Slov Vet Res 2017; 54 (2): 65 - 9
UDC 615.281.9:582.742:579.852.11:641.827 **Original Research Article**

EVALUATION OF ANTIMICROBIAL EFFECT OF IRANIAN SUMAC ON *Bacillus cereus* IN A COMMERCIAL BARLEY SOUP

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Abstract: The antimicrobial effect of different concentrations of water extract of sumac (*Rhus coriaria* L.) was studied on the growth of *Bacillus cereus* (ATCC 11778) by using sterilized samples and in a single incubation temperature (30 °C) during 6 days. After obtaining and powdering sumac, its water extract was prepared. After inoculation of bacteria and adding different concentrations of water extract, the antimicrobial effect of sumac was studied on *Bacillus cereus* at several concentrations. The results showed that concentrations of 0.3%, 0.5%, 1% and 2.5% had an inhibition effect on *Bacillus cereus*, and only concentration of 0.1% could not inhibit the growth of *Bacillus cereus*. According to the finding of this research, water extract of sumac (*Rhus coriaria* L.) has a retention effect against *Bacillus cereus* in soup and can be considered as a natural preservative in some food.

Key words: antimicrobial effect; sumac; *Bacillus cereus*; commercial barley soup

Introduction

Food antimicrobials are mostly synthetic chemicals, so they are limited to use in foods, because they may cause adverse effects on the health of consumers (Wetherilt and Pala, 1994). Therefore, much attention in recent years has been focused on extracts from herbs and spices, which have been used for many centuries to improve the sensory characteristics and to extend the shelf life of foods (Chung et al, 1998). Various tanniniferous plants, including sumac (*Rhus coriaria* L.), have been known to contain naturally occurring compounds with antimicrobial activities (Nasar-Abbas and Halkman , 2004).

The word of “Sumac” is derived from Aramaic word “Sumaqa” which means red (Zargari, 1996). Sumac *(Rhus coraria* L.*)* is a member of the Anacardiaceac family and it has many applications in different countries. Sumac is a shrub with a long history application in traditional medicine and Iranian cuisine. It is used as medical herb and spices as a condiment and sprinkled over kebobs, grilled meats, soups, and some salads. It is used in traditional medicine for treatment of indigestion, anorexia, diarrhea, hemorrhages, hyperglycemia,

Received: 26 May 2016
Accepted for publication: 24 February 2017

ocular trachoma and ear infection (Wetherilt and Pala, 1994; Chung et al, 1998; Nasar-Abbas and Halkman, 2004). It is grown wild in the region from the Canary Island over the Mediterranean area to Iran and Afghanistan (Rayne et al., 2007). In Iran sumac is grown in Mazandaran, Azarbayegan, Khorasan, Shiraz, Ghazvin, Ghom and Hamedan. Sumac is used as an antioxidant and food preservative. Sumac reduces blood sugar and uric acid; these studies have shown that sumac dramatically inhibits the alpha-amylase enzyme. Alpha-amylase is responsible for the breakdown of starch to simpler sugars. Inhibition of this enzyme by sumac increases glucose-tolerance in diabetic patients (Cowan, 1999; Chung, 1998). Sumac in powder form commonly used in a variety of foods in Iran. Since *Bacillus cereus* is one of the common foodborne pathogen that cause diarrhea and vomiting in Iran, so sumac as one of the inhibitors of the growth of *Bacillus cereus* has a considerable role.

B. cereus-associated foodborne illness occurs as two distinct intoxication syndromes: emetic and diarrhoeal. Recovery is rapid for both syndromes, usually within 12-24 hours. There are usually no long-term effects, but severe consequences, including fatalities, can occasionally occur (Ahani and Alipour Eskandani, 2013). Transmission is predominantly foodborne. Most raw foods will contain *B. cereus* spores, as do many dried herbs, spices and dehydrated foods. Emetic illness is frequently linked with raw starchy foods of plant origin (such as rice, pasta, potatoes, pastries and noodles) (Kosar et al, 2007). In 95% of emetic cases, fried or cooked rice is implicated (Razavilar, 2008). Diarrhoeal illness is often associated with meat products, soups, vegetables, sauces and milk/milk products. Dairy products may spoil through the growth of spores (including the spores of psychrotrophs) that survive pasteurization (Jenson and Moir, 2003).

According to the lack of available studies on the antimicrobial effects of Iranian Sumac against *Bacillus cereus* with considering the common use of this plant as food additives in Iran, the antimicrobial effect of water extract of this medicinal plant against *Bacillus cereus* was investigated.

