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Evaluating the Antimicrobial Effects of *Zataria multiflora* Essential Oils on Bacterial Growth of *Listeria monocytogenes* in Roast-Chicken Fillets

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The *Zataria multiflor*a belongs to the medicinal plants family, which urges the need for evaluating its Antimicrobial effects in food products.The aim of the study is evaluating the antimicrobial effects of *Zataria multiflora* essential oils on bacterial growth of *Listeria monocytogenes* in roast-chicken fillets at 10 °C. The roast-chicken fillets weighing 25 grams were prepared, sterilized, and different concentrations of *Zataria multiflora* essential oils of (0, 0.045, 0.135, 0.405 and 0.810%) and 0.2% Agar and 1% Lysitine were kept at 5 °C for 24 ± 2. Then, the roast-chicken fillets were separated with the condition that 1×10^3 bacteria would be present at the days 0, 1, 2, 3, 6, 9, 12, 15, 18, 21. The given essential oil had a significant effect on controlling and preventing the *Listeria monocytogenes* growth in roast-chicken fillets during the experiment. By increase the concentration to 0.405%, the inhibitory effect was significantly increased ($P< 0.05$). The Organolyptic analysis showed that the 0.405% essential oil content has resulted in an improved fish flavor. The *Zataria multiflora* essential oils can be used as a natural preservative (stabilizer) in the roast-chicken fillets.

Key Words: *Zataria multiflora*, *Listeria monocytogenes*, Roast-chicken fillets, Plant essential oil.

Plant essential oils are considered as the most natural stabilizers among which 30 kinds are highly important in business[1] and their components are also known to have antibacterial effects[2] and their antimicrobial effects have a long history in medicine and the ideology behind using the essential oils is seasoning and flavoring properties in spices all around the world[3,4]. Phenol properties in essential oils are responsible for the antimicrobial effects[1]. As a result, the more the phenol properties in essential oils, the higher the antimicrobial effects. Among the different existing components in the essential oils, the Carvacrol, Thymol and Eugenol can be stated[1,5].

Generally, the higher concentration of essential oils in proportion to laboratory ones could be certified due to the necessary antibacterial effect[6,7,8]. *Listeria monocytogenes* is a gram-negative, spore free and a non-acid fast bacterium, is a catalase-positive bacterium, and is able to ferment glucose and produce lactic acid[9,10]. Listerialaizine O is the most distinct *Listeria monocytogenes* pathogen[9]. For some people including infants, individuals with lack of immunity,

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elderly, organ-transplant recipients, dialysis patients, diabetics and people with AIDS, this bacterium can be really dangerous and may cause septicemia Meningo encephalitis, Meningitis, abortion or dead fetus[9,16] and may cause self limiting gastrointestinal diseases in healthy individuals with fever, nausea, diarrhea and vomiting[9,14]. Many of these bacteria are found in the raw and processed food such as milk, dairy product, meat including raw meat, poultry and seafood, vegetables and fruits. Freezing and cooling can serve as a barrier to stop the proliferation of pathogenic agents and in most foods prepared for being cooked , including roast chicken because they do not receive enough temperature to be cooked, natural additives can be used first for their anti bacterial effect to destroy pathogens and second to increase their keeping time which also give them a better and enhanced flavor[17,13,12,11,9].

This bacterium is cold-featured pathogen which can cause the contamination of foods in the refrigerators due to their resistance to cold and can be considered as an important cause of food borne diseases[9,10] the world health organization has estimated that 0 percent of the population in the industrialized countries suffer from the food-borne diseases annually and in 2000, 2 million people died all around the world because of the diarrhea[18].

Many foods might get infected during processing, preparation, storage and distribution, however, food trade and transport to remote areas have intensified the need for providing conditions for preventing them from being decayed and corrupted. Because consumers' awareness of the food additive and stabilizers has increased, they prefer natural preservers and stabilizers to the chemical ones. The *Zataria multiflora* belongs to the Lamiaceae family, which grows in Iran, Pakistan, and Afghanistan[19].

MATERIALS AND METHODS

Hypotheses

Evaluating the effect of oil *Zataria multiflora* on *Listeria monocytogenes* bacterium growth in roast chicken fillets with different concentrations of (0, 0.045, 0.135, 0.405, and 0.810%) at 10 °C (unfavorable refrigerated temperature).

Plants and essential oil preparation and its analysis

A botanist and plant expert at Jihad medicinal plant institute scientifically approved the *Zataria multiflora* was gathered from the Fars province and the name then the plants were dried in the sunshade and dried plant shoots were distilled using Clevenger apparatus for 3 hours.Then, the Essential oil analysis was performed by gas chromatograph connected to a mass spectrograph (GC/MS).

