

## Evaluation of antimicrobial and antioxidant activities of Syrian sumac fruit extract

Rima Kossah<sup>1\*</sup>, Consolate Nsabimana<sup>1</sup>, Hao Zhang<sup>1</sup>, Wei Chen<sup>1</sup>

<sup>1</sup>State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, Wuxi, Jiangsu, P. R. China

\*Corresponding Author

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### ABSTRACT

The fruits of sumac (*Rhus coriaria*) are consumed as a popular spice to flavor some meals and to treat diarrhea. This study focused on investigating the antimicrobial and antioxidant activities of *R. coriaria* growing in Syria. The antimicrobial activity of Syrian sumac fruit extract was tested against various Gram-positive and Gram-negative bacteria as well as yeasts. The extract exerted a strong and concentration-dependent inhibitory effect with a very broad spectrum. However, the extract showed better activity against the tested bacteria compared to the yeasts. Minimum inhibitory concentration (MIC) of the extract against Gram-positive bacteria ranged from 500 to 1500 µg/ml, whereas the MIC with Gram-negative bacteria was higher (1000-3500 µg/ml). Nevertheless, the MIC observed for yeasts varied between 5200 and 7000 µg/ml. *Bacillus cereus* and *Helicobacter pylori* were found to be the most sensitive Gram-positive and Gram-negative bacteria with their MIC being 500 and 1000 µg/ml, respectively. The antioxidant activity of Syrian sumac fruit extract was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging capacity and reducing power. Results showed that the IC<sub>50</sub> value obtained with DPPH (0.038 mg/ml) was lower than that observed for reducing power (0.074 mg/ml). To the best of our knowledge, this is the first report on the biological activities of the extract obtained from Syrian sumac fruit. Our findings indicated that Syrian sumac fruit extract might be used as a natural agent to prevent the growth of food spoilage bacteria, particularly, *H. pylori* leading to the reduction of gastroenteritis risk.

**Keywords:** Antimicrobial activity; antioxidant capacity; *Rhus coriaria*; Syrian sumac fruit.

### INTRODUCTION

Food poisoning originating from contaminated foods by both Gram-positive and Gram-negative bacteria causes concern to the society and the industry. Due to the world awareness on chemical preservatives, the food industry is now reflected by the consumer opinions for safer additives and thus focusing on natural food additives with less or no health hazards.

Spices are herbal products which have been safely used by people around the world to impart desirable flavors and aromas to the local foods. Several of these spices and their extracts have been reported to possess antimicrobial and antioxidant activities

including thyme, basil, laurel, mint, cumin and coriander (Arora and Kaur, 1999; Delgado, et al., 2004; Wangensteen, et al., 2004).

Sumac (*R. coriaria*) is a very popular spice in Mediterranean and Arabic countries, which is produced by grinding the dried fruits. 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, Israel, Palestine, and Jordan), *R. coriaria* was used against stomach, intestine and eye diseases, animals bites and poisons, hemorrhoids, sexual diseases and pains (Lev, 2002). Recently, the fruits of *R. coriaria* growing in Syria were found to contain a high amount of phenolic compounds (Kossah, et al., 2010), while being rich in oleic and linoleic acids, vitamins, minerals as well as organic acids (Kossah, et al., 2009).

Although some studies have reported on the antimicrobial and antioxidant properties of Turkish *R. coriaria* (Nasar-Abbas and Halkman, 2004; Kosar, et al., 2007), no reports exist on the biological properties of Syrian *R. coriaria*. Therefore, the objective of this study was to determine the antimicrobial and antioxidant activities of extract obtained from the fruits of *R. coriaria* growing in Syria.

## MATERIALS AND METHODS

**Plant material and chemicals:** Ripened and dried fruits of *R. coriaria* were collected in October 2007, from Burj Islam (Lattakia, Syria). The collected fruits were preliminarily separated from their stems and screened to remove foreign matters such as dust and leaf. The fruits were ground into powder using a household flourmill (Tianjin, China), passed through 1mm sieve and stored at 4°C until further use. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma (Sigma-Aldrich), whereas other chemicals and reagents were obtained from Shanghai Chemical Co. (China) and were of analytical grade.

