Original Article

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The Effect of Water Extract of Rhus Coriaria L. on the Pathogenic Bacteria at Different Temperatures

M. Pajohi-Alamoti (PhD)*[1], M. Yadollahi-Baghloyi (MSc)[1], B. Bazargani-Gillani (PhD)[1]

1.Department of Food Hygiene and Quality Control, Faculty of Para-Veterinary Science, Bu Ali Sina University, Hamedan, I.R.Iran

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ABSTRACT

BACKGROUND AND OBJECTIVE: Nowadays, natural preservatives are used in food industries rather than synthetic ones. Sumac fruit (*Rhus coriaria L.*) is widely used as an additive in meat products due to its antimicrobial effects. This study was conducted to examine antimicrobial properties of sumac at various temperatures.

METHODS: In this experimental study, the extract of sumac was prepared using soaking method. Different concentrations (3.12-50 mg/ml) of the extract were used against S. aureus, L. monocytogenes, S. typhimurium, and E. coli O157:H7 bacteria. Growth assessment curve, minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC) were evaluated using microplate method.

FINDINGS: The results of this study demonstrated that MIC (6.25 mg/ml) and MBC (12.5 mg/ml) belonged to *S. aureus* and *L. monocytogenes* bacteria, respectively. The extract could significantly attenuate growth of the four bacteria at 4°C and 25°C ($p<0.05$). The effect of the extract on Gram-positive bacteria was significantly more than Gram-negative ones ($p<0.05$). Temperature reduction also affected the growth of the bacteria; at 4°C bacterial growth was less than 25°C, that is, at 6.25 concentration, S. typhimurium, and E. coli populations reduced from 3.55 log and 3.31 log to 2.14 log and 1.06 log, respectively.

CONCLUSION: According to our findings, water extract of sumac is a viable alternative to chemical food preservatives, particularly at 4°C.

KEY WORDS: *Antimicrobial activity, Meat products, Natural preservatives, Pathogenic bacteria, Water extract of sumac fruit.*

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***Corresponding Author: M. Pajohi-Alamoti (PhD)**

Address: Department of Food Hygiene and Quality Control, Faculty of Para-veterinary Science, Bu-Ali Sina University, Hamedan, I.R.Iran.

Tel: +98 81 34227350

E-mail: pajohi@gmail.com

Introduction

Foodborne pathogenic agents are of great importance in general hygiene that annually cause great economic losses to societies. Among these pathogens, bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* typhimurium, *and Escherichia coli O157:H7* are of particular prominence (1). *S. aureus* is a Gram-positive, facultative anaerobe organism, and the enterotoxins this bacterium produces in foods are heat resistant and are less affected by the cooking process. *S. aureus* toxins in food cause nausea and vomiting. Direct hand contact with food is associated with this type of poisoning. *L. monocytogenes* is a Gram-positive, facultative anaerobic bacterium, and since it can be found everywhere, food contamination after the cooking process is a major concern. Infection with this bacterium was also reported in the packaging procedure of food products (2). Salmonella are rod-shaped and motile with peritrichous flagella, except for *S.* pullurum and *S.* gallinarum (3).

Intestinal salmonellosis is one of the most important foodborne diseases in humans, which annually affect a great number of people (4). Salmonella is a zoonotic disease, and approximately 3.1% of food poisonings in the world are caused by this organism.

The symptoms of salmonella poisoning include headache, abdominal pain, vomiting, diarrhea, and fever that may last for two-seven days (5). *E.coli* is a Gram-negative mesophilic bacterium; intestinal pathogenic *E. coli* is the cardinal cause of diarrhea in developing countries and areas with poor living conditions. Generally, four types of E. coli are associated with diarrheal diseases in humans: EPEC (*Enteropathogenic E.coli*), ETEC (*Enterotoxigenic E. coli*), EIEC (*Enteroinvasive E. coli*), and EHEC (*Enterohemoragic E. coli*) (3).