Material and methods

Sumac preparation

Sumac shrub fruits were collected from the orchards of the city of Ahar, Ahar Arsban, Hrand region and Rahim Bigelow village in East Azerbaijan province of Iran.

Preparations of water extracts of sumac

Crushed dried fruit after the well was crushed with a porcelain mortar to a powder income, then 5 grams of sumac powder was soaked in 95 ml of distilled water for 1 hour at room temperature with occasional stirring followed by gentle boiling for 2 min on a plate heater (the mixture was stirred intervals for one hour). Finally, the extract was obtained by cooling and filtration (Nasar-Abbasa and Kadir Halkman, 2004).

Preparations of food model (commercial barley soup)

Mahnam commercial barley soup (Mahnam Co., Karaj, Iran). to commodity number D891 was used in this study. According to the manufacturer's protocol, he contents of a packet (about 75 grams of powdered soup) dissolved in a liter of distilled water and to prepare for use were exposed in the boiling heat for 20 minutes. After cooling to 4 °C and pass the soup through a colander, in volumes of 80 ml were distributed in autoclavable glass containers and a magnet was added to each container (Alipour- Eskandani, 2009).

Microorganism and growth conditions

Bacillus cereus ATCC 11778, was donated from food microbiology laboratory of the Faculty of Veterinary Medicine of Tehran University, were used as test organisms. This bacteria in the lyophilized form were transferred into 10 ml Brain-Heart Infusion broth (BHI) and were prepared by culturing bacteria twice consecutively in BHI broth and incubating at 37 °C for 18 hours. Then the second 24 hour culture was mixed with 50% sterile glycerin and were distributed in the micro eppendorf and were stored inside the freezer (Alipour- Eskandani, 2009).

Preparation of samples to start incubation

To investigate the effect of water extract of sumac, the required quantities of water extract of sumac to produce the concentrations of 0%, 0.1%, 0.3%, 0.5%, 1% and 2.5% , was added to the glasses that contain 80 ml soup. After adding water extracts, samples were sterilized in an autoclave at 121 °C for 15 minutes. Then after autoclaved samples were cooled to ambient temperature, 10^3 *Bacillus cereus* ATCC in per ml soup (per 80 ml soup, 8×10^4 bacteria) were inoculated into the glasses that contain samples under sterile conditions under the hood. It should be noted, as mentioned bacteria in the Eppendorf microtubes - were stored inside the freezer, therefore, to use a solution containing bacteria after being thawed was transferred to a BHI broth and was stored at 37 °C for 18 hours. Again, second culture of this 18 hour culture was prepared in broth BHI (for 18 hours at 37 °C). After bacteria inoculation, the samples were transferred to a 30 °C incubator (Alipour- Eskandani, 2009).

Evaluation of samples for growth of bacteria

The growth of bacteria was assessed on day zero, 1, 3 and 6; in each of the days, 6 different dilutions (from 1-10 to 6-10) were made from each sample, then from each dilution on plates BHI agar surface culture (SPC) was performed. The amount of growth of bacteria in samples, by counting the number of colonies that is grown on the plate was seen and recorded by Colony Counter.

Statistical analysis

Because of the growth of bacteria is exponential, and over time the distribution of the number of bacteria in different samples is ousted from normal distribution. In order to normalize the data and avoid working with very large means and standard deviations, the logarithm of the number of bacteria on the basis of 10 was used in the analysis. To compare treatment means, Analysis of Variance was used in every day. To compare means changes on different days of tests Repeated measures was used. Significance level was considered P-Value>0/05. SPSS 18 statistical software was used to analyze the data.

Results and discussion

In Figure 1, the logarithm of the number of bacteria in 6 samples with different concentrations of sumac shown during testing. As this graph shows, sumac extracts have a bactericidal

