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Antibacterial effect of Bunium persicum, Eucalyptus globules, and Allium ampeloprasum extracts on STEC and MRSA in commercial barley soup

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Abstract

Background and aims: The antibacterial potency of *Bunium persicum* (BP), *Eucalyptus globules* (EG), and *Allium ampeloprasum* Var. *Iranicum* (AAI) hydroalcoholic extracts were evaluated against multidrug resistance (MDR) *Escherichia coli* and *Staphylococcus aureus* in vitro and in commercial barley soup during refrigerated and environmental storage.

Methods: In an experimental study, the BP, EG, and AAI extracts were prepared and their antibacterial activities were evaluated against MDR E. coli and S. aureus by micro dilution and disc diffusion methods. Then, the effects of BP and EG extracts were examined on microbial quality of commercial barley soup at 4±1°C and 22±2 °C in zero, 1, 3, 6, and 9 days in different concentrations (BP 1 and 2%; EG 1 and 2%).

Results: Two tested bacterial strains were susceptible to the BP and EG extracts and did not show susceptibility to the AAI extract. The count number of the bacteria in soup were was significantly reduced by the addition of the extracts. The lowest population of the S. aureus was determined in the samples treated with the EG at 1 and 2% during refrigerated and environmental storage. E. coli was suppressed by 1.54 and 3.4 log CFU/mL at 1 and 2 % BP and 1.57 and 3.5 log CFU/mL at 1 and 2 % EG, respectively, compared with the control on day 9 during environmental storage

Conclusion: According to our results, BP and EG extracts can be used as natural antimicrobial preservatives in commercial barley soup.

Keywords: *Bunium persicum*, *Eucalyptus globules*, *Allium ampeloprasum*, Drug resistant, Soup

original article

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Introduction

Shigatoxin-producing *E. coli* (STEC) as an important pathogen has been related to severe outbreaks since the 1980s in the world. Several foods such as raw milk, meat and their products, vegetables, fruits, and unpasteurised juices have been incriminated in STEC outbreaks [1, 2].

Contamination of various foods with *S. aureus* is an important cause of food intoxication. The expansion of disease after consumption, in the presence of bacterial toxins cells above a certain threshold (10^6 CFU/mL or g), as well as the production of it, is more concerning[3]. Methicillin-resistant *S. aureus* (MRSA) is currently present in the food and a potential hazard for public health. Previous studies have reported *S. aureus* contamination of raw and cooked ready to eat foods, meat, milk, vegetables, and their related products [3-5].

Commercial soups as cooked and packed dishes have high potential for the incidence of food infection and poisoning, due to the ways with which they are used in public sites and even houses, as well as their high consumption. These soups can be contaminated by people who carry the pathogenic bacteria. In the absence of personal and food hygiene, the growth of *E. coli* and *S. aureus* in the soups may accelerate and as a result lead to the increase of toxin, which can cause food intoxication in consumers [5].

Various extracts and essential oils of plants have confirmed antimicrobial properties. The bacteriostatic and bactericide effects of extracts on the food spoilage and pathogenic bacteria have been proved [6-8]. Therefore, they can be applied to control and inhibit the growth of pathogenic bacteria and the transmission of food spoilage as natural preservatives. The antibacterial activities of extracts are attributed to their phenolic and flavonoid compounds. Thus, the higher the amount of phenolic compounds such as hydroxycinnamic acids, hydroxybenzoic acids, apigenin, flavone, chlorogenic acid, and ellagic acid hexoside in extracts, the higher their antibacterial activities will be [9, 10].

Previous studies have examined the antibacterial activities of *Bunium persicum*, *Eucalyptus globules*, and *Allium ampeloprasum* Var. *Iranicum* in vitro to inhibit the most important clinical and foodborne pathogens such as *S. aureus*, *E. coli*, *E. faecalis*, *P. aeruginosa*, *S. agalactiae*, *L. monocytogenes*, and *B. cereus* [11-14].

Onto the best of our knowledge, the antibacterial activities of BP, EG, and AAI extracts against multi-drug resistant *S. aureus* and *E. coli* in barley soup have not been investigated. Therefore, the aim of this study was to investigate the antibacterial effects of *Bunium persicum*, *Eucalyptus globules*, and *Allium ampeloprasum* Var. *Iranicum* against multidrug resistant *S. aureus* and *E. coli* in commercial barley soup during refrigerated and environmental storage.