The use of chemical preservatives in food industry can improve food shelf life. The general concern over the effects of chemical preservatives leads consumers to use products that either are preservative-free or contain natural preservatives. In the recent years, many studies were conducted on the use of natural preservatives such as essential oils and plant extracts as alternatives to chemical preservatives in foods (6). Plant derivatives, which are widely used in traditional medicine, have great potential for growth of microbial pathogens. Medicinal plants have been broadly administered in the treatment of diseases since long ago, they were also used for improving food taste, and numerous studies demonstrated the antimicrobial effect of some traditional medicine plants (7). Sumac with the scientific name of *Rhus coriaria L.* is from Dodonaea (Sapindales) order and Anacardiaceae family, which is a 1-5 meters high shrub. Its fruit is small, hard, and red or purple that goes brown after drying out. Sumac is sour and astringent tasting, and after drying, it is commonly used with meat and salads as a spice (10). Sumac grows in the Mediterranean region, and in Iran, it commonly grows in Tehran, Karaj, Qazvin, Qom, Azerbaijan, Hamedan, and Guilan (9, 11).

In the Iranian traditional medicine, sumac acts as an astringent, and it is used in the treatment of diarrhea, ear infections, and hot trachoma as well as prevention of ocular complications of smallpox(12-15). Long-term consumption of sumac reduces blood cholesterol levels (16). All parts of the plant contain tannin, protein, fat, fiber, and minerals such as potassium, calcium, magnesium, phosphorus, iron, sodium, zinc, and vitamin C. Sumac contains significant amounts of antioxidants such as tannin and procyanidin C1 as well as organic acids (e.g., malic, citric, and tartaric). Sumac's taste is mainly due to two different types of components, that is, tannin and organic acids (17, 18).

Investigations on the derivatives and compounds of sumac indicate its antioxidant and antimicrobial properties (7). Given the widespread use of this plant in foods, its beneficial role in the creation of a favorable taste, and scarcity of studies on its antimicrobial activity against food poisoning organisms, this in-vitro study was conducted to evaluate the behavior of pathogenic bacteria at different temperatures and concentrations of water extract of sumac.

Method

Extraction: In this in-vitro experimental study, the fruit of sumac was obtained from distribution centers of Hamdedan, Iran. The extraction was performed using the soaking method; first, sumac was separated from the seed and powered completely. The powder was soaked in distilled water in 1:10 ratio for 24 hours. After filtering, the extract was concentrated by rotary evaporator, and finally, it was dried in the oven and kept in a cold and dry place for later use.

The bacteria: *L. monocytogenes, S. typhimurium, E. coli*, and *S. aureus* bacteria were provided from the Food Hygiene and Quality Control Department of Tehran University of Medical Sciences. Suspension of vegetative forms of the studied bacteria was prepared by transferring lyophilized bacteria into a sterile nutrient broth (Merck, KGaA, Darmstadt, Germany), incubating them at 37°C for 18 hours, and renewing their cultures for at least two consecutive times. Bacterial strains were cultured on a nutrient agar slope (Merck, KGaA, Germany), the culture was kept at 4°C for later use.

Preparation of the bacterial suspension: McFarland standard was used to make the bacterial suspension. A colony of bacterial cultures was transferred into a sterile nutrient broth under sterile conditions and was incubated at 37°C for 18 h. This process was performed for at least two consecutive times to achieve the desired level of bacterial suspension. In continuation, different dilutions were prepared from the suspension of bacterial cultures and each dilution was compared with McFarland standard turbidity. The equal dilution with this standard was calculated through the experiment as suspension with population of 1.5×10^8 CFU/ml.

Evaluation of the antimicrobial activity: To determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC), water extract of sumac fruit was employed in microtiter plates with 96 wells (SPL, Korea) using Broth Microdilution Susceptibility Assay. For this purpose, serial concentrations of the extract were prepared using distilled water (3.12-50 mg/ml.). To each well, 160 ml of nutrient broth, 20 ml dilution of sumac extract and 20 µl of bacterial suspension containing 5×10^6 CFU/ml were added. The positive control (wells containing bacterial culture and nutrient broth) and the negative control (wells containing water extract of sumac fruit and nutrient broth) were considered in each stage of the experiment. Microtiter plates, after the addition of serial dilutions of the extract and the bacteria, were briefly mixed and incubated at 37°C for 24 hours. Thereafter, for determination of MIC, we looked at the wells to find out any turbidity.