**Preparation of extract:** To 5g of sample were added 75ml of 20% (v/v) ethanol, and this mixture was left in a water bath at 40°C for 1h with occasional stirring. After cooling and filtration through a filter paper, the obtained extract was concentrated using a rotary evaporator (SBW-1, Shanghai Shenbo Instrument Co., China) under reduced pressure at 45°C to eliminate the solvent. The residual fraction was freeze-dried and stored at -20°C before analyses.

**Microorganisms and growth conditions:** The extract was tested against a panel of 10 microorganisms including four Gram-positive bacteria, three Gram-negative bacteria and three yeasts. Strains of *Bacillus cereus* ATCC 14574, *Bacillus subtilis*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Pichia pastoris* GS 115 and *Kluyveromyces lactis* GG 799 were obtained from Microbiology Laboratory Culture Collection, Jiangnan University. *Staphylococcus aureus* AS 1.72, *Escherichia coli* AS 1.543, and *Saccharomyces cerevisiae* AS 2.0164 were supplied by Chinese Academy of Sciences, Institute of Microbiology, while *Helicobacter pylori* SSI were donated by Shanghai Bright Dairy Company, China. Stock cultures of bacteria were kept at 4°C on Nutrient Agar slants (Glucose Peptone Yeast Agar slants for yeasts). Inocula were prepared by transferring a loopfull of each bacterial culture into 5ml of Mueller Hinton Broth and incubating at 37°C for 24h (48h for *H. pylori*). Active cultures of yeasts were generated by transferring a single pure activated colony of the yeast into a flask containing 50ml of Glucose Peptone Yeast Broth and incubated at 30°C for 48h in a flask shaker (200rpm).

**Agar diffusion assay:** A suspension of each microorganism (100µl) adjusted to 10<sup>6</sup>CFU/ml as final cell concentration was transferred onto the surface of Mueller Hinton Agar (Glucose Peptone Yeast Agar for yeasts) and spread evenly over the

entire surface of the plates. Blank discs (5mm, Oxoid, UK) were soaked in 30 $\mu$ l of extract dilution (0.5%, 1%, 3%, 5% and 7% (w/v)) prepared using 20% ethanol (v/v). The soaked discs were placed in the middle of inoculated plates and left for 1h at 4°C to allow better diffusion of the extract prior to incubation at 37°C for 24h (48h for *H. pylori*), and those spread with yeasts were incubated at 30°C for 48h. After incubation, the inhibition zones formed into the plates were measured in millimeter (mm), and each experiment was run in triplicate.

**Minimum inhibitory concentration (MIC):** The sample was first dissolved in distilled water to obtain a concentration of 1% (w/v) and serially diluted in Mueller Hinton Agar (Glucose Peptone Yeast Agar for yeasts) at about 55°C to achieve a concentration range of 300-8000 $\mu$ g/ml and the mixture was homogenized and poured immediately into plates. When the agar was solidified, a loopfull suspension of microorganism with concentration adjusted to 10<sup>4</sup>-10<sup>5</sup>CFU/ml was spot-inoculated onto the surface of agar-containing samples. The plates inoculated with bacteria were incubated at 37°C for 24h (48h for *H. pylori*), and those inoculated with yeasts were incubated at 30°C for 48h. Growth of microorganisms was visually monitored, and the MIC was determined as the lowest concentration of sample that ended with no growth of the tested microorganism. Each experiment was performed in triplicate.

**DPPH radical-scavenging activity:** The scavenging activity was tested using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical following the method of Havana et al., (1988) with slight modification. Extract dilutions were prepared (0.01-1mg/ml) and 0.3ml of each dilution was mixed with 2.7ml of methanolic DPPH solution (0.02g/L). The reaction mixture was left in the dark for 30min, and the absorbance was measured at 517nm. The DPPH scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

Where  $A_S$  is the absorbance of reaction mixture and  $A_{DPPH}$  is the absorbance of DPPH solution. The sample concentration providing 50% of inhibition (IC<sub>50</sub>) was calculated from the graph. Ascorbic acid was used as control, and all the tests were carried out in triplicate.