Methods

Plant material and preparation of the extracts

Samples of *Bunium persicum*, *Eucalyptus globulus*, and *Allium ampeloprasum* Var. *Iranicum* were purchased from a reputable grocery in Kashan, Iran. Voucher specimens of the collected samples were identified and stored at the herbarium of the Research Center for Biochemistry and Nutrition in Metabolic Diseases of Kashan University of Medical Sciences, Iran.

After cleaning, it was dried in shadow and ground by a mixer. To prepare ethanolic extracts, 100 g of the dried powder of the plants was mixed with 500 cc of 80 % ethanol and kept at room temperature (22ºC) for 24 h. The obtained extracts were filtered by filter paper and entered into rotary device (to remove solvent). The obtained alcoholic extracts were dried at the temperature of 40ºC in an incubator. For the antibacterial properties, several dilutions of the extracts were done using 5% (v/v) aqueous dimethyl sulfoxide (DMSO) (*Merck* Co., *Darmstadt*, *Germany*) and sterilized by filtration through a 0.45 µm membrane filter [15].

Total phenolic compounds (TPC)

The amount of total phenolic constituents was measured in ethanolic extracts by colorimetric method using Folin-Ciocalteu. Standard solutions with concentration of 12.5, 25, 50, 62.5, 100 and 125 ppm were prepared from Gallic acid in 60 % solution. 0.1 mL of each solution was transferred to a test tube and also 0.5 mL of reagent Folin-Ciocalteu solution 10% was added, along with 0.4 mL 7.5% sodium carbonate solution added to it 3 to 8 min. Then the tubes were kept at laboratory temperature for 30 min. The optical absorption was measured at a wavelength of 765 nm by a spectrophotometer (Unico UV-2100, USA) and a standard curve was prepared. Then 0.01 to 0.02 g of the dried extract was dissolved in 60% methanol up to the volume of 10 mL. Total phenol content was determined based on Folin-Ciocalteu method with the difference of adding 0.1 mL of the extract solution instead of the standard solution. The obtained absorbance rate was posed at the standard curve and thus the total phenol content of the extract in mg/g Gallic acid equivalent was estimated [16].

Total antioxidant activity (FRAP assay)

The determination of the total antioxidant activity of plant extracts was done by iron reduction (Ferric reducing antioxidant power assay). The stock solutions included 300 µM acetate buffer pH 3.6 (3.1 g C2H3NaO2_3H2O and 16 ml C2H4O2), 10 µM 2, 4, 6-tripyridyl-s-triazine (TPTZ) solution in 40 µM HCl, and 20 µM $FeCl_3$_$6H_2O$ solution. FRAP reagent was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3$_$6H_2O$ solution. The temperature of the solution was raised to 37°C before use. 200 µl of the plant extracts (1 mg/ml) was mixed with 2800 µl of the FRAP reagent. The absorbance was measured after 30 min incubation at 37ºC in the dark condition

at 593 nm. The values were calculated from a calibration curve obtained with FeSO4-7H2O (100 - 1000 µM). Final results were expressed as µM Fe^{2+}/g extract [17].

Screening of the antibacterial activity

Test microorganisms

In this study, *E. coli* and *S. aureus* isolated from different ready to eat food samples and confirmed by polymerase chain reaction (PCR) were used. *E. coli* had both *stx1* and *stx2* genes and also was resistant to amoxicillin/clavulanic acid, ceftriaxone, ceftazidime, ampicillin, nitrofurantoin, tetracycline, kanamycin, and gentamycin[18]. *S. aureus* had sea gene (enterotoxins A gene) and was resistant to ampicillin, amoxicillin, gentamycin, oxacillin, penicillin, tetracycline, vancomycin, and methicillin (HiMedia Laboratories Pvt. Ltd, Mumbai, India).

