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Research Article

The composition of sumac (*Rhus coriaria L.*) essential oil and its antimicrobial effect on some food borne bacteria

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ABSTRACT

Bacteria are responsible for a large part of the food poisoning. Application of natural preservatives for foods protection from microbial spoilage and to control the occurrence of foodborne pathogens has become an important issue worldwide. The aim of this study was to investigate the composition of sumac (*Rhus coriaria* L.) essential oil and its antimicrobial effect on some food borne bacteria. The antimicrobial activity of *R. coriaria* L. essential oil was tested against some foodborne bacteria including: *Pseudomonas aeruginosa, Yersinia enterocolitica, Klebsiella pneumonia, Listeria monocytogenes* and *Streptococcus pyogenes* by microdilution method. Furthermore, sumac fruit essential oil was also investigated to determine the chemical compositions by the gas chromatography (GC/MS) method. The essential oil showed a strong antimicrobial activity with concentration dependence and a broad antimicrobial spectrum for all tested bacteria species. *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were found to be the most sensitive gram positive and the most resistant bacteria with a minimum inhibitory concentration of 0.156 and 2.5 mg/ml, respectively. Sumac essential oil showed more antibacterial effect against *Streptococcus pyogenes* and *Listeria monocytogenes* isolates in comparison with other tested bacteria (p<0.05). Eleven constituents in the sumac fruit's essential oil were identified. The predominant compounds in the essential oil were trans-Caryophyllene (22.3%) and Butanedioic acid, and diethyl ester (21.01%). The current findings suggest the possibility of using the essential oil of *R. coriaria* L. as a novel source of natural antimicrobial agents for the food and pharmaceutical industries and herbal therapeutic.

1. Introduction

Food spoilage is one of the most important issues facing the food industry. In fact, food-borne illnesses are a global problem, even in developing and developed countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms. Many pathogenic microorganisms, including *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Candida* spp., *Zygosaccharomyces* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., and *Salmonella* spp. have been identified as the causal agents of

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food-borne diseases or food spoilage (Betts et al., 1999; Solomakos et al., 2008). There are many published reports that deal with the antimicrobial activities of plants essential oils. It was found that carvacrol (present in the essential oil of oregano, thyme and sumac) was active in reducing the growth of *B. cereus* and inhibiting its toxin production in brain-heart infusion (Ultee et al., 1999). Plants contain aromatic substances like phenolics, tannins, or their derivatives, which are used as defensive barriers against microbial infection or insect infestation (Cowan, 1999). Traditional uses of herbal medicinal plants to elongate shelf life of food are common in oriental countries. The natural antimicrobials and antioxidants derived from fruits, vegetables and most of the edible plants are believed to be of benefit in food preservation, therefore, the use of natural food additives is an attracting scientific field (Negi et al., 2003). Food antimicrobial compounds were added to or presented in foods that retard microbial growth or kill microorganisms. (Nasar-Abbas and Halkman, 2004). Sumac generally grows in non-agriculturally viable areas and indigenous people have utilized it for medical objectives; this shows that the bioactivity of these plants has a commercial potential without competing for food production land uses (Van Wyk and Wink, 2004). This spice is produced by grinding the dried fruit with salt, and it is usually used as a condiment; people sprinkle it over kebabs (grilled meat), salads and boiled broad beans (Nasar-Abbas and Halkman, 2004; Kossah et al., 2010). In traditional medicine, *R. coriaria* was also utilized as a herbal remedy because it has analgesic, antidiarrhetic, antiseptic, anorexic and antihypergylcaemic properties (Rayne and Mazza, 2007). The hydrophobic properties of the essential oils cause their penetration into the cell membrane lipids and increase their permeability, which disrupts all vital activity dependent on the cell membrane and ions secretion, vital compounds, and ultimately cell death (Palmer et al., 2001). Phytochemicals in *Rhus coriaria* include ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid; this content makes phytochemicals in *Rhus coriaria* useful for antibacterial, antidiarrhoea, antidysenteric, antihepatoxic, antiseptic, antispasmodic and antiviral objectivs (Abu–Shanab et al., 2005). The current research aimed at determining the composition of sumac (*Rhus coriaria* L.) essential oil and its antimicrobial effect on some food borne bacteria.