The minimum concentration that inhibits growth or obvious turbidity in comparison to the control group was reported as the MIC (19, 20). MBC was determined according to the results of MIC; from all the wells in which bacterial growth was inhibited, 100 µl was cultured in Brain Heart Infusion (BHI) agar plates. The plates were incubated at 37°C for 24 hours. Dilutions inhibiting bacterial growth were reported as MBC (19, 21-23).

Evaluation of the growth curve: The interaction of temperature and inhibitory and sub-inhibitory concentrations of water extract of sumac was investigated against *S. aureus, L. monocytogenes, S. typhimurium*, and *E. coli O157:H7* by evaluation of growth in trypticase soy broth (TSB broth). The extract was transferred into TSB flasks containing sterile broth and concentrations of MIC and sub-MIC.

Approximately 5×10^3 CFU/ml of the bacterial suspension was injected into each dilution of the extracts in the flask, and was incubated at 4°C and 25°C for 48 hours. Evaluation of the bacterial growth was conducted on flasks of colony count at 0, 0.5, 1, 2, 3, 4, 24, 48 hours using surface culture on BHI agar medium (24, 25).

Statistical analysis: Analysis of variance (ANOVA) was performed to evaluate the effect of water extract of sumac fruit on the bacterial count using SPSS, version 17. Each of the experiments was conducted three times, and the results were reported as mean. The means were compared by Tukey's test, and $p<0.05$ was considered statistically significant.

Results

The investigation of the effect of MIC and MBC of sumac extract on the studied bacteria indicates the higher sensitivity of Gram-positive bacteria, compared to the Gram-negative ones (table 1). Bacterial population in the control sample significantly increased, as compared to sample with sumac extract ($p<0.05$).

However, within 48 hours, bacterial growth at 4°C was at lower speed compared to 25° C. The use of sub-MIC at 4°C could exponentially reduce bacterial population, compared to 25°C.

Also, at 4°C and 25°C, the inhibitory concentration of the extract at the fourth hour could significantly decrease bacterial cultures, as compared to the concentration of sub-inhibitor ($p<0.05$), and except for *E. coli*, the growth of all the bacteria was inhibited (tables 2-5).

Table 1. Minimum inhibitory concentration and minimum bactericidal concentration extract of sumac fruit based on mg/ml bacteria

Bacteria	Minimum inhibitory concentration (MIC)	Minimum bactericidal concentration(MBC)
Staphylococcus aureus	6.25	12.5
Listeria monocytogenes	6.25	12.5
Salmonella typhimurium	12.5	25
Escherichia coli	12.5	25

Table 2. Evaluation of the growth of *Staphylococcus aureus* in the minimum and sub-minimum inhibitory concentrations of waters extract of sumac at 4˚ C and 25˚ C

Temp	Concentration	Hour							
		0	0.5	1	2	3	4	24	48
4	0.00	3.3±0.19^{a}	4.27±0.07^{a}	4.36±0.08^{a}	4.44±0.05^{a}	4.94±0.05^{a}	5.33±0.04^{a}	5.94±0.1^{a}	6.89±0.8^{a}
	3.125	3.27±0.06^{a}	3.33±0.07^{b}	3.29±0.01^{b}	3.24±0.07^{b}	3.14±0.08^{b}	2.88±0.04^{b}	2.26±0.05^{b}	0.37±0.64^{b}
	6.52	3.25±0.09^{a}	3.22±0.03^{b}	3.17±0.07^{b}	3.09±0.07^{b}	2.84±0.07^{c}	2.41±0.24^{c}	0±0^{c}	0±0^{c}
25	0.00	3.35±0.05^{a}	3.46±0.05^{a}	3.58±0.02^{a}	3.72±0.0 ^{a}	4.15±0.08^{a}	5.49±0.18^{a}	6.77±0.13^{a}	7.61±0.13^{a}
	3.125	3.39±0.05^{a}	3.47±0.08^{a}	3.44±0.12^{ab}	3.37±0.1^{b}	3.21±0.03^{a}	3.16±0.04^{b}	2.36±0.06^{b}	1.31±0.16^{b}
	6.52	3.36±0.09^{a}	3.45±0.06^{a}	3.34±0.04^{b}	2.33±0.1^{c}	1.94±0.08^{c}	1.33±0.17^{c}	0±0 ^{c}	0±0 ^{c}

Lower-case letters in each column indicate significant differences at the 0.05 level.