For extracting DNA from S. aureus isolates, 1mL overnight Brain Heart Infusion (BHI) (*Merck Co*., *Darmstadt*, *Germany*) broth culture (approximately 10^8 bacteria) was centrifuged at 4,000 rpm for 5 min. The pellets were then washed in 1 mL of PBS (Phosphate buffered saline), resuspended in 0.5 mL of H_2O, and boiled (100°C) for 15 min. The cell debris was pelleted by centrifugation at 12,000 rpm for 5 min, and the supernatant containing the released DNA was transferred into a fresh micro tube. The DNA purity and concentration was measured by a NanoDrop-1000 (NanoDrop Technologies, Wilmington, DE, USA). The presence of genes encoding for the 23S rRNA (23S-F1200, 5′- AGCTGTGGATTGTCCTTTGG-3′ and 23S-R1698, 5′-TCGCTCGCTCACCTTAGAAT-3′) and *sea* (SEA-F1170, 5′-TAAGGAGGTGGTGCCTATGG-3′ and SEA-R1349, 5′-CATCGAAACCAGCCAAAGTT-3′) was detected by Multiplex PCR using specific primers (*Takapo Zist* Company, Tehran, Iran) [19, 20]. The PCR reaction mix included 2 µL of DNA, 5 µL of 10X PCR buffer, 2 µM dNTPs, 20 µM of each primer, and 2U Taq DNA polymerase, and the volume of this mix was adjusted to 50 µl with sterile water. For multiplex PCR, the amplification was carried out in a thermal cycler (Eppendorf master cycler®, MA) with the following thermal cycling profile: an initial denaturation at 94°C for 5 min was followed by 30 cycles of amplification (denaturation at 94°C for 1 min, annealing at 57 °C for 1 min, and extension at 68°C for 1 min), ending with a final extension at 72°C for 7 min. All PCR amplification products were separated on 1.5% agarose gel and visualized by staining with ethidium bromide using a UV light transilluminator. The *S. aureus* strains ATCC 6538 and 25923 were used as control strains for PCR [19].

Then, these approved bacteria were cultured at 37°C in tryptic soy broth (TSB, Merck Ink, Darmstadt, Germany) and the standard of 0.5 McFarland was adjusted with densities of 1.5 × 108 CFU/mL in PBS.

Agar diffusion susceptibility testing

Susceptibility test was performed by agar disk diffusion method. Briefly, 100 µl of bacterial suspension (1.5 × 108 CFU/mL) was spread over the entire surface of the MHA plate using a sterile swab stick. Sterile 6-mm paper

discs impregnated with 25 µL of each tested extract (100 mg/mL) were placed on the surface of inoculated plates. Trimethoprim/sulfamethoxazole (10 µg) disc as positive control was used. A sterile disc impregnated with 25 µL of sterile distilled water was used as negative control. Then, plates were incubated at 37±2°C for 24h. The microbial inhibition zone (in millimeter) was measured by a caliper. The antimicrobial activities of extracts were divided into three ranges including strong activity (inhibition zone≥20 mm), moderate activity (12 mm<inhibition zone<20 mm), and weak activity (inhibition zone ≤12 mm) [21].

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assay

The broth microdilution method was used to determine the MIC and MBC. The 96-well plastic microdilution tray was prepared by dispensing into each 95 µL well of Mueller Hinton broth (MHB) (Merck Co., Darmstadt, Germany) and also into 5 µL of each isolate suspension equivalent to 0.5 McFarland. Finally, 100µL of consecutive dilution of (serial two-fold dilutions) each extract (0.156-80 mg/mL) was added to each well. Positive and negative controls were considered as follows: positive control: 195 µL MHB containing DMSO and 5 µL of bacterial suspension without extracts, and negative control: 200 µL of MHB containing DMSO without bacterial inoculum. After mixing the samples by shaker (with the rate of 300 rpm per 20 S), they were placed in an incubator for 18-24 h at 37°C. The wells were examined according to the presence or lack of turbidity. Dilution plate of the well containing the lowest concentration of plant extracts that inhibited growth of bacteria (lack of turbidity) was determined as the MIC. Furthermore, the lowest concentration that showed no visible growth on MHA was determined as the MBC [22].

Experimental Design and Microbiological Analysis

Commercial barley soup was purchased from a local market of Kashan, Iran. It contained barley, onion, parsley, vegetable oil, yeast extract, carrot, monosodium glutamate (MSG), salt, citric acid, and spices. In accordance with the instructions of the manufacturer, dispensing the appropriate soup powder in portions of 400 mL distillated water was done, and then the samples were sterilized at 121 °C for 15 min. After cooling, *Bunium persicum* (BP) and *Eucalyptus globules* (EG) extracts (1 and 2%) were added. The soups were divided into 5 treatments as follows: 1) control sample, 2) sample with 1% BP extract, 3) sample with 2% BP extract, 4) sample with 1% EG extract, and 5) sample with 2% EG extract. Then, the samples were inoculated with *E. coli* and *S. aureus* at 6 Log_{10} CFU/mL. All samples were stored in refrigerated (4±1°C) and environmental (22±2°C) storage. The microbial tests were analyzed in 0, 1, 3, 6, and 9 days. The experiment was conducted in triplicates.