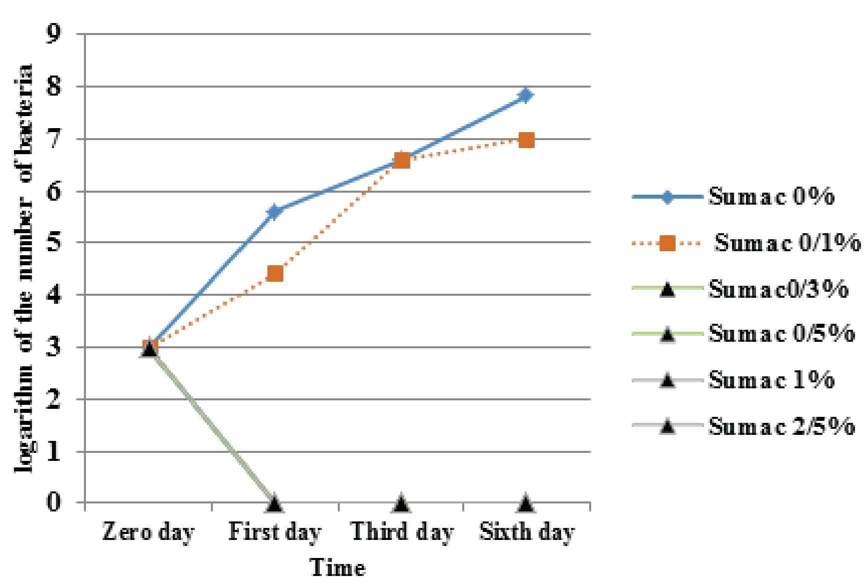

Figure 1: Comparison of effect of the different concentration of water extract of sumac on the logarithm of the number of *Bacillus cereus* during the storage period at 30 °C

effect in concentrations of 0/3%, 0/5%, 1% and 2/5%, but the bacteria have increasing growth only in concentration of 0/1% and there is not inhibitory effect. Analysis of Variance (ANOVA) showed that on the first, third and sixth, the logarithm of the number of bacteria in different treatments was statistically significant (in every three days the amount of P Value was less than 0/001), also Repeated Measures test showed logarithm changes of the number of bacteria in significant during the experiment (P Value <0.001).

Nasar-Abbas and Kadir Halkmanb in 2004 studied the antimicrobial effect of water extracts of sumac *(Rhus coriaria* L.*)* at different concentrations on the growth of 12 bacterial spp. (six Gram positive and six Gram negative bacteria), mostly food borne including pathogens. They found to be effective against all the test organisms with Gram positive bacteria being more sensitive than Gram negative bacteria. Among the Gram positive organisms, *Bacillus* species (*Bacillus cereus, Bacillus megaterium, Bacillus subtilis*, and *Bacillus thuringiensis*) were found to be the most sensitive followed by *Staphylococcus aureus*, while *Listeria monocytogenes* was found to be the least sensitive Of the Gram negative organisms, *Salmonella enteritidis* was found to be the most resistant. Bacteriostatic/bactericidal effects of sumac, as studied by enumerating survival by the viable count technique after 1 h direct contacts of each microorganism with various concentrations of sumac extract, revealed a 4–5 log cycle reduction in *Bacillus spp.* and 2–3 log cycle reduction in other bacteria tested with 1.0% sumac extract.

Moshtaghi *et al.*, 2013 investigated the antibacterial effect of ethanolic extract of Sumac *(Rhus coriaria* L.*)* on *Escherichia coli*, quantitatively and qualitatively. The results of well diffusion test showed that extracts of Sumac in a concentration of 0.5%, 1%, 2.5% and 5% could inhibit *E. coli*. The results from the evaluation of the antibacterial effects of the Sumac revealed that at 4 and 15°C, the growth of *E. coli* in test tubes containing meat extracts has increased throughout the 48 h incubation period. Results showed that the growth of this bacteria in different concentration of Sumac extract as decreased in the both tested temperatures in comparison to time zero (p<0.05). Furthermore, there was a significant difference in the number of microorganisms at various times between control and experimental groups in both tested temperatures (p<0.05). Gabr *et al.*, in 2014 studied the evaluation of *Rhus coriaria* (sumac) extracts as potential new sources of antimicrobial and antioxidant activity. The active constituents like, alkaloids, glycosides, phenol and terpenoids of sumac were extracted and separated using GC-MS. *R. coriaria* extract showed a higher content of Phenols compared to the other active constituents (glycosides, alkaloids and terpenoids). Total *R. coriaria* extract and its active constituent's phenol and glycosides were the most effective as antioxidant and antibacterial agents compared to alkaloids and terpenoids. The antibacterial activity of these compounds may relate to its total antioxidant activity. Therefore, *R. coriaria* extract and its constituents could act as bactericidal agents against bacterial infection and as a natural preservative in food against food borne diseases.

In this study antimicrobial effect of different concentrations (0%, 0.1%, 0.3%, 0.5%, 1% and 2.5%) of *Rhus coriaria* L. water extract on *Bacillus cereus* (ATCC 11778) was evaluated. The results showed this extract had an inhibition effect on the growth of *Bacillus cereus*. According to the finding of this research, water extract of sumac (*Rhus coriaria* L.) has a retention effect against *Bacillus cereus* in soup and can be considered as a natural preservative in some food.

