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The Antioxidant and Antimicrobial of Syrian Sumac (*Rhus coriaria*) Fruit Extracts

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Abstract
This study aimed at assaying the antioxidant activities of Syrian sumac (*Rhus coriaria*) . The proximate analysis revealed that Syrian sumac contains higher percentage of fat , carbohydrate , protein and ash 18.74 , 71.21 , 4.69 and 2.93 % respectively . All extracts 1,4 and 2 showed antioxidant effects compared with α- tocopherol . But the antioxidant effects of all extracts was low compared with BHT . While the extracts 3 has no effect in preventing lipid peroxidation 37.87% at 5 mg / ml after 1 day . The antimicrobial activity of *Rhus coriaria* extracts were tested against six strains including three Gram –positive and three Gram –negative . *Bacillus subtilis* was found to be the most sensitive Gram –positive with MIC of 0.5 mg / ml , while Gram –negative bacteria were affected by higher concentrations of sumac extracts ranging 10-20 mg / ml . Among bacteria , the inhibitory effects was increased with the increase of *R . coriaria* fruit extracts concentration from 0.1 to 20 mg / ml . The findings demonstrate that sumac can be used as a natural antioxidant and antimicrobial .

Introduction
One of the major advanes in human history is the ability to preserve food and inhibit food spoilage by using preservating technique (namely food antimicrobials and antioxidants) .The number of contributions to isolation methods , techniques and testing activity of plant – origin antioxidant have significantly increased in recent year (1 ; 2) . Food antimicrobials are compounds were added to or presented in foods that retared microbial growth or kill microorganisms . On the other hand the oxidation is one of the major causes of chemical spoilage , which resulting in rancidity and / or deterioration of the nutritional quality , colour , flavor , texture and safety of foods . There is an increasing interest a bout in the industry and in scientific research for spices and aromatic herbs an the recently because of their strong antioxidant and antimicrobial properties . Although many compounds have already approved for use in food as antimicrobials and antioxidants , but the researches about find greater number of these compounds are still interesting because most of the traditional , currently approved food antimicrobials and antioxidants have limited applications due to food compound interactions . The interaction with food components lower the ability of food antimicrobials , antioxidants to inhibit microorganisms and chemical spoilage of food products and a good food antimicrobial , antioxidant agent should have at least such interactions . It should be also non- toxic , non – allergenic , cheap , and stable to any process to which it is exposed (3; 1 ; 2).
Sumac is the common name for agenus (Rims) that contains over 250 individual species of flowering plants in the family Anacardiaceae , which is the name that as given to numerous shrubs and small trees . They have a milky or resinous juice , simple or compound leaves, small flowers , with the parts in fours or sixes and small dry , one seeded , often hairy , sometimes highly coloured fruits , usually in dense clusters 4 ; 5) . This plant is reported to posses hydrolysable tannins , gallotannins , volatile oil , flavonoids , anthocyanin , gallic acid , flavones , such as myricetin , quercetin and kaempferol , moisture , oil , protein, fiber and ash (6) .
Rhus coriaria L . commonly known as Sumac (also spelled as Sumach) grows wildly in the region that extending from the canary Island over the Mediterranean coastline to Iran and Afghanistan . It is native to Mediterranean and South eastern Anatolian region of Turkey . The name is derived from " Sumaga" which mean red in Syriac . The spice which produced by grinding the dried fruit with salt , is used as a condiment and sprinkled over kebabs (grilled meat) and salad as well as over the boiled broad beans . In addition , it is also a principal ingredient of Za'atar , the popular spice mixture of dried and ground leaves of Origanum Syriacum , powderd seed coats of *R .coriaria* as acidulant and responsible for the typical red color , roasted sesame seeds , salt and olive oil . Treatment of diarrhea is reported as the main medicinal use of this species in Gorden . In different historical records from the area of Bilad Al- sham (a historical geographical term by former Arab rulers that included significant parts of present – day Syria , Lebanon , Palestine and Gorden) . *R .coriaria* was used as antibacterial , antidiarrheic , antidysenteric , antihepatoxic , antiseptic , antispasmodic , antiviral , astringent , candidicide , hepato protective , hepatotonic , protisticide , analgesic , antigastric , anti – inflammatory , antioxidant , antulcer , fungicide , cyclo oxygenase – inhibitor and lipoxygenase inhibitor(3 ; 7 ; 8) .
The aim of this study is to determined the chemical composition of sumac , extracted sumac by using different solvents and studied the antioxidant and antimicrobial activities to establish the relationship between them the chemical composition and antioxidant , antimicrobial activities and sumac extracts .

