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Evaluation of the Antimicrobial Effects of Satureja Montana Essential Oil Alone and in Combination with Nisin on Escherichia Coli and Staphylococcus Aureus

Sajjad Babaei[1], Arezoo Moradi [2], Mahboobe Hosseinikia [3], Omid Mansouri zaveleh[4], Zohreh Amiri[5], Nasrollah Sohrabi[1*]

[1]Department of Laboratory Sciences, School of Paramedical Sciences, Kermanshah University of Medical Sciences, Kermanshah, Iran
[2]Department of Microbiology, School of Science and Research Sanandaj, Sanandaj Islamic Azad University, Sanandaj, Iran.
[3]School of Nutrition and food sciences, kermanshah university of medical sciences, Kermanshah, Iran
[4]Department of Biochemistry, School of Science and Research Sanandaj, Sanandaj Islamic Azad University, Sanandaj, Iran.
[5]Department of biology, Faculty of Science, Razi University, Kermanshah, Iran

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ABSTRACT

With ongoing use of preservative chemical compounds in the food industry, in addition to the challenge of microbial resistance, also there is growing concerns over side effects of these compounds. So, the use of herbal essential oils in food preservatives with much less side effects and sometimes even positive effects, has been considered. Therefore, in the present study, the antibacterial effects of Nisin and Satureja montana essential oil have been studied separately and in combination on standard strains of Escherichia coli and Staphylococcus aureus, that are important bacteria in food microbial contamination. Constituents analysis of Satureja essential oil was performed by GC/MS. Antimicrobial susceptibility testing as Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was performed by Broth micro dilution method and evaluation of interactions between the compounds, was performed by calculating FIC index (Fractional Inhibitory Concentration) method. Antimicrobial susceptibility test showed that MIC and MBC of Satureja montana essential oil and Nisin on S.aureus were 1.25mg/ml and ≤7.8I.U/ml, respectively. and MIC and MBC of Satureja montana and Nisin on E.coli were <10mg/ml and 1000 I.U/ml, respectively. Results of FIC index revealed that antibacterial effects of Satureja montana in combination with Nisin on E.coli O157 , synergistically increased. While, antibacterial effect of the above mentioned compounds on S.aureus was indifferent. Present study showed a significant improvement in the antibacterial effects of Satureja montana essential oil in combination with Nisin on E.coli compared to S.aureus. The effect of Nisin alone on gram-negative bacteria is weak and now faces the challenge of microbial resistance. However, obtaining a stable combination of herbal essential oils containing antimicrobial agents such as Carvacrol for use in food preservation, can be a good point for further research and development.

Key words: Food Preservative, Herbal Essential Oils, Nisin, Satureja Montana, MIC, MBC,FIC

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Corresponding author: Nasrollah Sohrabi
e-mail✉: na.sohrabi@gmail.com
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INTRODUCTION

Today, the use of chemical preservatives, is the most important way for a long-term food preservation [1]. Frequent use of these chemical compounds such as sodium benzoate, benzoic acid, sodium sorbate, sodium nitrate and potassium sorbate has been followed by some side effects including behavioral disorders in children such as Attention-deficit hyper activity disorder (ADHD) [2, 3]. Overuse of food chemical preservatives and their side effects as well as increasing rate of antibiotic resistance in the recent years, has

created a growing interest of using extracts and herbal medicines with antibacterial nature as the food preservatives. Extracts and the essential oils have antimicrobial, antifungal and antioxidant properties and are able to control the pathogens growth. One of the most important antimicrobial preservatives used in food industry is Nisin. Nisin is a kind of amphipathic polypeptide bacteriocin with 32 amino acids which are produced by specific strains of lactococcuslactis sub specious lactis[4]. Nisin prevents the growth of gram-positive bacteria including Clostridium and Bacillus spores [5]. Nisin disrupts the cell membrane function through creating pores in it. In order to increase the effect of Nisin as well as its efficacy on gram-negative bacteria, it is necessary to combine Nisin with other antimicrobial compounds [6]. Among the antimicrobial agents which are efficient in combination with Nisin, herbal essential oils such as Satureja essential oil can be mentioned. Today, special attention has been dragged to the use of essential oils as the natural antimicrobial agents for food preservatives. Satureja is a herbaneous, one year old plant which is considered as one of the peppermint family [7]. Satureja essential oil is obtained from steam distillation from leaves and fresh branches with leaves. This essential oil is a colorless or yellowish liquid with a nasty smell. Satureja has 0.8 to 1.5% essential oils associated with tannin, resin and mucilage. The summer Satureja includes substances such as carvacrol, thymol, betapinene, paracymon, limonene and camphon. Its other ingredients include different vitamins and minerals. Satureja compounds can be different depending on the weather conditions of plant area [8].