The bacterium under study

The Bacteria used in this study was *Listeria monocytogenes* ATCC 19118 whose Lyophilized culturing was conducted after its transfer to brain and heart broth at 37 ° C for 18 h, which were heated for two consecutive times . after the second culturing with the ratio of 1 to 5, the sterile glycerin was mixed and were kept at - 20 °C with in 500 micro-liter volumes in Apendrof micro-tubes.

Preparing the bacterium inoculation rate

In order to determine the bacterium inoculation rate for the study, the cultured bacteria kept in the Apendrof tubes were conducted at – 20 °C for 18 h, Then, 5 ml of the brain and heart broth was added to the Cuvett sterile tubes and various amounts were added to the Cuvett sterile tubes at the second 18 hours of culturing and by using a spectrophotometer (Milton Roy Company, USA) Nora absorption at 600 nm wavelength was determined. Simultaneously, bacterial counting was conducted using the sampling of Cuvett tubes contents and the Cuvett tube containing 1×10^7 CFU bacteria per ml was determined. Subsequently, 1 ml of the contents of the tube was added to 39 ml Cuvett diluents in Zimex sterile glass and to conclude into the Zimex glass content of up to 2.5×10^4 per 100 micro-liters of bacteria to be investigated (which was confirmed by culturing on agar for counting).

Now, at the time of inoculating the chicken fillets, 100 micro-liters of Zimex glass content were used to inoculate 1×10^3 bacteria per gram in chicken fillets.

Preparing chicken fillets

20 kg of chicken was prepared from slaughterhouse and their fillets were separated. The chicken pieces were cut into 3 × 8 cm square pieces average weighing about 25 g for each on

average. Total 12 pieces of sterilized roast-chicken fillets were selected randomly and were cultured after the dilution; the bacteria did not grow in the Pour plate medium method.

Different concentrations of *Zataria multiflora* essential oils of (0, 0.045, 0.135, 0.405 and 0.810% and 0.2% Agar and 1% Lysitine were used to keep the fillets at 5 °C for 24 ± 2. After this step, the fillets were taken out of the sterilized content and were kept under an air conditioner in a sterilized container for one minute to be dried in order to remove the extra water; subsequently; they were placed inside the sterilized plates to reach the desired weight. The given bacteria were kept under spot inoculation in ten points (total 10 drops), 10 micro-liters per drop (100 ml) in each Zimex sterile glass were inoculated with bacteria which were prepared so that up to 2.5×10^4 per 100 micro-liters of bacteria was investigated (which are confirmed by culturing). Using such a technique, 1×10^3 of *Listeria monocytogenes* bacterium was found in

Table 1. The results of *Zataria multiflora* essential oils compounds analysis using GC-MS instrument

Compound name	Inhibitory index	Percentage (%)
α-Thujene	932	0.29
α-Pinene	938	3.73
Octanol	995	0.28
Octanone	999	0.27
β-Pinene	1006	0.46
β-Myrcene	1013	1.52
δ-Caren	1017	1.49
Para - Cymene	1024	8.01
Dihydrocarveol	1036	0.99
γ-terpiene	1068	7.04
Linalool	1104	1.91
4-Terpineol	1176	0.74
α-Terpineol	1192	0.85
Cumucaldehyde	1197	0.86
Thymol methyl ether	1235	0.70
Carvacrol methyl ether	1240	1.94
Carvacrol	1297	59.72
Terans-caryophyllene	1418	0.36
β-Caryophyll ene	1426	1.73
Aromadendrene	1439	0.82
Spathylenol	1571	0.41
Caryophyll ene	1582	0.39
Total	-	94.51

Table 2. The growth rate (mean logarithm ± Standard deviation) of *Listeria monocytogenes* bacterium in chicken fillets at different concentrations of *Zataria multiflora* essential oils during 21 days storage at 10 ° C