Material and Methods

Plant material

Sumac (*Rhus coriaria* L ., *Anacardiaceae*) is commercially a vailable in the Basrah local markets .To avoid added salt the mature and dry fruits (brown red in color) were purchased from a local market and were cleaned by removing other plant debris . The fruits were ground into powder by using home mixer and the course pieces of plant material were reground and stored at 5c◦ for further use .

Chemical composition

The chemical composition (moisture , ash and fat) of sumac was determined according to A.O.A.C. (9) ,while the protein was determined according to semi micro kjeldahl (10) .

Extraction of crude phenols

The fractionation of crude phenols was carried out according to the methods of kosar *et al* . (11) . Plant material (10 g) was extracted with hexane and using a soxhlet apparatus for 8h . After drying , defatted plant material (3g) was extracted with 40 ml of 70 % (v/v) aqueous method using magnetic stirrer at 40 c◦ for 30 min and filtered . This extraction step was repeated three times using the same batch of starting material . The filterates were combined and methanol was evaporated at 40 c◦ using a rotavapor until dryness (extract 1) . The solid residue was dissolved in 75 ml of water and extracted with 75 ml ethyl acetate three times . The ethyl acetate phases were combined and evaporated under vaccum at 40 c◦ using a rotavapor until dryness (extract 2) . The aqueous phase remaining after ethyl acetate extraction was lyophilized (extract 3) .

Hydrolysis of phenols

Defatted plant material (5g) was mixed with 150 ml of 1.2 M HCl in 50% (v/v) aqueous methanol for 1 h using magnetic stirrer at 80 c◦ .The extract was cooled and filtered them methanol was evaporated .The aqueous phase was extracted with 75 ml of ethyl acetate three times . Ethyl acetate phases were combined and evaporated using a rotavapor at 40 c◦ until dryness (extract 4) . All samples were kept in freezer at (-18 +-2c◦) after preparation until assayed .

Total phenolics

Total phenolics of sumac fractions was determined according to the Folin – Ciocaltaeu procedure(12) . 0.5 ml of distilled water and 0. 125 g of the extracts were added to test tube , followed by addition of 0.125 ml of Folin – Ciocaltaeu reagent . They were mixed well and then allowed to stand 6 min before 1. 25 ml of a 7 % sodium carbonate solution was added . The mixture was diluted to 3 ml with distilled water . The colour was developed for 90 min at room temperature and the absorbance was measured at 760 min using a spectrophotometer . The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as mg of gallic acid equivalents per gram for the extracts

Determination of antioxidant activity

The antioxidant activity was estimated according to the method ferric – thiocyanate and was described by (11) . All extracts and commercial antioxidant BHT(butylated hydroxy toluene) l ml (1-5 mg / ml) , 4 ml of 2.5 % linoleic acid in 99% ethanol , 8 ml of 0.05 M phosphate buffer (pH 7.0) and 4 ml of distilled water were put in test tubes with a screw cap and placed in an oven at 40 c◦ in the dark for 24 hr . To 0.1 ml of samples a solution of 9.7 ml of 75 % (v/v) ethanol and 0.1 ml of 30% (w/v) ammonium thiocyanate were added . Precisely 3 min after the addition of 0.1 ml of 0.02M ferrous chloride in 3.5 % HCl to the reaction mixture , the absorbance of the mixture with red colour developed was measured at 500 nm using a spectrophotometer .

The % Inhibition of lipid peroxidation was calculated by the following equation

% Inhibition = 100[(A_1/ $A_\circ$) ×100]

Where $A_\circ$ is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample .

Determination of antimicrobial activity

The antimicrobial activity was determined according to the procedure (13). Six bacterial strains (three Gram positive and three Gram negative) were supplied by the department of Food Science and Biotechnology . Gram positive species were *Bacillus subtilis* , *Staphylococcus aureus* , *Micrococcus roseus* , while the Gram negative species were *Escherichia coli* , *Salmonella sp*., *pseudomonas aeruginosa*. Stock cultures were kept in a refrigerator (4c°) on Nutrient Agar slants . A loopful from the pure slant stock of cultures were transferred into tubes containing 5ml Muller Hinton Broth and incubated at 35 c◦ for 24 hr . Serial dilutions were made to reach an inoculum concentration of about 10^5 CFU / ml to be used as a working culture against the effect of sumac extracts on the growth of bacteria . 0.1 g from each extracts were prepared in 1 ml sterile Muller Hinton broth , 0.1 ml were poured into petri plates with 0.1 ml from each culture , then Muller Hinon agar was added and the mixture was homogenized immediately . When the agar was solidified , the plates were incubated at 37 c◦ for 24 hr . Control sample was the media plus the bacterium without extracts . The results were expressed as the percentage inhibition from the average number of the colonies without extract – the average number of colonies plus extract / the average number of colonies without extract × 100.