In previous studies such as Pol (et al.) study (1999), herbal essential oils with Nisin and di-glyceride fatty acids were used to inhibit Listeria monocytogenes bacterium. Among them, carvacrol and thymol were the most efficient agents against Listeria monocytogenes and then, eugenol, cinnamaldehyde and iso-ovogenol, respectively [9]. Also, the results of Zhang (et al..2004.) study showed that co-administration of Nisin and carvacrol acts as a synergist and reduces the number of live Listeria monocytogenes and Bacillus cereus [10].

Staphylococcus aureus and E.coli bacteria are important for the creation of microbial contamination in food industries. Staphylococcus aureus is a gram positive coccus which is the main cause of staphylococcal food poisoning and extra-intestinal infections such as ulcer, abscess, pneumonia, meningitis, bacteremia and toxic shock syndrome [11]. On the other hand, the E.coli bacterium which is a gram-negative bacillus from Enterobacteriaceae family, is seen abundant in microbial contamination in food industries. The main source of E.coli bacteria is human and animal intestines. Humans are known as the major reservoir for enterotoxin-produced E.coli and invasive intestinal E.coli strains [12]. Therefore, the importance of combating microbial contamination in food industries as well as the use of effective compounds with the least disadvantages to achieve this goal, make it essential to achieve new antimicrobial compounds in food industries. In the present study, the antimicrobial effect of Nisin and Satureja montana have been investigated separately and in combination on Staphylococcus aureus and E.coli bacteria.

MATERIAL AND METHODS

2-1-Preparation of bacterial strain: For this study, standard strains of Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) bacteria were used.

2-2-Preparation of Satureja montana essential oil: Satureja montana plant was gathered from the hillsides of the Zagros Mountains in Iran and then, its genus and specious were identified by the experts at the Faculty of Agriculture of Razi University in Kermanshah. The plant essential oil was prepared by steam distillation using the Clevenger apparatus. Then, it was dehydrated by anhydrase sodium sulphate and passed through $22_{\mu m}$ filters and kept at 4ºC in special dark glasses with closure until use.

2-3- Analysis of the chemical compounds of Satureja montana essential oil using the GC Mass: The essential oil was constantly injected into a column of gas chromatography (Thermo Finnigan, USA) with a mass spectrophotometry (Trace 2000/EI quadrupole) which was equipped by a thin column of DB1-MS ($30_m \times 0.25_{mm}$) that is chemically linked to the constant phase of DB1-MS 0.25_m. The injection temperature was 260ºC and the flow rate of clearing walls was $20_{ml/min}$. Clearing was activated after 60 seconds. The flow rate of gas in the columns was $1_{ml/min}$. The primary temperature of columns was kept at 70ºC for 2 minutes and then was increased to 180ºC with the rate of $3^{º}{}_{C/min}$ (for 15 seconds) and finally increased

to 250ºC at the rate of $10^{ºC/min}$. The temperature of ion sources was 290ºC. Ionization was carried out with a 70_{eV} electron beam at a flow of 2.0_{mA}. The masses were collected at TIC when the accelerated voltage after a 190 seconds delay. All data were analyzed by an Xcalibur (Thermo Finnigan, USA). The inhibitory index for each compound was calculated using comparison of its inhibition time with the inhibition time of alkane sets. The specification of all substances was obtained by comparing both spectra with those that were collected from the library data including Wiley, NIST and internal libraries.

2-4- Preparation of Nisin Solution: The main solution of Nisin ($10^{4}_{IU/ml}$) was prepared by dissolving 20_{mg} (0.02_{g}) of pure Nisin (Merck-Germany) in 1_{ml} of Chloridric acid $0.02_{mol/L}$. Then, this solution was centrifuged at 1500_{rpm} for 20 minutes and the supernatant solution was removed. Filtration was carried out using a syringe filter with a pore diameter of $0.45_{\mu m}$ and the obtained solution was stored in a captive tube at the refrigerated temperature of +4ºC.