Table 3. Evaluation of the growth of *Listeria monocytogenes* by the minimum and sub-minimum concentrations of water extract of sumac at 4°C and 25˚C

Temp	Concentration	Hour							
		0	0.5	1	2	3	4	24	48
4	0.00	4.34±0.2 ^{a}	4.45±0.24 ^{a}	4.51±0.19^{a}	4.57±0.17^{a}	5.23±0.11^{a}	5.75±0.09^{a}	6.17±0.15^{a}	6.90±0.07^{a}
	3.125	4.31±0.11 ^{a}	4.45±0.06^{b}	4.53±0.07^{b}	4.62±0.08^{b}	4.37±0.08^{b}	4.26±0.08^{b}	3.08±0.09^{b}	2.17±0.07^{b}
	6.52	4.23±0.16 ^{a}	4.45±0.09^{b}	4.48±0.07^{b}	4.57±0.08^{b}	3.74±0.06^{c}	3.45±0.06^{c}	0±0^{c}	0±0^{c}
25	0.00	3.63±0.03 ^{a}	3.7±0.02^{a}	3.85±0.08^{a}	4.68±0.13^{a}	5±0.017^{a}	5.59±0.2^{a}	6.71±0.36^{a}	8.22±0.26^{a}
	3.125	3.55±0.06 ^{a}	3.58±0.09^{a}	3.56±0.14^{ab}	3.51±0.06^{b}	3.32±0.03^{a}	3.27±0.05^{b}	2.26±0.06^{b}	1.17±0.11^{b}
	6.52	3.57±0.11 ^{a}	3.62±0.09^{a}	3.37±0.11^{b}	3±0.01 ^{c}	1.19±1.06^{c}	0.79±0.69^{c}	0±0 ^{c}	0±0 ^{c}

Lower-case letters in each column indicate significant differences at the 0.05 level.

Table 4. Evaluation of the growth of *Salmonella* typhimurium using minimum and sub-minimum inhibitory concentrations of water extract of sumac at 4°C and 25°C

Temp	Concentration	Hour							
		0	0.5	1	2	3	4	24	48
4	0.00	3.59±0.1^{a}	4.35±0.11^{a}	4.97±0.12^{a}	5.25±0.14^{a}	5.53±0.1^{a}	5.88±0.04^{a}	6.36±0.05^{a}	7.15±0.12^{a}
	6.25	3.55±0.07^{a}	3.51±0.03^{b}	3.47±0.04^{b}	3.38±0.03^{b}	3.32±0.03^{b}	3.25±0.06^{b}	3.07±0.03^{b}	2.14±0.06^{b}
	12.5	3.47±0.05^{a}	3.34±0.07^{b}	3.34±0.04^{b}	3.23±0.01^{b}	3.19±0.05^{b}	2.53±0.1^{c}	0±0^{c}	0±0^{c}
25	0.00	3.74±0.09^{a}	3.86±0.1^{a}	4.1±0.12^{a}	4.64±0.1^{a}	5.54±0.026^{a}	6.59±0.16^{a}	7.69±0.34^{a}	8.56±0.12^{a}
	6.25	3.67±0.02^{a}	3.62±0.1^{b}	3.54±0.07^{b}	3.44±0.05^{b}	3.37±0.06^{b}	3.28±0.08^{b}	2.18±0.05^{b}	1.15±0.07^{b}
	12.5	3.66±0.07^{a}	3.49±0.01^{b}	3.39±0.08^{b}	2.82±0.11^{c}	2.66±0.12^{c}	1.23±0.27^{c}	0±0 ^{c}	0±0 ^{c}

Lower-case letters in each column indicate significant differences at the 0.05 level.

