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Antimicrobial and Antioxidant Activities of Iranian Sumac (*Rhus coriaria* L.) Fruit Ethanolic Extract

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Abstract

Rhus coriaria L. otherwise known as sumac *R coriaria*, extracts are important in drug development with numerous pharmacological reputations in the South-Eastern Anatolia, Mediterranean area and Western Asia, especially in Iran. This study aimed at assaying the antimicrobial and antioxidant activities of the Iranian sumac (*Rhus coriaria* L.) fruit ethanolic extract. The antimicrobial activity of *R. coriaria* L. fruit ethanolic extract was tested against bacterial strains, including gram positive and gram negative bacteria, derived from foodstuff by micro dilution method. Furthermore, the antioxidant activity of the ethanolic extract was investigated, including scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals. Sumac fruit essential oil was also investigated to determine the chemical compositions by the gas chromatography (GC/MS) method. The extract showed a strong antimicrobial activity with concentration dependence and a broad antimicrobial spectrum for all tested bacteria species. *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%. Sumac ethanolic extract showed a high antioxidant effect. The antioxidant property of sumac ethanolic extract was higher than BHT in all of the examined concentrations. Eleven constituents in the fruit's essential oil were identified. The predominant compounds in the essential oil were malate (39.7%), Butanedioic acid, and diethyl ester (22.01%). Our findings suggest the possibility of using the fruit of *R. coriaria* L. as a novel source of natural antimicrobial and antioxidant agents for the food and pharmaceutical industries.

Keywords: Antimicrobial; Antioxidant; GC/MS; *Rhus coriaria* L.

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Introduction

A renewed interest has occurred in the last decade to search for phytochemicals for pharmaceutical and nutritional purposes [1]. In fact, the extracts of several medicinal plants have been reported to exhibit antioxidant properties [2], as well as protective effects against the degradation of deoxyribose and DNA and hepatic oxidative stress [3]. Antioxidants that retard the oxidation process may additionally exhibit an antimicrobial activity [4]. Food antimicrobial compounds were added to or presented in foods that retard microbial growth or kill microorganisms. On the other hand, oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, color, flavor, texture and safety of foods [5]. The antimicrobial compounds found in plants are of interest because antibiotic resistance is becoming a worldwide public health concern, especially in terms of food-borne illness and nosocomial infections [6]. In general, sumac can grow in non-agriculturally viable regions and various species have been used by indigenous people for medicinal and other purposes, suggesting a potential for commercializing the bioactivity of these plants without competing for food production land uses [7]. *Rhus coriaria* L., commonly known as sumac, grows wildly in the region, which extends from the Canary Island over the Mediterranean coastline to Iran and Afghanistan. The spice, which is produced by grinding the dried fruit with salt, is used as a condiment and is sprinkled over kebabs (grilled meat) and salads, as well as over boiled broad beans [5,8]. Its sour taste

is due to the citric and malic acids content of its juice [9]. *R. coriaria* is also used as a herbal remedy in traditional medicine due to its analgesic, anti-diarrhetic, antiseptic, anorexic and anti-hypergylcaemic properties [10]. However, the extract of *R. coriaria*, which protectshumans against oxidative DNA-damage, is most notable for its antimicrobial and antioxidant activities [11,12]. Ethanol extracts of the ripe and unripe fruits of the plant have exhibited a broad range of antimicrobial activity by inhibiting the growth of Gram positive and Gram negative species, such as *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* [13]. Phytochemicals in *Rhus coriaria* are being used as antibacterial, anti-diarrhoea, anti-dysenteric, anti-hepatoxic, antiseptic, antispasmodic and antiviral purposes due to their contents, which include ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid [9]. The present work focused on determining the antimicrobial and antioxidant activities of *R. coriaria* fruit ethanolic extract.

Materials and Methods

Plant materials and chemicals

Ripened and dried fruits of *R. coriaria* L. were collected in July 2015 from Maragheh city (East Azarbaijan province, Iran). The fruits were ground into powder using a household flourmill (Asantoos, model 4000, Iran), passed through a 1 mm sieve and stored at 5°C in plastic bags. 2,2- diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (USA), whereas Butylated hydroxytoluene (BHT) and other chemicals and reagents were obtained from Merck (Darmstadt, Germany), all of which were of analytical grade. The carrier gas used in GC-MS was of the highest purity.