2-5- Disk diffusion test: To do this test, 24-hours culture of the bacteria was incubated in BHI broth (Merck-Germany) at 37ºC, at first. Then, the turbidity of bacterial culture was visually compared and adjusted using the 0.5 McFarland standard and the inoculum was finally adjusted using a spectrophotometer (Jenway 6305) with wavelength of 600_{nm}, so that the number of bacteria was obtained 1.5×10^{8} $_{CFU/ml}$. Then, each of the studied bacteria was cultured on a Muller-Hilton Agar medium (Merck, Germany) using a sterile swab. In the next step, $20_{\mu l}$ of Satureja essential oil was loaded on standard sterilized disks (BaharAfshan, Iran), then disks were put on Muller-Hilton Agar medium and incubated at 37ºC for 24 hours. Also, the loaded sterilized disks with DMSO (Merck, Germany) on Muller-Hilton Agar medium were used as the control negative. After 24 hours, the diameter of inhibition zone around the disks were measured under the study light and using a colis and reported as mm unit. This test was performed for each bacterium, separately.

2-6- Determination test of MIC and MBC: To determine MIC and MBC, Broth Microdilution MIC testing was used. The microplate containing 96-wells (SPL-South, Korea) was used in this method. To adjust the amount of bacterial inoculation and using the 0.5 McFarland standard, the number of studied bacteria was first obtained $1.5\times10^{8}_{CFU/ml}$. Then, optical density of bacterial suspension was determined using a spectrophotometer at 600_{nm}. Then, to achieve the bacterial inoculation of $5\times10^{6}_{CFU/ml}$, the suspension was diluted with BHI broth medium by one-twentieth.

2-6-1- Determination of essential oil and Nisin concentration: To dissolve the essential oil, DMSO 10% solution (Merck, Germany) was used. So that, 0.1_{g} of the studied essential oils wad added to the test tubes containing 1_{ml} of DMSO 10% solution at first and the test tubes were shaken using a shaker to create a dye suspension. Then, two-times to seven-times dilutions of essential oil solutions were prepared in the test tubes containing BHI broth medium. To obtain the desired concentration of Nisin, 8 sterile tubes containing 0.5_{ml} of sterile distilled water in any of them were prepared. Then, 0.02_{g} of pure Nisin powder + 1_{ml} HCL (Merck, Germany) were taken and diluted to the eighth tube in two-fold way. Adding Nisin to the wells, the tube number 1 which was contained $10^{4}_{IU/ml}$, was diluted to 10^{-1} and reached to 10^{3} $_{IU/ml}$. This process went up to well number H, so that the concentration in wells reached from 10^{3} to 7.8 $_{IU/ml}$.

2-6-2- MIC and MBC determination of Nisin and Satureja montana essential oil, separately: First, $160_{\mu l}$ of BHI broth and then, $20_{\mu l}$ of the prepared Nisin and Satureja montana concentrations were separately added to the microplate wells in two separate microplate and finally $20_{\mu l}$ of bacterial inoculation was added into microplate wells. Thus, the volume of contents in the wells was reached to $200_{\mu l}$ and according to the dilution of 0.1 in wells, the concentration of the substances in wells was diluted to 10 times and the approximate number of bacteria in the wells reached to $5\times10^{5}_{CFU/ml}$. For positive and negative controls, $200_{\mu l}$ and $180_{\mu l}$ BHI broth with $20_{\mu l}$ bacterial inoculation were added to the wells, respectively. The microplates were shaken for 20 seconds at the speed of 300_{rpm} on the plate-thermoshaker machine after cap-covering and then incubated at 37ºC for 24 hours. After 24 hours, the microplates were removed from the incubator and the wells were examined visually for the appearance or absence of turbidity and the first and second wells were considered as MIC and MBC, respectively. This test was performed for each bacterium and each combination, separately.

2-6-3- MIC and MBC determination of Nisin in combination with Satureja montana: To do this test, first, $140_{\mu l}$ of BHI broth and then $20_{\mu l}$ of the

prepared concentration of Nisin solution and $20_{\mu l}$ of the prepared concentration of essential oil and finally $20_{\mu l}$ of bacterial inoculation were added to the wells of microplate. So that, the volume of contents in the wells reached to $200_{\mu l}$ and according to the created dilution of 0.1 in the wells, the concentration of the substances was diluted 10 times and the approximate number of bacteria in the wells was reached to 5×10^{5} $_{CFU/ml}$. For positive and negative controls, $200_{\mu l}$ of BHI broth and $180_{\mu l}$ of BHI broth with 20µl of bacterial inoculation were added to the wells, respectively. After cap-covering, microplates were shaken for 20 seconds at the speed of 300_{rpm} on the plate-thermoshaker machine and then, were incubated at 37ºC for 24 hours. After 24 hours, microplates were removed from the incubator and the wells were examined visually for the presence or absence of turbidity and the first and second wells were considered as MIC and MBC, respectively. This test was performed for each bacterium, separately.