**Reducing power:** The reducing power was determined according to the method described by Oliveira et al., (2007). Extract dilutions were prepared (0.05-1mg/ml) and 2.5ml of each dilution were mixed with 2.5ml of 0.2M sodium phosphate buffer (pH 6.6) and 2.5ml of 1% (w/v) potassium ferricyanide. After incubation of the mixture at 50°C for 20min, 2.5ml of 10% trichloroacetic acid (w/v) were added. A volume of 5ml taken from each reaction mixture was mixed with 5ml of distilled water and 1ml of 0.1% (w/v) ferric chloride in a test tube. The absorbance was measured spectrophotometrically at 700nm. Ascorbic acid was used as control, and all the tests were performed in triplicate. The sample concentration at which the absorbance is 0.500 (IC<sub>50</sub>) was estimated by interpolation.

**Statistical analysis:** Results were subjected to the analysis of variance (ANOVA) using the SAS System for Windows, and Duncan's multiple-range test was used to compare means with a significance level of 5%.

## RESULTS

**Antimicrobial activity:** The inhibitory activity of Syrian sumac fruit extract at different concentrations against several microorganisms was assayed using the agar diffusion method and MIC. The extract showed different inhibitory capabilities towards the tested bacterial strains, with Gram-positive bacteria being more sensitive than Gram-negative bacteria. Moreover, the inhibitory effect on all bacterial strains increased significantly ( $P < 0.05$ ) with the increase of extract concentration from 0.5 to

7% (Table 1). Among Gram-positive bacteria, *B. cereus* was the most sensitive with MIC of 500µg/ml, whereas *H. pylori* were the most sensitive among Gram-negative bacteria with MIC of 1000µg/ml (Table 2). However, the MIC observed for fungal strains varied between 5200 and 7000µg/ml.

**Antioxidant activity:** The antioxidant activity of Syrian sumac fruit extract was measured by DPPH radical-scavenging capacity (Table 3) and reducing power (Table 4). The extract showed a concentration-dependent behavior with IC<sub>50</sub> value of 0.038mg/ml for DPPH scavenging activity and 0.074mg/ml for reducing power.

## DISCUSSION

**Antimicrobial activity:** Alcohol extracts from Turkish sumac fruit have been reported to show different inhibitory capabilities towards the tested bacterial strains, with Gram-positive bacteria being more sensitive than Gram-negative bacteria (Nasar-Abbas, et al., 2004). Results obtained from the present study revealed a similar trend for Syrian sumac fruit extract. Moreover, the inhibitory effect on all bacterial strains increased significantly ( $P < 0.05$ ) with the increase of extract concentration from 0.5 to 7% (Table 1). The extract exhibited a relatively low inhibitory activity against the tested fungal strains, with *P. pastoris* being the most sensitive yeast. In contrast, the inhibitory effect on fungi did not increase significantly ( $P > 0.05$ ) with the increase of extract concentration. Among Gram-positive bacteria, *B. cereus* was the most sensitive (widest inhibition zones), whereas *H. pylori* was the most sensitive among Gram-negative bacteria. These findings are of great importance as it is reported that *H. pylori* plays a casual role in chronic gastritis and peptic ulcer in humans (Waytt and Dixon, 1988; Graham, 1991). Similarly, a strong inhibitory effect on the growth of *H. pylori* was recorded recently in Chinese sumac (*Rhus typhina*) fruit extract (Kossah, et al., 2011).