References

1. Wetheritlt H, Pala M. Herbs and spices in digenous to Turkey. Dev Food Sci 1994: 258-307.

2. Nasar-Abbasa S.M, Kadir-Halkman. Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. Int J Food Microbiol 2004; 97: 63–9.

3. Zargari A. Medicinal plants. 6th ed. Teheran: University Press, 1992: 556-61.

4. Chung KT, Wong T, Wei CI, Huang YW, LinY. Tannins and human health: a review. Crit Rev Food Sci Nutr 1998; 38: 421–64.

5. Rayne S, Mazza G. Biological activities of extracts from sumac (*Rhus spp.*): a review. Plant Foods Hum Nutr 2007; 62: 165–75.

6. Cowan M. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4): 564–82.

7. Ahani N, Alipour Eskandani M. Detection of enterotoxigenic isolates of *Bacillus cereus* from nonenterotoxigenic in *Schizothorax zarudnyi* by PCR. Mod Genet 2013; 9: 245–9.

8. Kosar M.B, Bozan F, Temelli K. Antioxidant activity and phenolic composition of sumac (*Rhus coriaria* L.) extract. Food Chem 2007; 103(1): 952–9.

9. Jenson I, Moir CJ. *Bacillus cereus* and other *Bacillus* species. In: Hocking AD, ed. Foodborne microorganisms of public health significance. 6th ed. Waterloo, Australia : Australian Institute of Food Science and Technology Incorporated, 2003: 445–78.

10. Razavilar V. Pathogen microbial in food and epidemiology of food born intoxication. Tehran : University Press, 2008: 153–4.

11. Alipour-Eskandani M, Misaghi A, Akhondzadeh-basti A, Zahraei-Salehi T, Bokaie, S, Noori N. Effect of *Zataria multifloraboiss* essential oil on the growth of *Bacillus cereus* ATCC 11778 in a commercial barley soup. J Vet Res 2009; 64: 29–32.

12. Fazeli M.R, Ashtiani H, Ahmadian-Attari M.M, Jamalifar H, Zaheri A. Antimicrobial effect of *Rhus coriaria* L. (Sumac) total extract on skin isolates *Staphylococcus epidermidis* and *Corynebacterium xerosis*. J Med Plants 2006; 5: 27– 31.

13. Ahmadian-Atari MM, Amin Gh, Fazeli M, Jamalifar H. A review on antimicrobial activities of sumac fruit (*Rhus coriaria* L.). J Med Plants 2008; 7: 1–9.

14. Gabr SA, El-Metwally MM, Al-Gadir AH. Antioxidant and antibacterial active constituents of *Rhus coriaria*. J Biotechnol 2014; 13(2): 37–45.

15. Moshtaghi H, Abbasvali M, Mohammadi E, Safian A.R, Adel M. Investigation of antimicrobial effects of ethanolic extract of sumac (*Rhus coriaria* L.) against *Escherichia coli* in vitro. J Food Hyg 2013; 10: 1–8.

UGOTAVLJANJE PROTIMIKROBNEGA VPLIVA IZVLEČKA IRANSKEGA RUJA NA RAST BAKTERIJ *Bacillus cereus* V INDUSTRIJSKO PRIPRAVLJENI JEŠPRENJEVI JUHI

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Povzetek: Proučevan je bil protimikrobni učinek različnih koncentracij vodnega izvlečka ruja (*Rhus coriaria* L.) na rast bakterij *Bacillus cereus* (ATCC 11778) v industrijsko pripravljeni ješprenjevi juhi (ričet) z uporabo steriliziranih vzorcev pri inkubacijski temperaturi 30 °C v času 6 dni. Po pridobitvi in uprašitvi ruja je bil pripravljen njegov vodni izvleček. Po inokulaciji bakterij in dodajanju različnih koncentracij vodnega izvlečka je bil proučevan protimikrobni učinek različnih koncentracij ruja na *Bacillus cereus*. Rezultati so pokazali, da je imelo dodajanje izvlečka ruja ješprenjevi juhi v koncentracijah 0,3 %, 0,5 %, 1 % in 2,5 % zaviralni vpliv na rast bakterij *Bacillus cereus*, pri koncentraciji 0,1 % pa izvleček ruja rasti bakterije *Bacillus cereus* ni zaviral. Glede na ugotovitve raziskave ima vodni izvleček ruja (*Rhus coriaria* L.) učinek zadrževanja rasti bakterije *Bacillus cereus* v juhi in bi ga lahko uporabili kot naravni konzervans v različnih vrstah hrane.

Ključne besede: protimikrobni učinek; ruj; *Bacillus cereus*; ješprenjeva juha; ričet