2-7- Evaluation of the effect of Nisin and Satureja montana combination: To determine the interaction between studied essential oil and Nisin, FIC index (Fractional Inhibitory Concentration) was used which is calculated using the below formula. If the FIC index of antimicrobial compounds is less than 0.5, interaction of antimicrobial compounds is synergistic. If the FIC is between 0.5 and 1, the interaction is additive. If the FIC is between 1 and 4, the interaction is indifference and finally if the FIC is greater than 4, the interaction of antimicrobial compounds is antagonistic.

$$FIC\alpha = \frac{\text{MIC } \alpha \text{ in participation}}{\text{MIC } \alpha \text{ alone}}$$

$$FICI : FIC_{\text{nisin}} + FIC_{\text{essential oil}}$$

RESULTS

3-1- Analysis of Satureja montana essential oil: According to GC-MS system analysis, 18 compounds and a total of 99.87% was identified in essential oil. Major compounds include: π-Cymene (29.47%), γ-Terpinen (28.02%), carvacrol (25.97%) that the above compounds contain more than 83.46% of essential oil. Of course, other compounds such as 1R-alpha-pinene, 3-Thujene, alpha-pinene, 3-carene, beta-bisabolene also formed much less essential compounds. In table-1, the results of the analysis of Saturej montana essential oil have been listed.

Table 1. Essential oil analysis of Satureja by GC-MS

Chemical compositions	Percent (%)
π-Cymene	29.47
γ-Terpinen	28.02
Carvacrol	25.97
1R-α-Pinene	4.53
3-Carene	2.72
α-Pinene	1.91
β-Bisabolene	1.39
3-Thujene	1.24
Terpinenol-4	0.86
1,2,5,5,8,a-Pentamethyl-1,2,3,5,6,7,8,8a-octahydronaphthalen-1-ol	0.68
(+)-4-Carene	0.64
Acetylthymol	0.62
Caryophyllene oxide	0.52
Limonene oxide, cis-	0.41
Camphene	0.28
(−)-Alloaromadendrene	0.25
α-Myrcene	0.19
α-Phellandrene	0.17

3-2- Disk diffusion test results of Satureja montana: This test was performed in three replications and the results have been presented in the table-2.

Table 2. Disk diffusion test results of Satureja montana

Bacteria	Mean±SD	Neg. Control
E.coli (ATCC25922)	18±2 mm	0
S.aureus (ATCC 25923)	27±2 mm	0

3-3- The results of MIC and MBC determination test of Nisin and Satureja montana essential oil: The results of MIC and MBC determination showed that Nisin and Satureja montana essential oil have significant antibacterial effects on Staphylococcus aureus, so that, MIC and MBC of Satureja montana and Nisin on S.aureus are obtained $1.25_{mg/ml}$ and $\leq7.8_{I.U/ml}$, respectively. Also, the results of MIC and MBC determination showed that Satureja montana and Nisin have a little antimicrobial effect on Escherichia coli, so that, MIC and MBC of Satureja montana and Nisin on E.coli are obtained $<10_{mg/ml}$ and $1000_{I.U/ml}$, respectively. The results of MIC and MBC determination of Satureja montana essential

oil and Nisin on E.coli and S.aureus have been presented in table-3.

3-4- Evaluation of the interactions of Satureja montana with Nisin using the FIC index: The results of FIC index calculation showed that the antimicrobial effects of Satureja montana essential oil in combination with Nisin is increased on E.coli, synergistically while these effects have no changes on S.aureus such that antimicrobial effect was Indifferent. The results of FIC index calculation has been mentioned in table-3.

Table3. The average comparison of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Satureja montana essential oil alone and in combination with Nisin on the E.coli and S.aureus bacteria and calculating Fractional Inhibitory Concentration (FIC) index to evaluate their interaction.