10 mL of each treatment was diluted in 90 cc of a sterile saline solution (0.9%) and homogenized in a Mixer (Seward Stomacher 400, Seward

Medical, London, UK). Then, in saline solution, decimal dilutions of soup homogenates were made and cultured on selective media for determination of *E. coli* and *S. aureus*. 100 µl of dilutions was subsequently surface cultured on Eosin-methylene blue (Merck, Germany) and Mannitol salt agar (Merck, Germany) in duplicate. Finally, plates incubated for 24-48 h at 37ºC and populations of the pathogens were determined and expressed as $\log_{10}$ CFU/mL [5, 23].

Sensory Evaluation

The overall acceptability of soup treatments was evaluated by a panel of ten trained people. A five-point hedonic scoring scale, ranging from 1 (dislike very much) to 5 (like very much), was used for overall acceptability [23].

Statistical Analysis

Data were analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) and descriptive statistics were used. The results of all experiments were expressed as the mean ± standard deviation (SD) of triplicates.

Results and Discussion

The total phenolic compounds and total antioxidant activity identified in the extracts from the leaves of *Bunium persicum*, *Eucalyptus globulus*, and *Allium ampeloprasum* Var. *Iranicum* are presented in Table 1. The three measured plants revealed significant difference in total phenolic contents ($p<0.05$). TPC in the plant extracts ranged from 4.55 to 24.05 mg gallic acid equivalents/g. *Eucalyptus globulus* had the highest phenolic content as compared with other extracts. Evaluated with FRAP method, antioxidant activity of tested extracts ranged from 11.88 to 169.33 µM Fe^{2+}/g and *Eucalyptus globulus* had the highest antioxidant activity as compared with other extracts ($p<0.05$). Ražná *et al.* (2018) reported total polyphenol content in evaluated cumin extract achieved the value of 4.22 mg GAE/g and also antioxidant activity was 1.18 mg TEAC/g when evaluated with DPPH method and 45.23 mg TEAC/g by phosphomolybdenum method [24]. Dos Santos *et al.* (2016) showed that TPC in ethanolic extract of eucalyptus was 1.93± 0.03 mg GAE/g. The extract from eucalyptus leaves contained chlorogenic acid, rutin, quercetin 3-glucuronide, and ellagic acid derivatives. They demonstrated a direct relationship between the amounts of total phenolics and the antioxidant activities in various extracts[9]. Karamian and Hosseini (2014) reported that TPC in the extract of *Allium ampeloprasum* was 6.25 ± 0.5 mg GAE/g, and results from the antioxidant activity showed that the extracts studied in DPPH radical scavenging assay are less active than ascorbic acid and BHT as synthetic antioxidants [25]. The different chemical compositions and antioxidant activities of the plant extracts might be related to agronomic and environmental conditions, genetic factors, and various types of analysis methods [6].

Antibacterial activities of the BP, EG, and AAI extracts against multi-drug resistant *S. aureus* and *E. coli* were evaluated by disc diffusion and microdilution methods in 100 mg/mL of extracts concentration. Our results indicated that BP and EG extracts have a moderate antibacterial activity against

S. aureus and *E. coli* (Table 1). Maximum antibacterial activities were seen by EG extract. But AAI extract had no effect on multi-drug resistant *S. aureus* and *E. coli.*

In agreement with our study, Karamian and Hosseini (2014) demonstrated that the leaves extract of *Allium ampeloprasum* in 100 mg/mL concentration had no inhibition zone on *H. influenza*, *P. aeruginosa*, and *S. aureus*[25]. Previous studies have demonstrated the antibacterial activities of *Cuminum cyminum*, *Eucalyptus globules*, and *Bunium persicum* against clinical and foodborne pathogens such as *S. aureus, S epidermidis, E. coli* , *E. faecalis, P. aeruginosa, S. agalactiae, L. monocytogenes,* and *B. cereus*[11, 12, 14].

Previous studies reported that various chemical compounds such as phenolic compounds and their derivatives are present in ethanolic extracts of plants, and thus these chemical components can affect multiple target sites against the bacterial cells [23, 24]. Also, these compounds could bind to membrane proteins by hydrophobic and hydrogen bonding, thus changing the permeability of the membranes [27].

