 1 mL of the prepared bacterial inoculum was added to the meat. Once prepared, treatments were transferred into sterile lidded plastic containers. Of these five containers, one contained 1 cc of bacterial inoculum with no essential oil, and the other four contained essential oil and bacterial inoculum. Savory essential oil combined with 1% sodium chloride and 1% salt at the above concentrations was added to the minced meat, and kept refrigerated at 8°C. Bacterial count was carried out on days zero, three, five, and seven. Two control groups were used at this stage for confirmation of data (19).

2.7. Statistical Analysis

Tests were performed with three repeats, and data obtained were analyzed in SPSS-17 using ANOVA, Duncan's test (if required), and t-test at a significance level of $P < 0.05$.

3. Results

3.1. Constituent Compounds of Savory Essential Oil

Table 1 presents the percentage of constituent compounds of Savory essential oil. Based on GC/MS analysis, 18 compounds comprising a total of 99.87% were identified in this essential oil. The main compounds included P-Cymene (29.47%), Gama-Terpinene (28.02%), and Carvacrol (25.97%), which comprise 83.46% of this essential oil. Savory essential oil also contains much lower levels of other compounds, including 1R-α-pinene, 3-tugun, alpha-pinene, 3-caren, Beta-Bisabolene.

3.2. Disc Diffusion Test Results

In disc diffusion test, mean Savory essential oil obtained on *Salmonella typhimorium* was 40 ± 2 mm (Table 2).

Table 1. Constituent Compounds of Savory Essential Oil

Row	Chemical Composition	Percentage
1	3-Thujene	1.24
2	1R-α-Pinene	4.53
3	Camphene	0.28
4	α-Pinene	1.91
5	3-Carene	2.72
6	α-Myrcene	0.19
7	α-Phellandrene	0.17
8	(+)-4-Carene	0.64
9	π-Cymene	29.47
10	γ-Terpinen	28.02
11	Terpinenol-4	0.86
12	Limonene oxide, cis-	0.41
13	Carvacrol	25.97
14	Acetylthymol	0.62
15	(-)-Alloaromadendrene	0.25
16	β-Bisabolene	1.39
17	Caryophyllene oxide	0.52
18	1,2,5,5,8,a-Pentamethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	0.68

Table 2. Disc Diffusion Results

Replication	Diameter of Inhibition Zone Region, mm
1	38
2	40
3	42

3.3. MIC and MBC Test Results

MIC and MBC of Savory essential oil found on *Salmonella typhimorium* are shown in Table 3.

Table 3. MIC and MBC of Savory Essential Oil in mg/mL

Bacterium	Type of Bacterium	Savory Essential Oil	
		MIC	MBC
Salmonella typhimorium	-	1.25	2.5

3.4. The Effect of Savory Essential Oil Alone on Salmonella typhimorium Count in Minced Poultry Meat

The two-way variance analysis results showed that different concentrations of Savory essential oil significantly reduced bacterial count compared to the control group ($P < 0.05$), such that it had the most effect on *Salmonella typhimorium* count at 400 ppm and 800 ppm concentrations,

and compared to control group, this reduction was about logarithm of 1 and 1.2. Different concentrations of this essential oil significantly reduced bacterial count on storage days five and seven, while the results were not significant on days zero and three. Table 4 shows the effect of different concentrations of Savory essential oil on *Salmonella typhimorium* count in minced meat on different days.

3.5. The effect of Savory Essential Oil with 1% Sodium Chloride on Salmonella typhimorium Count in Minced Poultry Meat

The results of Duncan ad hoc test showed that among concentrations tested, 800 ppm was the most effective in reducing *Salmonella typhimorium* count, such that compared to control group, reduction in Salmonella count was about log 1.5. Different concentrations of this essential oil significantly reduced bacterial count on storage days zero, three, five and seven. Significant and negative correlations were observed between Salmonella count and different concentrations of Savory essential oil ($P < 0.05$). Table 5 shows the effect of different concentrations of Savory essential oil with 1% sodium chloride on *Salmonella typhimorium* count in minced poultry meat on different days.