Gas chromatography–Mass spectroscopy

The Gas chromatography-Mass spectroscopy (GC/MS) analysis of the essential oil of sumac was carried out on a Shimadzu gas Chromatograph (Shimadzu-2010, Japan) 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. High purity helium served as the carrier gas role 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. The comparison of the mass spectrum method was used for the identification of ethanolic extract components. The quartz index of components was identified by standard matters presented in references, while the analogy of mass spectrum of components was recognized by a mass spectrum of standard materials presented in the database of the device.

Preparation of bacterial strains

Three bacterial strains from each Gram positive bacteria, including *Staphylococcus aureus* and *Bacillus cereus* and Gram negative bacteria including *Escherichia coli* and *Salmonella enteric*, isolated from food were used for the investigation of the antimicrobial effect of sumac ethanolic extract. *Staphylococcus aureus* PTCC 1112, *Bacillus cereus* ATCC 11778, *Escherichia coli* PTCC 1270 and *Salmonella enteric* PTCC 1709 were used as standard strains.

Determination of the antimicrobial effect of sumac ethanolic extract by microdilution method

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) methods were used for the determination of the antimicrobial effect of sumac ethanolic extract [14]. 100 µl of sterile Brain Heart Infusion (BHI) was poured into each micro pellet (from No. 2-10). Then, 100 µl sumac ethanolic extract was poured into the first and the second micro pellet and 100 µl extract was poured from the second well to a third well, this continued to the 10th well. Therefore, a dilution of 100-0.39% of extract was prepared. 100 µl of new bacterial culture (the equivalent of concentration of 0.5 McFarland test) with 1:100 diluted ratio was added to each well. Then, 30 µl of resazurin index was added to all of the wells. The well that showed a color change was the extract MIC. The well had changed its color with two wells, before and after it had been cultured in BHI agar medium, was incubated in 37°C for 24 h. The first plate associated with the well that didn´t show bacterial colony was considered as the extract MBC. The MBC was defined as the concentration in which no microorganism growth was observed.

Evaluation of antioxidant activity of sumac ethanolic extract (free radicals inhibition) by DPPH method

Evaluation of total antioxidant activity was performed via an inactivation of the produced free radicals by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) and vanishment of dark violet color of this matter. 500 µM of methanolic solution of DPPH was prepared. Then, different concentrations of BHT as the reference antioxidant were prepared and 4 ml from each concentration was transferred to a glass tube and mixed with 1mL DPPH solution. At the end, after 30 min, the absorption of the solution was performed in 517 nm wavelength by a spectrophotometer device. This test was also performed for the synthetic antioxidant of BHT. Different concentrations of extract were prepared and a radical scavenger antioxidant (RSA%) was calculated by using the above-mentioned method and according to the formula below:

$RSA\% = (A_c - A_s)/A_c \times 100$

A_c = Control absorption

A_s = Sample absorption

Results and Discussion

Sumac ethanolic extract showed a strong antimicrobial effect against the investigated bacteria. *Staphylococcus aureus*, *Salmonella enteric*, *Bacillus cereus* and *Escherichia coli* isolates were the most to the least sensitive bacteria shown toward the ethanolic extract, respectively. *E. coli* PTCC1270 showed the most resistance toward ethanolic extract among the examined standard strains **(Table 1)**.

Sumac ethanolic extract showed a high antioxidant effect. The antioxidant properties of the sumac ethanolic extract were higher than BHT in all of the examined concentrations. The antioxidant effect increased with the increase of concentration in both ethanolic extract and BHT **(Table 2)**.

The analysis of the components of sumac ethanolic extract by the GC/MS method was as follows **(Table 3).**

The results showed that *R.coriaria* L. fruit ethanolic extract had an appreciable activity against the examined bacteria, with an MIC of lower than 1% (0.78%). A comparison of MIC of the sumac fruit ethanolic extract against the examined bacteria showed that there were no significant differences between the investigated bacteria($P>0.05$). *E. coli* was found to be more resistant than the Gram positive bacteria, and similar observations were made on the *R. coriaria* fruit extract [5,15].This result was in accordance with the results of Abu-Shanab [9], who also reported that *R.coriaria* has strong antibacterial activity. Similar observations were also shown by Nasar-Abbas and Halkman [5].