2. Materials and Methods

2.1. Plant materials and chemicals

Ripened and dried fruits of *R. coriaria* L. were collected in July 2015 from Maragheh city (East Azarbaijan province, Iran). Using a household flourmill (Asantoos, model 4000, Iran), the fruits were ground into powder; the powder was passed through a 1 mm sieve and was stored at 5°C in plastic bags. Sumac essential oil was extracted by using of 40 g of dried sample 3 h after boiling by hydrodistillation method (Saei Dehkordi et al., 2009).

2.2. Gas chromatography–mass spectroscopy

A Shimadzu gas Chromatograph (Shimadzu-2010, Japan) was used for the analysis of Gas chromatography–Mass spectroscopy (GC/MS) of the essential oil of sumac; the Shimadzu gas Chromatograph used for the analysis was equipped with a Shimadzu QP2010 Plus mass selective detector in the electron impact mode (ionization energy: 70 eV). The injection mode was split/splitless (ratio 1:50), operating at 260°C. The carrier gas role was performed by high purity helium with a flow-rate of 0.46 ml min^{-1}; the capillary column used was ZB-WAX (20 m × 0.18 mm, film thickness 0.18 µm) phenomenex, USA. The column temperature was kept at 50°C for 4 min, then heated to 240°C with a 10°C min^{-1} rate and kept constant for 2 min. The effluent of the GC column was introduced directly into the source of the MS. The column temperature programming was the same as the GC analysis. The ion source temperature was set at 200°C and the interface temperature was set at 240°C. MS was taken at 70 eV (E1), electron multiplier voltage 1800 eV; the mass range was over the range 35-530 amu and event time was 0.15 sec. Scan speed was 5000. In order to identify the essential oil components, the comparison of the mass spectrum method was employed. Standard matters presented in the references were utilized to identify the quartz index of components, whereas a mass spectrum of standard materials presented in the database of the device was employed to recognize the analogy of mass spectrum of components.

2.3. Preparation of bacterial strains

Five bacterial strains from each Gram positive bacteria, including *Listeria monocytogenes* and *Streptococcus pyogenes*, and Gram negative bacteria including *Pseudomonas aeruginosa*, *Yersinia enterocolitica* and *Klebsiella pneumonia* isolated from food (They were previously identified by specific biochemical tests and Gram staining) were used for the investigation of the antimicrobial effect of sumac essential oil. *Listeria monocytogenes* PTCC 1298, *Streptococcus pyogenes* PTCC 1240, *Pseudomonas aeruginosa* PTCC 1310, *Yersinia enterocolitica* PTCC 1151 and *Klebsiella pneumonia* PTCC 1290 were used as standard strains.

2.4. Evaluation of antimicrobial activity by microdilution method

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) methods were used for the determination of the antimicrobial effect of sumac essential oil. For this purpose, the tested bacteria were cultured in sterile Brain Heart Infusion (BHI) (Merck company, Germany) for 24 hours at 37^{oC}. The serial dilution was considered using Müller Hinton Broth (MHB, Merck company, Germany) medium from 20 to 0.39 mg/ml. MIC test was performed by microdilution method. 100 µl of Müller Hinton Broth was added to each well. Then, 100 µl of essential oil with a concentration of 20 mg/ml was poured into the first well and 100 µl was transferred from the contents of the first well to the second well; this continued to the 10th well. Therefore, dilutions of 20 to 0.39 mg/ml of essential oil were prepared. 100 µl of new bacterial culture (the equivalent of concentration of 0.5 McFarland turbidity) was added to each well and they were incubated in 37°c for 24 h. After 24 hours, the presence of turbidity (compared to the control row) indicated bacterial growth and transparency, indicating no growth of the bacteria. The lowest concentration in which no bacterial growth was observed (lack of turbidity caused by bacterial growth) was determined as the Minimum inhibitory concentration (MIC). A higher and lower concentration with a Minimum inhibitory concentration (MIC) were cultured in BHI agar medium and incubated in 37°c for 24 h. The first plate associated with the well that didn´t show bacterial colony was considered as the MBC of essential oil. The MBC was defined as the concentration in which no microorganism growth was observed (Oroojalian et al., 2010). All of tests were repeated in 3 replicates and the mean of gained results were shown as MIC and MBC. The results were analyzed by Duncan's multiple range test at 95% confidence level with SPSS software (version 21.0).