3.6. Comparing the Effect of Savory Essential Oil Alone and in the Presence of 1% Sodium Chloride on Salmonella typhimorium Count in Minced Poultry Meat

The results of comparison of *Salmonella typhimorium* count at different concentrations of Savory essential oil alone and in the presence of 1% sodium chloride showed no significant difference between these groups ($P > 0.05$) (Table 6).

3.7. The Effect of Savory Essential Oil in the Presence of 1% Sodium Chloride on pH of Minced Poultry Meat on Different Days of Storage in Refrigerated Conditions

The two-way variance analysis results showed that different concentrations of Savory essential oil in the presence of 1% sodium chloride have a significant effect on pH of minced poultry meat compared to control group ($P < 0.05$). Moreover, the mutual effect of different refrigeration days and different concentrations of the oil in the presence of sodium chloride on pH was also significant ($P < 0.01$). In other words, the effect of different concentrations of the essential oil in reducing pH of the minced meat depended on different refrigeration days. Pearson correlation test showed no significant correlation between pH of the meat and storage days ($P < 0.05$) (Table 7).

4. Discussion

Many researchers have proposed that Savory genus is mainly composed of phenolic monoterpenes such as Thymol and Carvacrol, often together with Gama-terpinene, Para-cymene, and Linalool, all of which have antibacterial properties (20-23). Phenolic compounds cause cell membrane dysfunction, inhibition of cell functional characteristics, and ultimately leakage of intracellular contents. The chemical structure of essential oils and their volatile compounds have the highest effect in antimicrobial mechanisms (10). The antimicrobial effects of essential oils depend on extraction method, growth phase and bacterial count, type of culture medium used, external and internal factors, incubation duration and temperature, and food packaging and physical structure, and therefore, the results obtained may be different in different studies (1, 24). In the chemical analysis of *Satureja bachtiarica* bunge performed in flowering stage in Ardebil province, the main compounds included Carvacrol (26.4%), Thymol (20.6%), Linalool (14.19%), Alpha-terpinene (5.94%), Mircen (3.56%), and Gama-terpinene (2.3%), which are similar to those found in the present study, but different in terms of percentage of compounds (25). According to the chemical decomposition, the antimicrobial compounds of essential oils were mainly terpenes and other compounds of phenolic nature or free hydroxyl group, all of which have been identified as the most active antimicrobial compounds, which were also identified in plant in the present study. The phenolic compounds of the essential oil in phospholipid layer of the plant's cell membrane are produced, and the higher the amount of compounds and the more phenolic the essential oil is, the greater its antimicrobial property will be (26). The results obtained by Yaghoubi et al. also confirmed the antimicrobial effects of phenolic and flavonoid compounds (27).

Mahboubi and Kazempour (2011) studied and compared the chemical composition and antimicrobial effects of *Satureja hortensis* and *Trachyspermum copticum* essential oil on a number of gram-positive and gram-negative bacteria. The results showed that Thymol, Gama-terpinene and P-cymene were the main compounds in these essential oils. The phenolic compound of Thymol in both oils has a major role in their antimicrobial effect. The antimicrobial effect of Thymol damages cell membrane integrity, which changes pH homeostasis and disturbs ionic balance. P-cymene alone has no antimicrobial property, but enhances antimicrobial effects of Thymol and Carvacrol (28).

The study of the antimicrobial effects of *Satureja montana* and *Satureja cuneifalia* essential oils on nine microorganisms showed that *Satureja montana* has a greater antimicrobial effect than *Satureja cuneifalia*, and it mostly af-

Table 4. The Effect of Different Concentrations of Savory Essential Oil on *Salmonella typhimorium* Count in Minced Meat[a,b,c]

Concentration, ppm	Mean Logarithm of *Salmonella typhimorium* Count ± Standard Error				
	Production Day (Zero)	Day Three	Day Five	Day Seven	P Value
Control (zero)	3.75 ± 0.15A#	5.25 ± 0.08B#	5.82 ± 0.14C×	6.18 ± 0.04C§	0.000
100	3.68 ± 0.13A#	4.80 ± 0.08B#	4.99 ± 0.08B&	5.71 ± 0.06C×	0.001
200	3.64 ± 0.13A#	4.58 ± 0.05B#	4.63 ± 0.06B$	5.51 ± 0.02C&	0.000
400	3.59 ± 0.13A#	4.42 ± 0.51AB#	4.00 ± 0.04AB#	4.84 ± 0.05C$	0.104
800	3.54 ± 0.13A#	4.35 ± 0.56A#	3.79 ± 0.02 A#	4.56 ± 0.03 A#	0.181
P Value	0.845	0.438	0.000	0.000	

[a]Upper case English letters denote the effect of a specific concentration of Savory essential oil on bacterium (comparing table rows) on different storage days.
[b]Symbols denote the effect of different concentrations of Savory essential oil on the bacterium (comparing table columns) on a specific day.
[c]Common English letters indicate the absence of a significant difference (P< 0.05).