Results of the inhibitory effects of BP and EG extracts at 1 and 2% on *S. aureus* and *E. coli* in commercial barley soup samples in refrigerated and environmental storage are shown in Figures 1 to 4. Based on our results, the samples treated with BP and EG extracts (1 and 2%) had population of *S. aureus* and *E. coli* significantly lower than ($P<0.05$) the control sample in

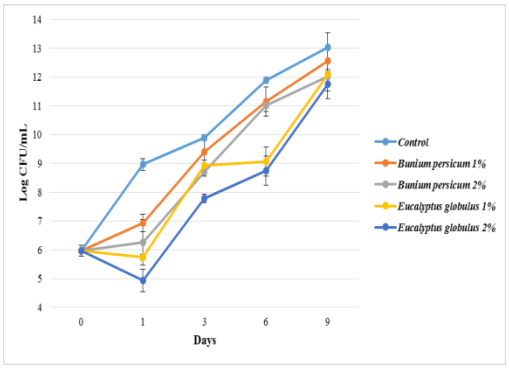

Fig 1. Antibacterial activity of *Bunium persicum* and *Eucalyptus globulus ethanolic* extracts against *E. coli* in commercial barley soup during refrigerated storage (4±1°C).

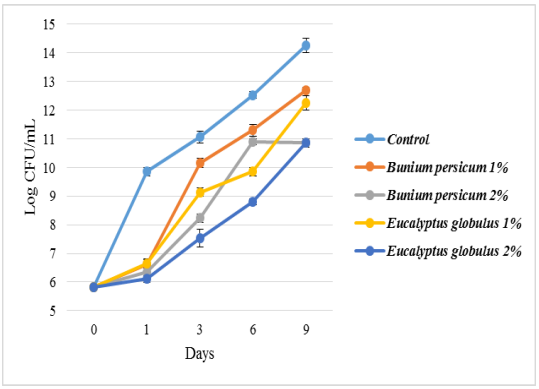

Fig 2. Antibacterial activity of *Bunium persicum* and Eucalyptus *globulus ethanolic* extracts against *E. coli* in commercial barley soup during environmental storage (22±2 °C).

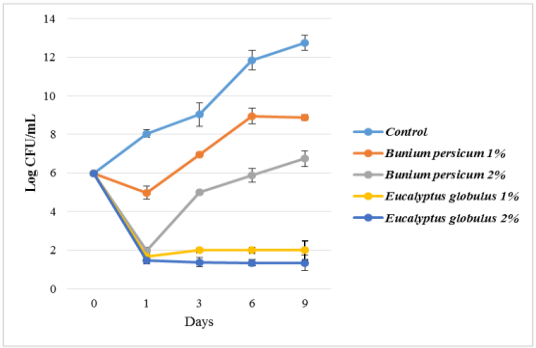

Fig 3. Antibacterial activity of *Bunium persicum* and Eucalyptus *globulus ethanolic* extracts against *S. aureus* in commercial barley soup during refrigerated storage (4±1 °C).

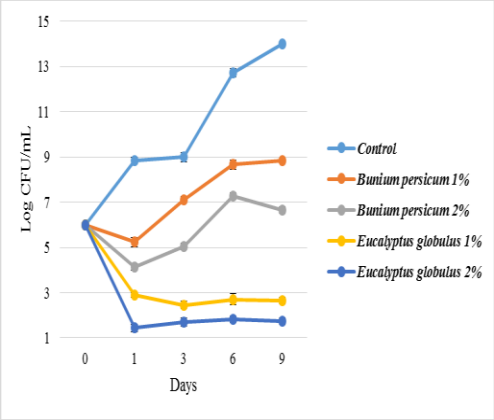

Fig 4. Antibacterial activity of *Bunium persicum* and *Eucalyptus globulus ethanolic* extracts against *S. aureus* in commercial barley soup during environmental storage (22±2 °C).

refrigerated and environmental storage. Moreover, the highest inhibition effect was found in the samples containing *S. aureus*, which were treated with EG extract (1 and 2%) in both storage. In the present study, BP extract at 1% concentration showed minimum inhibition effect on the growth of *E. coli* in both storage. From the 1st to the 9th day, *S. aureus* number at the studied temperatures in the treatments was significantly lower than that in the control ($p<0.05$). EG extract at 1 and 2% concentrations had highest inhibition effect against *S. aureus* than BP extract in both storage ($p<0.05$). from the 1st to the 9th day, *E. coli* number at the studied temperatures in the treatments was significantly lower than that in the control ($p<0.05$). However, the inhibitory effect of BP and EG extracts on the number of *E. coli* was lower as compared with *S. aureus* in treatment groups. EG extract at 2% concentration had highest inhibition effect against *E. coli* than other treatments in both storage ($p<0.05$).