3. Results

3.1. Antimicrobial effects

Sumac essential oil showed a strong antimicrobial effect against the investigated bacteria. *Streptococcus pyogenes* and *Listeria monocytogenes* isolates were the most sensitive bacteria shown toward the essential oil with MIC of 0.156 and 0.312 mg/ml, respectively. *Pseudomonas aeruginosa* isolates were the most resistant bacteria with a MIC of 2.5 mg/ml (Table 1). Sumac essential oil showed more antibacterial effect against *Streptococcus pyogenes* and *Listeria monocytogenes* isolates in comparison with other tested bacteria (p<0.05).

3.2. Phytochemical components

The analysis of the components of sumac essential oil by the GC/MS method was showed in Table 2. The most compounds in the essential oil were trans-Caryophyllene (22.3%) and Butanedioic acid, and diethyl ester (21.01%), respectively.

Table 1. Antimicrobial activity (MIC and MBC in term of mg/ml) of essential oil of Rhus coriaria L. by microdilution method.

Bacteria	*P. aeruginosa*/ *P. aeruginosa* PTCC 1310		*Y. enterocolitica*/ *Y. enterocolitica* PTCC 1151		*K. pneumonia*/ *K. pneumonia* PTCC 1290		*L. monocytogenes*/ *L. monocytogenes* PTCC 1298		*S. pyogenes*/ *S. pyogenes* PTCC 1240	
Essential oil (mg/ml)	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Rhus coriaria L.	2.5/ 1.25	2.5/ 1.25	1.25/ 0.625	1.25/ 0.625	1.25/ 0.625	1.25/ 0.625	0.312/ 0.156	0.312/ 0.156	0.156/ 0.156	0.156/ 0.156

PTCC= Persian Type Culture Collection

Table 2. The components of sumac essential oil gained by GC/MC.

No	Name of component	Percent of Total	Retention Time (min)
1	trans-Caryophyllene	22.3	12.858
2	Butanedioic acid, diethyl ester	21.01	13.87
3	1,7-Nonadien-4-ol, 4,8-dimethyl	1.73	15.869
4	Malate	19.8	17.873
5	Tricyclo[6.3.1.02,5]dodecane-8-ol	2.03	17.941
6	Cembrene	4.93	19.198
7	Palmitate	8.01	19.808
8	9-Octadecenoic acid	2.53	21.78
9	Ethyl Linoleic acid	3.86	22.187
10	Ethyl Linoleolate	5.62	22.743
11	Phytol	6.4	22.858

4. Discussion

The findings of the current research revealed that *R. coriaria* L. fruit essential oil performed considerably well against the examined bacteria, with a MIC of ≤ 2.5 mg/ml. The results of current study showed that Gram negative bacteria were more resistant than Gram positive bacteria toward the essential oil of *R. coriaria* L (p<0.05). A comparison of MIC of the sumac fruit essential oil against the examined bacteria indicated no significant differences between the investigated Gram negative bacteria (p>0.05). The structural difference of the bacteria plays an important role in their susceptibility. A possible explanation for these observations may lie in the significant differences in the outer layers of Gram-negative and Gram-positive bacteria. Gram-negative bacteria possess an outer membrane and a unique periplasmic space not found in Gram-positive bacteria (Duffy and Power, 2001). The resistance of Gram-negative bacteria towards antibacterial substances is related to the hydrophilic surface of their outer membrane which is rich in lipopolysaccharide molecules, presenting a barrier to the penetration of numerous antibiotic molecules. It is also associated with the enzymes in the periplasmic space, which are capable of breaking down the molecules introduced from outside (Gao et al., 1999). Gram positive bacteria do not possess this type of outer membrane and cell wall structure. Antibacterial substances can easily destroy the bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm and its coagulation (Kalemba and Kunicka, 2003). Sohrabi et al (2018) showed that *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* were the most resistant to most sensitive bacteria toward essential oil of *Rhus coriaria* L. fruits (Sohrabi et al., 2018). Sanchooli et al (2012) reported that *Listeria monocytogenes* and