Table 5. The Effect of Different Concentrations of Savory Essential Oil with 1% Sodium Chloride on *Salmonella typhimorium* Count in Minced Poultry Meat on Different Refrigeration Days[a,b,c]

Concentration, ppm	Mean Logarithm of *Salmonella typhimorium* Count ± Standard Error				
	Production Day (Zero)	Day Three	Day Five	Day Seven	P Value
Control (zero)	3.81 ± 0.02A#	5.22 ± 0.05B$	5.58 ± 0.03C§	6.07 ± 0.03D§	0.000
100	3.76 ± 0.02A#	4.26 ± 0.06B#	4.77 ± 0.02C$	5.36 ± 0.02D$	0.000
200	3.70 ± 0.03AB#	4.16 ± 0.09B#	4.56 ± 0.03C#	5.12 ± 0.05D#	0.000
400	3.60 ± 0.03AA&	3.73 ± 0.06A&	3.92 ± 0.03B&	4.29 ± 0.04C&	0.001
800	3.52 ± 0.06A×	3.51 ± 0.12A×	3.70 ± 0.08AB×	3.95 ± 0.02B×	0.050
P Value	0.017	0.000	0.000	0.000	

[a]Upper case English letters denote the effect of different storage days at a specific concentration of the essential oil on bacterial count (comparing rows).
[b]Symbols denote the effect of different concentrations of Savory essential oil on the bacterium (comparing table columns) on a specific day.
[c]Common English letters indicate the absence of a significant difference (P< 0.05).

Table 6. Comparing the Effect of Savory Essential Oil Alone and in the Presence of 1% Sodium Chloride on *Salmonella typhimorium* Count in Minced Poultry Meat[a]

Concentration, ppm	Mean Logarithm of *Salmonella typhimorium* Count ± Standard Error		
	Savory Essential Oil	Savory Essential Oil * 1% Sodium Chloride	P Value
Control (zero)	5.25 ± 0.35c	5.17 ± 0.31 e	0.867
100	4.79 ± 0.27b	4.54 ± 0.22d	0.480
200	4.59 ± 0.25b	4.38 ± 0.19c	0.532
400	4.21 ± 0.20a	3.88 ± 0.09b	0.170
800	4.06 ± 0.18a	3.67 ± 0.07a	0.076
P-value	0.000	0.000	

[a]English letters show comparison of two groups

fects *E. coli* (29). The antimicrobial effects of *Satureja hortensis* on 11 bacteria and three fungi were assessed using MIC and MBC disc diffusion method, and with equal MIC and MBC of 0.09 μg/mL, it had growth inhibitory effects on gram-positive and gram-negative bacteria and fungi (30). In studying the effect of different essential oils including eucaluptus, Savory, Oregano, and Marjoram on *E. coli*, *Salmonella typhimorium*, *Aspergillus niger*, and *Aspergillus flavus*, oregano, followed by Marjoram, and Savory essential oils showed the most inhibitory effects, and these antimicrobial effects were due to Thymol and Carvacrol (31). Mehdizadeh et al. (2012) studied antibacterial, antioxidant, and optical properties of edible starch-chitosan composite film containing essential oil of *Thymus kotschyanus*, and their results showed a significant increase in the concentration of phenolic compounds of the film

Table 7. The Effect of Savory Essential Oil in the Presence of 1% Sodium Chloride on pH of Minced Poultry meat on Different Days of Storage in Refrigerated Conditions[a,b,c]