Determination of the minimum inhibitory concentration (MIC)

Dilutions of sumac extracts were prepared in sterile Muller Hinton broth to a range concentration range of 0.1 -

20 mg / ml . Diluted bacterial cultures about 10^5 CFU / ml were added to the extract preparation , while the control was free of the extract , the mixture was homogenized and 1 ml from each mixture poured into plates . Muller Hinton agar was added , when the agar was solidified , the plates were incubated at 37c◦ for 18 hr . The MIC was defined as the concentration at which no growth of microorganism was observed

Results

A proximate composition : The proximate composition of sumac fruits presented in Table 1: The results showed that the percentage of moisture and ash were 2.43and 2.93 % respectively . The sumac found to be rich in protein, fat and carbohydrate which were 4.69 , 18.74 and 71.21 % respectively .The pH of sumac was 3.02 .

Table 1 : proximate composition of sumac fruits (% dry weight)

Moisture	Ash	Fat	Protein	Carbohydrate
2.43	2.93	18.74	4.69	71.21

The extraction yield obtained by methanol (70%) of defatted sumac was 25.77 % and the percentages (%) of ethyl acetate and water soluble fraction of total methanolic extract were 18.80 and 55.23 % Table 2. To obtain phenolics as aglycone structure , defatted sumac sample was extracted with 50% methanol containing 1.2 M HCl . After removal of methanol , the remaining aliquot phase was partitioned with ethyl acetate. The yield of ethyl acetate soluble matter of hydrolysed extract was 10.15 % . Total phenolic content of the methanol extract was 151.71 mg/g extract as gallic acid equivalent. Although ethyl acetate soluble fraction of total extract was decreased compared to that of water of fraction , total phenolic content 65 .31 mg / g extract was almost 10 times higher than that of water fraction 6. 10 mg / g extract Table 2 . Almost all phenolic compounds were extracted with ethyl acetate from total methanolic extract .

Table 2: The yield and total phenolic of Sumac extracts

extract	Yield %	Total phenolic mg /g
1	25.77	151.71
2	18.80	65.31
3	55.23	6.10
4	10.15	45.5

The effect of sumac extracts on the prevention of linoleic acid peroxidation was investigated by ferric – thiocyanate method . As seen in Fig 1 it showed that the antioxidant activity was increased with the increased of sumac extracts concentration, BHT and α - tocopherol after 1 day . Extracts 1, 4, 2 were more effective than α-tocopherol in lipid peroxidation assay , but they were not as good as BHT . 86.70 , 84.97 , 80.97 and 97 .10 % respectively at 5 mg / ml . Extract 3 has no effect in preventing lipid peroxidation 37.87% at 5 mg / ml after 1 day .