Moradi and Sadeghi (2017) reported that all concentrations of *Satureja edmondi* essential oil with or without nisin significantly reduced the number of *S. aureus* at 25°C as compared with the control. In agreement with our study, they demonstrated that with increasing concentrations, the log reduction of *S. aureus* became more significant and the effect of the essential oil without nisin was stronger [5].

Shahbazi *et al.* (2016) showed the antibacterial activity of nisin, *Ziziphora clinopodioides* essential oil (0.1 and 0.2%), and their combination against *B. cereus* and *E. coli O157:H7* in commercial barley soup during refrigerated storage for 9 days. Two bacteria were significantly affected by the addition of the essential oil and nisin. In contrast with our results, they showed in control group that *E. coli O157:H7* and *B. cereus* were reduced to the value of 2.30 and 3.95 $\log_{10}$ CFU/mL, respectively, during refrigerated storage [23]. In the present study, the population of *S. aureus* and *E. coli* increased to the values of 6.77 and 5 $\log_{10}$ CFU/mL, respectively, during refrigerated storage. The results of the two mentioned studies were consistent with our results; in addition, they found that with increasing concentration of essential oil, the growth inhibitory effect increased.

Habibian Dehkordi et al. (2013) demonstrated antibacterial activities of alcoholic extract of Satureja bactiarica on food-borne pathogenic bacteria,

including *S.aureus* and *E. coli*. Moreover, the effect of this extract on meat extract at two different temperatures was evaluated. They showed a significantly different number of bacteria upon changing the temperature from 4°C to 15°C [28]. This finding is also consistent with the results of our study.

Therefore, our results showed that the combination of ethanolic BP and EG extracts and refrigerator temperature can be utilized to improve the retention and storage time of barley soup.

Similarities and differences in results of previous studies are duo to large differences between the different types of foodborne pathogenic bacteria, and there is diversity in their sensitivity to different preservatives; moreover, a wide range of food products is susceptible to bacterial contamination [5].

Table 1: The chemical components, antioxidant and antibacterial activities of *Bunium persicum*, *Eucalyptus globulus* and *Allium ampeloprasum ethanolic* extracts

Antimicrobial agent	Dry weight (%)	Total phenolic (mg Gallic acid equivalent /g)	FRAP[1] (µM Fe^{2+}/g)	MIC (mg/mL)		MBC (mg/mL)		Inhibition zone (mm) in 100 mg/mL of extracts	
				E. coli	*S. aureus*	*E. coli*	*S. aureus*	*E. coli*	*S. aureus*
Bunium persicum	7.94±0.03[a]	4.55±0.43[a]	49.67±2.08[a]	19.8	9.9	39.6	19.8	14.33±1.53[a]	12±1.73[a]
Eucalyptus globulus	11.69±0.13[b]	24.05± 0.51[b]	169.33±1.53[b]	7.22	7.22	14.44	14.44	18.5±2.12[b]	15±1.41[b]
Allium ampeloprasum	15.27±0.25[c]	9.22±0.16[c]	11.88±2.8[c]	NE	NE	NE	NE	NE	NE
Trimethoprim/sulfamethoxazole (10 µg)	-	-	-	-	-	-	-	19±1[b]	16.6±1.53[c]

1; Ferric reducing antioxidant power

NE; Not effect

Different letters a, b and c in the column indicates significant differences ($p < 0.05$).

Overall, acceptability scores of soup samples for various treatments are shown in Table 2. There were significant differences ($P<0.05$) in the acceptability of treated samples with 2% BP and EG extracts as compared with other groups. It should be noted that increase in concentrations of extracts caused a bitter taste in samples. In agreement with our results, Moradi and Sadeghi (2017) showed that among the studied samples with different concentrations of the *S. edmondi* essential oil, the soup containing essential oil with a concentration of 0.01% obtained good and fairly good scores, and its acceptability had the highest mean score 5.