Escherichia coli were more resistant than *Vibrio alginolyticus* toward essential oil of *Rhus coriaria* (Sanchooli et al., 2012). Mahdavi et al (2018) showed that *Rhus coriaria* L. ethanolic extract possessed a strong antimicrobial activity with concentration dependence and a broad antimicrobial spectrum for all tested bacteria species. They showed that *Staphylococcus aureus* and *Salmonella enteric* were found to be the most sensitive Gram positive and Gram negative bacteria respectively, with a minimum inhibitory concentration (MIC) of <0.78% (Mahdavi et al., 2018). These differences can relate to differences between the examined strains of geographical or ecological origin. Naghdi Badi et al. (2004) reported that the activity of this plant may be attributed to different contents of compounds found in this plant, such as ellagic acid, gallic acid, isoquercitrin, myrecitrin, myricetin, quercitin, quercitrin and tannic acid (Naghdi Badi et al., 2004). Each of these groups has antibacterial effects against bacteria due to their toxicity and effects on bacterial enzymes (Cowan, 1999). It seems that the antimicrobial effect of *Rhus coriaria* L. is associated with high amounts of citric acid and malic acid (Wetheritlt and Pala, 1994). Radmehr et al (2011) showed that Caryophyllene is probably the most important antimicrobial component in sumac essential oil that can justify its antimicrobial properties well (Radmehr et al., 2011). The main factors that determine antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, and temperature of the environment (Syed Abdul Rahman et al., 2010). In this study, the components of the sumac fruit essential oil were determined by a GC/MS apparatus and it has been shown that trans-Caryophyllene was the predominent compound. The most important effective component in the antimicrobial effect of sumac essential oil is probably related to caryophyllene. Kurucu et al (2011) reported that the major components of *Rhus coriaria* L. leaf oil and branch/bark oil were 16.95% and 21.91% β-caryophyllene, respectively (Kurucu et al., 2011). Morshedloo et al (2018) showed that (E)-caryophyllene (5.9-50.3%) and cembrene (1.9-21.7%) were identified as the main constituents of *Rhus coriaria* L. essential oil (Morshedloo et al., 2018). Sohrabi et al (2018) demonstrated that β-caryophyllene (34.3%) and cembrene (23.8%) were the most frequently found constituents in *R. coriaria* (Sohrabi et al., 2018). Legault and Pichette (2007) showed that plant extracts containing caryophyllene have high antimicrobial activity (Legault and Pichette, 2007). Furthermore, other aldehyde components found in sumac essential oil may also have antimicrobial activities. The different results of the antimicrobial effects of *R. coriaria* L. in various studies could be related to the style of preparation of the essential oil, their used concentrations, used solvent, differences in microbial strains and the method of evaluation of the antimicrobial effect. The antibacterial properties of essential oils seem to depend on their hydrophobic properties and on the walls of the plasmid membrane of the microbes. Increasing the amount of certain ions on or inside the plasma membrane has a large impact on the driving force of the protons, the amount of intracellular ATP, and the overall activity of the microbial cells (including controlling the inflation of live cells, transferring the solubilizing material and regulating the metabolism) (Iscan et al., 2002).

Conclusion

According to the results of the current study, the sumac fruit can be used as a natural preservative and is a suitable antibacterial for use in foodstuff, such as meat. It seems that more investigations in this field need to be carried out, particularly on other pathogenic bacteria and foodstuffs.

Conflict of interests

The authors declared no competing interests.

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Refereces

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