The growth rate *Zataria multiflora* essential oils (%)	The growth rate (eamLogN ± SD)										
	Days										
	0	1	2	3	6	9	12	15	18	21	
0	2.97±0.42	3.42±0.015	4.19±0.023	5.75±0.002	8.98±0.001	>8	>8	>8	>8	>8	
0.045	2.96±0.025	3.43±0.005	4.31±0.009	5.51±0.009	7.88±0.003	8.32±0.002	>8	>8	>8	>8	
0.135	2.96±0.025	3.26±0.014	4.16±0.028	5.20±0.026	6.86±0.051	7.95±0.001	>8	>8	>8	>8	
0.405	2.94±0.047	2.54±0.012	2.97±0.044	3.67±0.008	5.81±0.003	6.95±0.003	7.06±0.002	>8	>8	>8	
0.810	4.85±0.061	2.58±0.055	2.96±0.11	2.98±0.042	5.57±0.013	7.26±0.012	7.95±0.002	>8	>8	>8	

every one gram of inoculated fillets. The inoculated fillets remained to be dried for one hour at room temperature (25 °C) under a Biosafety Cabinet and then the fillets were placed in sterile Stomacher bags by maintaining the sterile conditions and were transferred to a warm chamber at 10 °C. the inoculated fillets culturing and the given bacteria counting were conducted on the days 0, 1, 2, 3, 6, 9, 12, 15, 18 and 21. It should be noted that all experiments were performed with three replicas.

Statistical Analysis

Different concentration effects of *Zataria multiflora* essential oils on *Listeria monocytogenes* bacterium counting was performed using three-way ANOVA using SPSS Version 15 software. The significant differences at the significance level; of α=0/05 level was conducted by Tuley test using the same software program.

RESULTS

Table 1 shows the results of *Zataria multiflora* essential oils compounds analysis using GC-MS instrument. The highest compound was the Carvacrol with 59.72%. The essential oil effect on bacterial growth (bacterial count) is shown in Table 2. The essential oil at different concentrations had a significant effect in preventing the growth of Monocytogenes bacterium during chicken fillets storage. By increasing the essential oil concentration to 0/405%, the inhibitory effect was significantly increased ($P < 0.05$), but no significant difference was observed at 0.405% concentrations compared with the 0.810% in the growth inhibition ($P < 0.05$) (See Table 2). Organolyptic analysis indicated that using essential oils concentrations of 0.405% not only decrease the product flavor but also improves the taste and flavor.

DISCUSSION

Plant essential oils are the potential sources of antimicrobial compounds, which serve as useful inhibitors of microbial growth[2,1,20]. In recent years, due to concerns on the use of chemical stabilizers and their possible harmful effects, the trend towards using vegetable oils has increased[19,6,1].

Future research on antimicrobial materials used in food is almost facing two main approaches. The first one is developing the information on the natural antimicrobial materials and the second is using the natural antimicrobial materials in a mixed condition in traditional or modern processing approaches to determine the possible synergistic effect of the compounds[2]. Therefore, different evaluations of the plant essential oils effect or their combination compounds were conducted on some food borne pathogenic microbes such as *Listeria monocytogenes* bacterium, *Staphylococcus aureus, Escherichia coli, Salmonella, Shigella, Clostridium perfringenes* and *Bacillus cereus* all of which represent the researchers' attempts to achieve plant stabilizers which can replace the chemical ones[21,7-1].

Munoz *et al*., conducted a study in 2009 on antimicrobial properties of Oregano, Rosemary and Lourel extracts on the growth and survival rate of *Listeria monocytogenes* bacterium in vitro and Broccdi extract which showed that the combination of these three extracts inhibited the growth of *Listeria monocytogenes* bacterium in the given conditions[22].

Singh *et al* (2003) investigated the Thyme, Clove and Pimenta essential oils effects in vitro peptone water and hot dog food model with different concentrations of 1, 5 and 10 ml per liter on *Listeria monocytogenes* bacterium and made it clear that Clove and Thyme essential oil significantly reduced the bacterial population in peptone water and (hotdogs) , they also stated that the clove essential oil concentration of 1 ml per liter compared with 5 ml concentration of *Zataria multiflora* essential oils per liter was more effective, it should also be noted that the effect of the given essential oils at the same essential oils concentration level in peptone water was higher than that of the hotdogs[24].

In research conducted by Paparella*et al* 2008, the antimicrobial effects of essential oils of Oregano, Thyme and Cinnamon was evaluated against Bisteria Monocytogenes ATCC 19114 in the presence of large amounts of salt. They showed that by increasing the salt concentration, the antimicrobial effects of plants essential oils will be strengthened, they also found that essential oil of Oregano and Thyme was more effective than Cinnamon[23].

In another study conducted by Solomalas *et al* (2007), they used the essential oil of Thyme,

Nisin and their combination against *Listeria monocytogenes* on ((TSB) Tryptic soy broth) and grated cow meat condition.

The antimicrobial effects of different essential oil concentrations of 0.3, 0.6, 0.9% on grated cow meat and TSB medium against *Listeria monocytogenes* showed that by increasing the concentration of essential oils, the antimicrobial effects have increased, but regarding the Organolyptic characteristics of the 0.9 concentration was not acceptable and based on this study, 0.6% concentration level of essential oil had the acceptable antibacterial effects on *Listeria monocytogenes* and its appropriate Organolyptic effects were confirmed[26].