	E.coli (ATCC 25922)			S.aureus (ATCC 25923)		
	MIC mg/ml	**MBC** IU/ml	**FIC**	**MIC** mg/ml	**MBC** IU/ml	**FIC**
Essential oil	> 10	> 10	-	1.25	2.5	-
Nisin	1000	> 1000	-	≤7.8	≤15.6	-
Essential oil + Nisin	15.6+0.156	31.2+0.31	0.03 **(S)**	≤7.8+≤0.078	≤15.6+≤0.156	1.06 **(I)**

S: Synergism, I: Indifference

DISCUSSION AND CONCLUSION

The results of this study showed that Satureja montana essential oil have more antimicrobial effects on gram-negative bacteria compared to the gram-positive bacteria. In addition to that, the antimicrobial effects of Satureja montana essential oil in combination with Nisin act as synergism and are increased on E.coli while have no change on Staphylococcus aureus (Indifferent). Nisin, as a preservative in food industries, has a little effect on gram-negative bacteria. Gram-negative bacteria are resistant to Nisin due to having the lipopoly saccharide composition (LPS) in their outer layer which acts as a barrier against Nisin on cytoplasmic wall and in addition to that, peptide antibiotics like Nisin are not able to pass the bilayer wall in the absence of wall potential [13]. On the other hand, some of gram-positive bacteria are also resistant to Nisin because of having nisinase enzyme, changes in the cell wall and membrane phospholipids, regulatory networks as well as ABC transfers [14]. Therefore, combination of Nisin with other substances with antimicrobial properties which increases the antimicrobial function of Nisin and have low side effects is important. To achieve such this compound, herbal essential oils such as Satureja essential oil are considered appropriate. Many studies have shown that the main components of the specious of Satureja genus, have contained phenolic monotherapines such as thymol and carvacrol which there are usually associated with γ-Terpinen, paracymene and linalool and this group of phenolic compounds has antimicrobial properties [15,16]. Phenolic compounds lead to impair the cell membrane function and inhibit the functional properties of the cell and finally cause the leakage of intracellular materials and thus, they act their bactericidal role. The chemical structure of herbal essential oil and their volatile compounds have the greatest effect on the antimicrobial mechanisms of essential oils. The antimicrobial effects of herbal essential oils are highly dependent on extraction methods, growth phase, bacterial count, type of culture medium, pH, incubation time and temperature, packaging method and the physical structure of foods, so the results obtained from different studies are sometimes associated with differences [17,18]. In a similar study to the present study, the combination effect of Nisin and carvacrol on the bioavailability of different strains of Bacillus cereus was investigated in different levels of pH and heat. The results indicated that five studied strains showed significant difference in the sensitivity to Nisin and when the combination of Nisin and carvacrol was used, the bioavailability of the cells was lower than the time when Nisin was used, alone. On the other hand, carvacrol concentration has had an important role on the antimicrobial effect of Nisin and has a synergistic role in this combination [19]. In another study in this regard, which was carried out on the antimicrobial effect of herbal essential oil on Satureja hortensis, the inhibitory effects of thymol compound derived from the essential oil were confirmed on 25 bacteria and 8 fungi [20]. The results of our research, aligned with the previous studies, indicate the antibacterial effects of Satureja essential oil and according to the previous studies [21,22] about the antibacterial effect of carvacrol, it seems that carvacrol which contains 26% of Satureja montana essential oil, has had the greatest role in the antimicrobial effects of essential oil in this research. Therefore, it is recommended to continue this research to achieve a stable and natural compound of Satureja essential oil to use in the preservatives of the food industry. Since, Satureja essential oil is a natural substance and in addition to antimicrobial effects, has other desirable effects such as antioxidant effect, many

side effects of the essential oil are not anticipated for human. Although, it seems that investigation of the effects of long-term use of this substance alone or in combination with the other preservatives like Nisin, can be a good topic for the further research.

Conflict of interest

The authors of this research declared no conflict of interest.