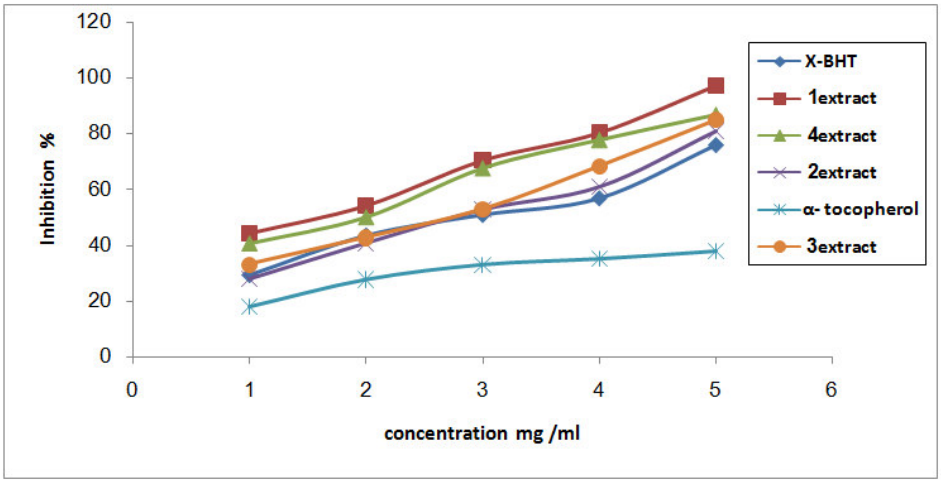

Fig 1: The antioxidant activity of Sumac extracts

Inhibitory effect

The inhibitory activity of *R. coriaria* fruit extract are shown in Table 3 . The inhibition is vary depending on bacterial species and type of extract . Methanolic extract showed excellent antibacterial activity and was remarkably greater than the other extracts . The largest percentage of inhibition was observed on extract 1 and 2 then 4 on the growth of *B .subtilis* 98.2 , 86.1 and 83.8 % respectively . No effects were detected for water extract 3 on the growth of microorganisms . *B .subtilis* was the most sensitive food –borne bacteria to sumac extracts . *Salmonella spp*. showed less sensitive to *R. coriaria* extracts 1, 2 and 4 51.7 , 50.2 and 51.4 %

respectively .

The minimum inhibitory concentration (MIC) of *R .coriaria* fruit extracts were determined against several strains including Gram – positive and Gram – negative bacteria Table 3 . Among Gram – positive bacteria *B . subtilis* was found to be the most sensitive with MIC of 0.5 mg / ml . In addition *S. aureus* and *Micrococcus roseus* ranked next with MIC of 6 gm / ml for extract 1 . While the extract 2 and 4 were found less effective on both Gram – positive and Gram – negative bacteria . Among bacteria , the inhibitory effect was increased with the increased of *R . coriaria* fruit extract concentration from 0.1 to 20 mg / ml.

Table 3: Minimum inhibitory concentration (MIC) and the antibacterial activity of sumac extracts against food – borne bacterial

Microorganism	MIC mg / ml			Inhibition activity %		
	Extract1	Extract2	Extract4	Extract1	Extract2	Extract4
Staphylococcus aureus	6	8	10	70.7	69.5	65.6
Bacillus subtilis	0.5	4	4	98.2	86.1	83.8
E. coli	10	14	12	60.7	51.5	54.3
Micrococcus roseus	6	10	10	67.8	60.7	65.9
Salmonella spp.	12	18	20	51.7	50.2	51.4
Pseudomonas aeruginosa	10	12	18	59.5	54.2	53.8

Discussion

The sumac found to be rich in protein , fat and carbohydrate . However , the fiber , fat , and protein contents exhibited by sumac fruits were higher than those reported by(5) on Syrian and Chines sumac respectively. The results indicated that the sumac fruits can be considered as potential source of dietary fiber which is helpful in alleviating gastrointestinal disorders (5) . The yield of fractions were lower than those reported by (11) on fruit sumac .The selection of suitable extraction procedure can increase the antioxidant concentration relative to the plant material . For poly phenols and other antioxidant in plant material there are three principal extraction techniques may be used extraction using solvents , solid – phase extraction and supercritical extraction . Several extraction techniques have been patented by using solvents with different polarities , such as petroleum ether , toluene , acetone , ethanol , methanol , ethyl acetate and water . However the yield of extract and resulting antioxidant activities of the plant materials are strongly depend on the nature of extracting solvent , due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent (1 ; 14) .

The results of antioxidant activities indicated that the antioxidant effects are due to phenolic OH groups . The antioxidative activity displayed by spices on other antioxidants was depend on several factors , such as the concentration , the temperature , the hydrophobic or amphipathic character, the presence of synergists , and the chemical nature of the food or medium to which they are added (15) . Soluble phenolics are present in higher concentrations in the outer tissues (epidermal and sub epidermal layers) of fruits and grains than in the inner tissues (mesocarp and pulp) (14) .

Gram – positive bacteria were generally found to be more sensitive than Gram – negative bacteria , and a similar observation was made on *R . typhina* fruit extract (16) . This tendency of phenolic compounds could be explained by that the structures of cell envelope , including cytoplasmic membrane and cell wall component , are different from Gram –positive and Gram- negative bacteria . Gram negative bacteria possess an outer membrane surrounding the cell wall , which restricts diffusion of hydrophobic compounds through its lipo polysaccharides covering . Without outer membrane , the cell wall of Gram – positive can be permeated more easily and phenolic compounds can disturb the cytoplasmic membrane , disrupt the proton motive force , electrom flow , active transport and coagulation of cell contents . Therefore , the structural difference of bacteria plays an important role in their susceptibility (17 ; 18) .

CONCLUSION

Rhus coriaria extracts showed antimicrobial and antioxidant activities , this plant might be utilized as a raw material to produce natural antioxidants and / or preservatives for the food industry . Sumac extracts can be considered as good sources of additives and / or ingredients for the food industry . Those findings would be useful for food scientists and nutritionists interested in the nutritive value of non conventional plants such as Sumac .

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