Table 2. Mean rating for the overall acceptability of barley soup containing various concentrations of *Bunium persicum* and *Eucalyptus globulus* ethanolic extracts.

	Mean rating
Control	3.2 ± 0.45^{a}
1% *Bunium persicum*	2.4 ± 0.55^{a}
2% *Bunium persicum*	1.2 ± 0.45^{b}
1% *Eucalyptus globulus*	2.6 ± 0.55^{a}
2% *Eucalyptus globulus*	1.5 ± 0.55^{b}

Conclusion

Our results confirmed the antibacterial activities of *Bunium persicum* and *Eucalyptus globules* extracts to inhibit the growth of *S. aureus* and *E. coli* in commercial barley soup during storage at 4±1 °C and 22±2 °C. The highest inhibition effect of the bacteria was found in the soups treated with the 2% EG extract. Nevertheless, BP and EG extracts with 1% concentration had not adverse effects on sensory properties of barley soup. In addition, future studies are necessary to examine the accurate mechanism of action and toxicology of the mentioned extracts.

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Conflict of Interest

There is no conflict of interest in this study.

References

1. Chaleshtori FS, Arani NM, Aghadavod E, Naseri A and Chaleshtori RS. Molecular characterization of Escherichia coli recovered from traditional milk products in Kashan, Iran, Vet World. 2017; 10: 1264-1268.

2. Bier D, Silva M R, Ramos C A D N, Moriningo G D A and Silva T A D S, et al. Survey of verotoxin-producing Escherichia coli and faecal coliforms in beef carcasses destined for export at slaughterhouses in Brazil, *J Food Sci Technol.* 2018; 31: 60-66.

3. Kluytmans J. Methicillin resistant *Staphylococcus aureus* in food products: cause for concern or case for complacency, *Clin Microbiol Infect*, 2010; 16: 11-15.

4. Shafizi A, Ridzuan M, Ubong A, New C and Mohhiddin O, et al. Assessing *Staphylococcus aureus* in ready to eat (RTE) food and risk assessment of food premises in Putrajaya, *Int Food Res*. 2016; 23: 1761-1766.

5. Moradi S and Sadeghi E. Study of the antimicrobial effects of essential oil of Satureja edmondi and nisin on *Staphylococcus aureus* in commercial

soup, *J Food Process Preserv*. 2017; 41: e13337.
6. Sharafati Chaleshtori F, Taghizadeh M, Rafieian Kopaei M and Sharafati Chaleshtori R. Effect of chitosan incorporated with cumin and eucalyptus essential oils as antimicrobial agents on fresh chicken meat, *J Food Process Preserv*. 2016; 40: 396-404.
7. Ramli S, Radu S, Shaari K and Rukayadi Y. Antibacterial Activity of Ethanolic Extract of Syzygium *polyanthum L*. (Salam) Leaves against Foodborne Pathogens and Application as Food Sanitizer, *Biomed Res Int*. 2017; 2017, 9024246. doi: 10.1155/2017/9024246.
8. Mostafa AA, Al-Askar A A, Almaary KS, Dawoud TM, Sholkamy E N, et al. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases, *Saudi J Biol Sci*. 2018; 25: 361-366.
9. Dos Santos Ferreira CI, Pereyra A, Patriarca AR, Mazzobre MF, Polak T, et al. Phenolic Compounds in Extracts from Eucalyptus globulus Leaves and Calendula officinalis Flowers, *J Nat Prod Plant Resour*. 2016; 2: 53-57.
10. Ouerghemmi I, Bettaieb Rebey I, Rahali FZ, Bourgou S, Pistelli L, et al. Antioxidant and antimicrobial phenolic compounds from extracts of cultivated and wild-grown Tunisian Ruta chalepensis, *J Food Drug Anal*. 2017, 25: 350-359.
11. Takahashi T, Kokubo R and Sakaino M. Antimicrobial activities of eucalyptus leaf extracts and flavonoids from *Eucalyptus maculata*, Lett Appl Microbiol, 2004, 39, 60-64.
12. Saee Y, Dadashi M, Eslami G, Goudarzi H and Taheri S, *et al.*, Evaluation of antimicrobial activity of *Cuminum cyminum* essential oil and extract against bacterial strains isolated from patients with symptomatic urinary tract infection, *Novelty in Biomedicine*, 2016, 4: 147-152.
13. Saeedi M, Yeganegi M, Alizadeh B B, Vasiee A and Tabatabaei Y F, Antimicrobial effects of leek (*Allium ampeloprasum* l. Subsp. *Iranicum*) extract on some food-borne pathogens in vitro, *J, Food Sci, Technol*, 2017; 14: 73-82.
14. Sharafati Chaleshtori F, Saholi M and Sharafati Chaleshtori R. Chemical composition, antioxidant and antibacterial activity of Bunium persicum, Eucalyptus globulus, and rose water on multidrug-resistant Listeria species, *J Evid Based Integr Med*. 2018; 23: doi: 10.1177/2515690X17751314.
15. Hajhashemi V, Dashti G, Saberi S, Malekjamshidi P. The effect of hydroalcoholic extract and essential oil of *Heracleum persicum* on lipid profile in cholesterol-fed rabbits. *Avicenna J Phytomed*. 2014; 4: 144-150.
16. Sharafati Chaleshtori R, Rafieian Kopaei M, Rokni N, Mortezaei S, Sharafati Chaleshtori A. Antioxidant activity of *Zataria multiflora* hydroalcoholic extract and its antibacterial effect on *Staphylococcus aureus*, *J Mazandaran Univ Med Sci*. 2013; 22: 87-94.
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem*. 1996; 239, 70-76.