REFERENCES

1. Lück E. Chemical preservation of food. Zentralbl Bakteriol Mikrobiol Hyg B. 1985.180(2-3):311-8.
2. McCann D, Barrett A, Cooper A, Crumpler D, Dalen L, Grimshaw K,(et al). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomized double-blinded, placebo-controlled trial. Lancet .2007; 370: 1560–67. DOI:10.1016/S0140-6736(07)61306-3.
3. Pelsser L .M, Frankena K, Toorman J, Savelkoul H.F, Dubois A.E, Rodrigues Pereira R,(et al). Eff ects of a restricted elimination diet on the behaviour of children with attention-defi cit hyperactivity disorder (INCA study): a randomised controlled trial. Lancet. 2011. 377: 494–503.
4. Bryan EM, Bae T, Kleerebezem M, Dunny GM. Improved vectors for nisin-controlled expression in gram-positive bacteria. Plasmid 44. 2000 . 44(2):183-90.
5. Liu W, Hansen J.N. Some Chemical and Physical Properties of Nisin, a Small-Protein Antibiotic Produced by Lactococcus lactis American Society for Microbiology. 1990. p. 2551-2558.
6. Kleerebezem M, Beerthuyzen, M.M, Vaughan, E. E, Vos, W.M. de, Kuipers O.P. Controlled Gene Expression Systems for Lactic Acid Bacteria: Transferable Nisin-Inducible Expression Cassettes for Lactococcus, Leuconostoc, and Lactobacillus spp. American Society for Microbiology. 2008. p. 4581–4584.
7. Hamidpour R, Hamidpour S, Hamidpour M, Shahlari M, Sohraby M. Summer Savory: From the Selection of Traditional Applications to the Novel Effect in Relief, Prevention, and Treatment of a Number of Serious Illnesses such as Diabetes, Cardiovascular Disease, Alzheimer's Disease, and Cancer. J Tradit Complement Med. 2014. 4(3): 140–144.
8. Pfefferkorn A, Kruger H, Pank F. Chemical Composition of Satureja hortensis L Essential Oils Depending on Ontogenetic Stage and Season. Journal of Essential Oil Research. 2008. Vol 20, - Issue 4.
9. Pol IE, Smid EJ. Combined action of nisin and carvacrol on Bacillus cereus and Listeria monocytogenes. Lett. Appl. Microbiol.1999. 29: 166 – 70.
10. Chi-Zhang Y, Yam K, Chikindas MS. Synergistic Antimicrobial Effect of Lactobacillus plantarum with Extracts of Satureia hortensis and Anethum geravolens on Salmonella typhimurium; in vitro and in Animal Model. Int. J. FoodMicrobiol.2004. 90: 15 - 22.
11. Le Loir Y, Baron F ,Gautier M. Staphylococcus aureus and food poisoning. http://www.funpecrp.com.br/gmr/year2003/vol1-2/sim0009_full_text.htm.
12. Lim J.Y, W. Yoon J, J. Hovde C. A Brief Overview of Escherichia coli O157:H7 and Its Plasmid O157. J Microbiol Biotechnol. 2010.20(1): 5–14.
13. Kordel M, Schiiller F , G. Sahl H. Interaction of the pore forming-peptide antibiotics Pep 5, nisin and subtilin with non-energized liposomes. Federation of European Biochemical Societies.1989. Vol 244, number 1, 99-102.
14. Zhou, H., Fang, J., Tian, Y. et al. Mechanisms of nisin resistance in Gram-positive bacteria. Ann Microbiol .2014. 64: 413. https://doi.org/10.1007/s13213-013-0679-9.
15. Svoboda K., Greenaway R. Investigation of volatile oil glands of Satureja hortensis L. (summer savory) and phytochemical comparison of different varieties. Int J Aromather.2003. 13: 196-202.
16. Sefidkon F., Jamzad Z. Chemical composition of the essential oil of three Iranian Satureja species (S. mutica, S. macrantha and S.intermedia). Food Chem. 2005.91: 1-4.
17. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International Journal of Food Microbiology .2004. Vol 94, 223–253.
18. Tajkarimi M.M., Ibrahim S.S., Cliver D.O. .Antimicrobial herb and spice compounds

in food. Food Control.2010.Vol 21, 1199–1218.
19. Periago P.M. Palop A. Fernandez P.S. Combined Effect of Nisin, Carvacrol and Thymol on the Viability of Bacillus Cereus Heat-Treated Vegetative Cells. Food Science and Technology International.2001. Vol: 7 : 6, p 487-492. https://doi.org/10.1106/JE3P-NYKQ-4UDQ-TJC6.
20. Adiguzel A., Ozer H., Kilic H., Cetin B. Screening of antimicrobial activity of essential oil and methanol extract of Satureja hortensis on foodborne bacteria and fungi. Czech J Food Sci.2007. 25: 81-89.
21. ULTEE A, A. SLUMP R, STEGING G, SMID E.J. Antimicrobial Activity of Carvacrol toward Bacillus cereus on Rice. Journal of Food Protection.2000, Vol. 63, No. 5, pp. 620-624.
22. Ben Arfa A, Combes S, Preziosi-Belloy L, Gontard N,Chalier P. Antimicrobial activity of carvacrol related to its chemical structure.