Concentration, ppm	Mean pH ± Standard Error				
	Production Day (Zero)	Day Three	Day Five	Day Seven	P Value
Control (zero)	5.69 ± 0.02A#	5.69 ± 0.02A#	5.69 ± 0.02B#	6.15 ± 0.03C#	0.001
100	5.67 ± 0.02A$#	5.69 ± 0.03A$#	5.64 ± 0.04A#	5.67 ± 0.03A$	0.753
200	5.66 ± 0.03A$#	5.68 ± 0.03A$#	5.63 ± 0.03A$	5.66 ± 0.03A$	0.718
400	5.64 ± 0.03A$#	5.63 ± 0.04A$#	5.61 ± 0.03A$	5.63 ± 0.04A$	0.952
800	5.62 ± 0.02A$#	5.61 ± 0.03A$#	5.58 ± 0.02A$	5.59 ± 0.01A$	0.644
P Value	0.166	0.169	0.008	0.000	

[a] Upper case English letters denote the effect of different storage days at a specific concentration of the essential oil on pH (comparing table rows).
[b] Symbols denote the effect of different concentrations of Savory essential oil on pH (comparing table columns) on a specific storage day.
[c] Common English letters indicate the absence of a significant difference ($P < 0.05$).

with increasing concentration of essential oil ($P < 0.05$), such that at 1% and 2% concentrations of the essential oil, the amounts of phenolic compounds were 10 mg and 13.3 mg Gallic acid in every gram of the film, and this increase in concentration significantly increased antimicrobial effects ($P < 0.05$) (32). These results concur with the present study results.

Other studies conducted on *Satureja hortensis* essential oil confirmed antimicrobial effect of Thymol on 25 bacteria and 8 fungi (33). Savory essential oil, at concentrations higher than 400ppm, was reported to completely inhibit *Alternaria citri* in the culture medium, and prevented its growth, while it slowed but did not stop fungal growth at lower concentrations (34). Since in the present study, π-Cymene was the main compound and the largest constituent in the essential oil extracted (29.47%), it can be argued that antimicrobial effect of the oil may have been due to the high level of π-Cymene. The present study investigated the effects of Savory essential oil alone and in the presence of 1% sodium chloride on the growth rate and survival of *Salmonella typhimorium* in minced poultry meat. The diameter of the inhibition zone of Savory essential oil was found 40 ± 2 mm and its MIC was 1.25 mg/mL. Maghsoudlou et al. investigated the antimicrobial effects of *Satureja Khosestanica* essential oil compared to sodium nitrite in Frankfurter sausages, and showed that 600 ppm of Savory has greater antimicrobial effects on gram-positive bacteria than 500 ppm of sodium nitrite (35). In the present study, the highest concentration of Savory essential oil reduced Salmonella count significantly more than that in the control group ($P < 0.05$). Thus, the present study results agree with those of the above studies.

Mahmoodi et al. investigated the antimicrobial effects of different essential oils on *Pseudomonas aeruginosa*, *Pseudomonas Carranza*, and *Xanthomonas arburicola*, and showed that mint, sage, cumin, cacti, and marigold essential oils have the most bacterial growth inhibitory effects, and yarrow, coriander, and angelica essential oils have the least effects. They also showed that concentrations of 0.015 and 0.03 percent of *Mentha longifolia* L. have antimicrobial effect on *Staphylococcus aureus* (36), which is greater than antimicrobial effect of Savory essential oil, and this can be due to different amounts of phenolic compounds contained in these essential oils. Dadfar et al. studied antibacterial properties of *Saturejaba chtiarica* essential oil on *Pseudomonas aeruginosa* count in minced beef, and showed that this essential oil has the highest antibacterial effect at a concentration of 0.1% (37), which agrees with the present study results.

The effects of Savory essential oil on Salmonella count significantly increased with increasing storage days of minced meat ($P < 0.05$). Thus, this essential oil can be used as a preservative against bacteria in meat. The effects of Savory essential oil on Salmonella count did not significantly increase in the presence of 1% sodium chloride ($P < 0.05$), and low concentrations of salt may explain ineffectiveness of 1% sodium chloride.

4.1. Conclusions

It can be inferred from the results that essential oils as natural and new preservatives can reduce the microbial count in foods including meat products. Therefore, Savory essential oil as a natural preservative is suitable for increasing shelf-life of minced poultry meat.

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