18. Chaleshtori FS, Arani NM, Aghadavod E, Naseri A, Chaleshtori RS. Molecular characterization of *Escherichia coli* recovered from traditional milk products in Kashan, Iran. *Vet World*. 2017; 10(10): 1264-1268.
19. Cremonesi P, Luzzana M, Brasca M, Morandi S, Lodi R, Vimercati C, et al. Development of a multiplex PCR assay for the identification of *Staphylococcus aureus* enterotoxigenic strains isolated from milk and dairy products. *Mol Cell Probes.* 2005; 19: 299-305.
20. Rahimi F, Karimi S. Characteristics of virulence factors in methicillin-resistant *Staphylococcus aureus* strains isolated from a referral hospital in Tehran, Iran. *Arch Clin Infect Dis*. 2016; 11: e33220.
21. Rota MC, Herrera A, Martínez RM, Sotomayor JA, Jordán MJ. Antimicrobial activity and chemical composition of Thymus vulgaris, Thymus zygis and Thymus hyemalis essential oils, *Food control*. 2008; 19: 681-687.
22. Al-Mariri A, Safi M. *In Vitro* Antibacterial Activity of Several Plant Extracts and Oils against Some Gram-Negative Bacteria, *Iran J Med Sci*, 2014; 39: 36-43.
23. Shahbazi Y, Shavisi N, Mohebi E. Potential application of Ziziphora clinopodioides essential oil and nisin as natural preservatives against Bacillus cereus and *Escherichia coli* O157: H7 in commercial barley soup, *J Food Saf*. 2016; 36: 435-441.
24. Ražná K, Khasanova N, Ivanišová E, Qahramon D and Habán M, Antioxidant properties of cumin (Bunium persicum Boiss.) extract and its protective role against abiotic stress tested by microRNA markers, *Potr S J F Sci*. 2018; 12: 11-19.
25. Karamian R and Hosseini DA, Screening of total phenol and flavonoid contents, antioxidant and antibacterial activities of the methanolic extract of *Allium ampeloprasum L*. (Alliaceae) from Iran, *J Sci Kharazmi Univ*. 2014; 14: 225-238.
26. Oonmetta-aree J, Suzuki T, Gasaluck P, Eumkeb G. Antimicrobial properties and action of galangal (Alpinia galanga Linn.) on *Staphylococcus aureus*. LWT Food Sci. Technol. 2006; 39: 1214–1220.
27. Gonelimali FD, Lin J, Miao W, Xuan J, Charles F, Chen M, Hatab SR. Antimicrobial Properties and Mechanism of Action of Some Plant Extracts Against Food Pathogens and Spoilage Microorganisms. Front Microbiol. 2018; 9: 1639. doi: 10.3389/fmicb.2018. 01639.
28. Habibian Dehkordi S, Gholipour S, Moshtaghi Broojeni H, Fallah A. Evaluating antibacterial effects of alcoholic extract of Satureja bactiarica on some foodborne pathogenic bacteria of meat. *Veterinary Researches & Biological Products.* 2014; 27(3): 28-37.