The structural difference of the bacteria plays an important role in their susceptibility. Gram negative bacteria possess an outer membrane surrounding the cell wall, which restricts the diffusion of hydrophobic compounds through its covering of lipo polysaccharides [14]. In the current study, *S.aureus* showed the most sensitivity toward the *R.coriaria* L. fruit ethanolic extract among the examined bacteria. This result is not in agreement with the experimental results of Fazeli et al. [16], who reported that *B.cereus* was the most sensitive bacterium toward the hydroalcoholic extract of *Rhus coriaria* L. These differences can

Table 1 Antimicrobial effect of sumac ethanolic extract against examined bacteria.

Extract(%) / Bacterium	≥25	12.5	6.25	3.12	1.56	0.78	0.39
E.coli PTCC 1270	-	-	-	-	-	+	+
E.coli	-3	-3	-3	-3	-3	+2	+2
S.enteric PTCC 1709	-	-	-	-	-	-	-
S.enteric	-3	-3	-3	-3	-3	-3	+2
S.aureus PTCC 1112	-	-	-	-	-	-	-
S.aureus	-3	-3	-3	-3	-3	-3	+1
B.cereus ATCC11778	-	-	-	-	-	-	-
B.cereus	-3	-3	-3	-3	-3	+1	+1

(+) Growth of bacterium, (-) No growth of bacterium. ATCC= American Type Culture Collection. PTCC= Persian Type Culture Collection

Table 2 Comparison of antioxidant effect of sumac ethanolic extract with BHT.

Concentration (ppm) / Sample	50	100	200	300	400	500	1000
Extract(%)	79.66	93.1	93.65	94.08	94.64	94.88	95.25
BHT(%)	77.97	90.41	92.53	92.86	93.24	93.51	93.75

Table 3 The components of sumac ethanolic extract gained by GC/MC.

No	Name of component	Percent of Total	Retention Time(min)
1	trans-Caryophyllene	7.84	12.858
2	Butanedioic acid, diethyl ester	22.01	13.87
3	1,7-Nonadien-4-ol, 4,8-dimethyl	1.06	15.869
4	Malate	39.7	17.873
5	Tricyclo[6.3.1.02,5]dodecane-8-ol	1.18	17.941
6	Cembrene	5.84	19.198
7	Palmitate	7.64	19.808
8	9-Octadecenoic acid	2.61	21.78
9	Ethyl Linoleic acid	4.04	22.187
10	Ethyl Linoleolate	6.32	22.743
11	Phytol	1.76	22.858

relate to differences between the examined strains of geographical or ecological origin. Badi et al. [17] 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. Each of these groups has antibacterial effects against bacteria due to their toxicity and effects on bacterial enzymes [18]. It seems that the antimicrobial effect of *Rhus coriaria* L. is associated with high amounts of citric acid and malic acid [19]. The naturally occurring compounds in spices, such as sulphur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides, have shown antimicrobial functions. 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 [20]. In the current study, the antioxidant effect of *R.coriaria* L. fruit ethanolic extract was more than BHT. This result is not in agreement with the results of Raodah et al. [21], who reported the antioxidant activity of *R.coriaria* was lower than BHT. The DPPH free radical scavenging activity of the extract increased in accordance with the increase of the extract concentration. This result is in agreement with the results of Raodah et al. [15,21]. Kosar et al. [22] reported that the sumac fruit extract contains various amounts of metabolites with strong antioxidant properties, such as phenolic compounds and anthocyanin. In this study, the components of the sumac fruit ethanolic extract were determined by a GC/MS apparatus. The most important effective component in the antimicrobial effect of sumac ethanolic extract is probably related tocaryophyllene. Legault and Pichette [23] showed that plant extracts containing caryophyllene have high antimicrobial activity. Furthermore, other aldehyde components found in sumac ethanolic extract may also have antimicrobial activities.

Conclusion

The different results of the antimicrobial effects of *R.coriaria* L. in various studies could be related to the style of preparation of the extract, their used concentrations, used solvent, differences in microbial strains and the method of evaluation of the antimicrobial effect. 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.

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