Table 5. Evaluation of the growth of *Escherichia coli* using minimum and sub-minimum inhibitory concentrations of water extract of sumac at 4°C and 25°C

Temp	Concentration	Hour							
		0	0.5	1	2	3	4	24	48
4	0.00	3.33±0.04^{a}	3.81±0.14^{a}	4.5±0.04^{a}	4.52±0.07^{a}	4.69±0.05^{a}	5.04±0.05^{a}	5.85±0.08^{a}	6.87±0.06^{a}
	6.25	3.31±0.05^{a}	3.38±0.03^{b}	3.33±0.03^{b}	3.24±0.03^{b}	3.24±0.04^{b}	3.2±0.07^{b}	2.2±0.04^{b}	1.06±0.06^{b}
	12.5	3.26±0.06^{a}	3.23±0.02^{b}	3.14±0.03^{c}	3.15±0.02^{b}	3.05±0.04^{c}	2.48±0.17^{c}	0±0^{c}	0±0^{c}
25	0.00	3.6±0.11^{a}	3.94±0.07^{a}	4.37±0.09^{a}	4.84±0.15^{a}	5.56±0.022^{a}	6.7±0.25^{a}	7.67±0.15^{a}	8.69±0.21^{a}
	6.25	4.49±0.09^{a}	3.46±0.05^{b}	3.39±0.13^{b}	3.28±0.08^{b}	3.25±0.03^{b}	3.23±0.07^{b}	2.20±0.04^{b}	1.10±0.05^{b}
	12.5	3.51±0.19^{a}	3.6±0.19^{b}	3.63±0.04^{b}	2.81±0.36^{c}	2.38±0.25^{c}	1.76±0.41^{c}	0.33±0.57^{c}	0±0^{c}

Lower-case letters in each column indicate significant differences at the 0.05 level

Discussion

The results of the study showed that the Gram-positive bacteria (*S. aureus and L. monocytogenes*) were more sensitive to water extract of sumac fruit, compared to the Gram-negative ones (*E. coli and S. typhimurium*). Pandit et al. (1983) demonstrated that Gram-positive bacteria are more sensitive to herbal extracts compared to Gram-negative ones. Moreover, herbal extracts not only have inhibitory activities against bacteria, but also exhibit bactericidal properties (26). Similar studies indicated that effect of the extracts derived from plants of the traditional medicine is more intense on Gram-positive bacteria rather than Gram-negative ones (27).

In addition, Qussalah et al. reported that *S. aureus* is more sensitive to herbal derivatives, compared to microorganisms such as *E. coli* and *S. typhimurium*, due to its single-layer cell wall (28), which is in accordance with our results.

Resistance of Gram-negative bacteria to herbal extracts can be due to complexity of the bilayer cell wall of these bacteria, compared to the glycoprotein-teichoic acid cell wall of Gram-positive bacteria. In addition, the resistance of microbial cells could be secondary to the speed and the level of solution of antimicrobial compounds in lipid part of the cell membrane.

Although, this problem cannot be a definitive explanation for the difference in sensitivity of Gram-positive and Gram-negative bacteria; however, cell surface hydrophobicity can also be proposed as an effective factor (29, 30). Researchers have concluded that shelf life is improved with increasing the concentration of sumac extract and oregano; however it attenuates population of Gram-positive bacteria more than the Gram-negative ones (31). Moreover, Nasar-Abbasa et al. concluded that higher concentrations of sumac extract could eliminate pathogenic bacteria, including Gram-positive ones, more effectively (32). Considering the current results, by increasing the concentration of water extract of sumac fruit, the growth of Gram-positive reduces more, as compared to Gram-negative ones. Studies on the effective compounds of the sumac fruit showed that antimicrobial activity of the extract is due its considerable amounts of antioxidants such as tannin and procyanidin C1 (17, 18). Gulmez et al. performed a study on the effect of water extract of sumac and lactic acid on shelf life of chicken wings. They concluded that extract of sumac and lactic acid significantly diminished pathogenic bacteria, especially *psychrotrophs*, *enterobacteriaceae*, and *coliforms* in chicken wings, and the antibacterial effect of water extract of sumac was mainly due to its high level of tannin (33).

According to the former studies and the current results, it can be concluded that water extract of sumac has significant antibacterial effects and considering its tastefulness, it can be used in different types of foods as a natural preservative instead of its harmful chemical counterparts. Temperature also had a significant effect on the antimicrobial properties of the extract. Therefore, at 4°C its antimicrobial properties were much higher than 25°C, which accentuates the role of simultaneous use of various factors such as temperature for control of pathogen growth, which is known as hurdle technology.

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