In another study conducted by Singh *et al* 2001, the synergistic effects of garlic and Nisin extract was evaluated through the Minimum Inhibitory Concentration (MIC) on six strains of *Listeria monocytogenes* in Triptose phosphate medium culturing and both plants have shown to have a synergistic effect on bacterial growth inhibition[2]. Sivropoulou *et al* (1996) and Karman *et al* 2001 reported that plant essential oils which are rich in phenol compounds (Carvacrol and Thymol) have higher antimicrobial activities[25,18].

Consequently the review of literature for the conducted studies in the given field have revealed that lots of studies were conducted on the antimicrobial effects of the plants essential oils and their inhibitory growth effects on the food borne pathogenic bacteria which were performed in vitro condition. On the one hand, the researchers on grated cow meat, hotdog and cheese have shed lights on the fact that the antimicrobial effects of the plants essential oils in vitro condition was higher than that of the food models and on the other hand, by increasing the essential oils concentration, the antimicrobial effects will increase too which is congruent with the research on the roast chicken fillets. The essential oils compounds belong to the geographic area, variety and the plant age and the way the plant is dried and distilled[5,26,27,28]. Carvacrol and Thymol are the most are important compounds in the *Zataria multiflora* essential oil, which showed inhibitory effects on *Listeria monocytogenes* in vitro condition[29].

In the studies conducted by Shafiee and Javadnia, the main components of Yazd *Zataria multiflora* were reported to contain 61.29% Carvacrol phenol compounds and 25.18% Thymol[30]. However, in the current study, as it is shown in Table 1. The analyzed essential oils, which were collected from the Fars province, contained 59.72% Carvacrol and low amounts of Thymol (0.7%), which makes the analysis somewhat difficult. It should be considered that the increase in the essential oil concentration must not cause the unwanted and unsatisfactory Organolyptic effects and if such a problem occurs, using the high concentration is not recommended.

In this study, by increasing the concentration level to 0.405%, the inhibitory effect on *Listeria monocytogenes* was significantly increased ($P< 0.05$), but no significant difference was observed between 0.405% and 0.810% concentrations. High concentrations of *Zataria multiflora* essential oil (0.810%), as it can be seen in Table 2, the essential oils not only prevents bacteria from growing, but it also partly had stretching effect on *Listeria monocytogenes* bacterium and has reduced the amount of bacteria in the early days of inoculation, but the concentration level of 810% has resulted in unacceptable Organolyptic effects, while the %0/405 concentration level provided an improved product taste.

REFERENCES

1. Burt S. Essential oils: Their antibacterial properties and potential applications in foods-a review. *International J. Food Microbial*, 2004; **3**: 223-53
2. Bhurinder S,Bernadette F, Martin R. Synergistic inhibition of *Listeria monocytogenes* by nisin and garlic extract. *Food Microbial*, 2001; **18**: 133-9
3. Fazeli MR, Amin G, Ahmadian Attari MM, Ashliani H. Jamalifar H and samadi N. Antimicrobial activities of Iranian sumac and avishan- e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Control* 2007; 6: 646-9
4. Juneja Vijay K, Xuetong F, Pena Ramos A, Diaz – Cinco M and Pacheco – Aguilar R. The effect of grapefruit extract and temperature abuse on growth of Clostridium perfringens from spore inocula in marinated Sous- vides chicken products. *Innovative Food Science & Emerging Technologies* 2006; **7**: 100-6

5. Bagambula CF, Ultendaele M, Debevere J. Inhibitory effect of thyme and basil essential oils, Carvacrol, thymol, estragol, linalool and p-Cymene towards Shigella sonnei and S. flexneri. *Food Microbial*, 2004; **21**: 33 – 42
6. Chi-zhang Y,Yam K L and chikindas M L. Effective control of *Listeria monocytogenes* by Combination of nisin formulated and slowly released into a broth system. *Inter. J. Food Microbial*, 2004: **90**; 15 – 22
7. Etlayebi K, Yamani J El, Rossi – Hassani BD. Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. *FEMS Microbiology Letters* 2000; **18**: 131 – 5
8. Holley R A, Pated D. Improvement in shelf – life and safety of perishable foods by plant essential oils and smoke antimicrobials. *Food Microbiol*, 2005; 272 – 92.
9. Jey J M, Loessner M J Golden D A. Modern Food Microbiology. Seventh Edition, Springer science and Business media 2005, pp: 591 – 617.
10. Pouch D P, Ito K. Compendium of Methods for the Microbiological Examination of Foods. Fourth Edition American Health Association, Washington D. C. 2001, pp: 343 – 53
11. Garcia, M.T., Canamero, M.M., Lucas, R., Omar, N.B.,Pulido, R.P., Galvez, A., Inhibition of *Listeria monocytogenes* by enterocin EJ97 produced by *Enterococcus faecalis* EJ97. *Int. J. Food Microbiol*. 2004; **90**, 161–17.
12. Dorman, H.J.D., Deans, S.G., Antimicrobial agents from plants: antimicrobial activity of plant volatile oils. *J.Appl. Microb*. 2000; **88:** 308-316
13. Gondhi M and Chikinds M L. *Listeria*: A Foodborne pathogen that knows low to survive. *Inter. J. Food Microbial*, 2007; 113: 1-15
14. Daganany M, Listeriosis: Clinical presentation. *FEMS Immunology and Medical Microbial*, 2003; **35**: 173-5.
15. Rocourt J, Jacquet ch, Reilly A. Epidemiology of human listeriosis and seafood, *Inter. J. Food Microbial*, 2000; **6**: 197 – 209
16. Gasanov U, Hughes D, Hansbro P M. Methods for the isolation and identification of *Listeria* spp. And *Listeria monocytogenes* – a review, *FEMS Microbial, Eev*. 2005; **29**: 851 – 75
17. Karunasager I, Karunasagar I. *Listeria* in tropical fish and fishery products. *Inter. J. Food Microbial*, 2000; **62**: 177 – 81
18. WHO 2002. Food safety and Food borne Illness, World Health Organization. Fact sheet 237, revised January 2002 – Geneva
19. Hosseinzadeh H, Ramezani M, Salmani A. Antinociceptive anti – inflammatory and acute toxicity effects of *Zataria multiflora* Doiss. Extracts in mice and rats, *J. Ethanopharmacol*. 2000; **73**: 379 – 85
20. Karaman S, Digrak M, Ravid U, Ilcim A. Antimibacterial and antifungal activity of essential oils of thymus revolutus celak from Turkey. *J. Ethopharmacol.* 2001; **76**: 183 – 6
21. Misaghi A and Akhandzadeh Basti A. Effects of *Zataria multiflora* Boiss. Essential oil and nisin on Bacillus cereus ATCC 11778, *Food Control* 2007; 1**8**: 1043-9.
22. Munoz M, Guevara L, Palop A, Jabera J, Fernandez PS. Determination of the effect of plant essential oils obtained by supercritical fluid extraction on the growth and viability of *Listeria monocytogenes* in broth and food systems using flow cytometry. *LWT Food Science and Technol*, 2009; **42**: 220 -7.
23. Paparella A, Taccogna L, Aguzzi I. Clavesgopez C, Serio A. Marsilio F. Suxxi G. Flow cytometic assessmentof the antimicrobial axtrvity of essential oils against *Listeria monocytogenes*, *Food Control* 2008; **19**: 1147-82.
24. Singh A, Singh R H, Bhunia A K, singh N. Efficacy of plant essential oils as antimicrobial agents against *Listeria monocytogenes* in hotdogs. *Lebensm – wiss Technol*. 2003; **36**: 787-94.
25. Sivropoulou A, Papinkolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M. Antimicrobial and Cytotoxic activities of Oreganum essential oils. *J. Agriculture and Food Chem.* 1996; **4**: 1202-5.
26. Sollomakos N, Govaris A, koidis P, Botsogloun N. The antimicrobial effect of thyme essential oil nisin and their combination against *Listeria monocytogenes* in minced beef during refrigerated storage, *Food Microbial*, 2008; **80**: 159 – 66,
27. Nanasombat, S., Lohasupthawee, P., Antimicrobial activity of crude ethanolic extracts andessential oils of spices against salmonellae andother enterobacteria. *KMITL Sci. Tech. J.*, 2005; **5**, 527 -538.
28. Arras, G., Grella, G. E., Wild Thyme, Thymuscapitatus, essential oil seasonal changes andantimyotic activity. *J. Horticultural Sci*., 1992; **67**: 197-202.
29. Mcgimpsey, J. A., Douglas, M. H., Seasonalvariation in essential oil yield and compositionfrom natural Thymus vulgaris L. in New Zealand. *Flavour Fragrance Journal*, 1994; **9**, 347-352.
30. Shaffiee A and Javidnia K. Composition of essential oil of *Zataria multiflora*, *Planta Medicine* 1997; **63**: 371-2.