The MIC values exhibited by Syrian sumac fruit extract for Gram-positive bacteria (500-1500µg/ml) were found to be lower than those observed for Gram-negative bacteria (1000-3500µg/ml) and for yeasts (5200-7000µg/ml) (Table 2). Among Gram-positive bacteria, *B. cereus* was found to be the most sensitive with MIC of 500µg/ml, which was lower than the MIC values observed for Chinese sumac (Kossah, et al., 2011) and similar to that of Iranian sumac (Fazeli, et al., 2007). However, the MIC values obtained with Syrian sumac for all tested bacteria seemed lower than those reported on the water extract of Turkish sumac (Nasar-Abbas and Halkman, 2004). Gram-negative bacteria showed less sensitivity to Syrian sumac fruit extract, except for *H. pylori*, whose MIC value was not significantly different ( $P > 0.05$ ) from that of *L. monocytogenes*. In contrast, Syrian sumac exhibited lower MIC value against *S. typhimurium* when compared to those reported on some spices and herbs (Weerakkody, et al., 2010). The antimicrobial activities of Syrian sumac fruit extract might be attributed to the synergistic action of organic acids (Kossah, et al., 2009) and phenolic compounds (Kossah, et al., 2010).

**Antioxidant activity:** DPPH (2,2-diphenyl-1-picrylhydrazyl), a stable free radical with a characteristic absorption at 517nm, has been used to evaluate the radical scavenging effects of plant extracts (Goze, et al., 2010; Ozcan, et al., 2010). As antioxidants donate protons to these radicals, the absorbance decreases. The decrease in absorbance is taken as a measure of the extent of radical scavenging capacity. As shown in Table 3, the DPPH radical-scavenging activity of Syrian sumac fruit extract increased with the increase of extract concentration. In addition, the scavenging capacity of Syrian sumac fruit extract seemed to be lower than that reported by Kossah et al., (2011) on Chinese sumac fruit extract (48.91, 88.67 and 95.42% at 0.01,

0.04 and 0.4mg/ml, respectively). It is interesting to mention that when the concentration was as low as 0.01mg/ml, the scavenging activity of Syrian sumac fruit extract was not significantly different ( $P>0.05$ ) from that of ascorbic acid. In contrast, the extract showed high  $IC_{50}$  (0.038mg/ml) compared to that of ascorbic acid used as control (0.019mg/ml). Moreover, the  $IC_{50}$  value of Syrian sumac (0.038mg/ml) was found to be lower than that of Turkish sumac (0.050mg/ml) (Bozan, et al., 2003).

As it can be seen in Table 4, the reducing power of Syrian sumac fruit extract increased with the increase of sample concentration. A similar observation was made for Chinese sumac fruit extract (Kossah, et al., 2011). In addition, a significant difference ( $P<0.05$ ) could be detected between the reducing power of Syrian sumac fruit extract and that of ascorbic acid. Nevertheless, when the concentration was 0.40mg/ml, the two reducing powers did not differ significantly ( $P>0.05$ ) from each other. Furthermore, the  $IC_{50}$  value obtained with Syrian sumac fruit extract (0.074mg/ml) was found to be higher than that of ascorbic acid (0.030mg/ml) used as control. The antioxidant and radical scavenging activities of Syrian sumac fruit extract might be due to the abounding presence of polyphenols (Kossah, et al., 2010).

### CONCLUSION

The fruit extract showed a remarkable inhibitory activity against the growth of food spoilage and/or poisoning bacteria, especially *B. cereus*. More interestingly, the extract could inhibit strongly the growth of *H. pylori*, which is regarded as a major etiological concern in gastro-duodenal disorders. On the other hand, Syrian sumac fruit extract exhibited a good antioxidative capacity. Therefore, the extract of Syrian sumac fruit can be used as a natural source of antimicrobial and antioxidant agents to preserve foodstuffs against a range of food related microorganisms.

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**Table- 1: Inhibitory effect of Syrian sumac fruit extract at different concentrations on some bacteria and yeasts.**

Microorganism	Diameter of inhibition zone (mm)				
	Concentration of Syrian sumac fruit extract (% w/v)				
	0.5	1	3	5	7
<b>Gram-positive bacteria</b>					
<i>B. cereus</i>	12.5 ± 0.5 <sup>abD</sup>	15.5 ± 1.5 <sup>aC</sup>	18.5 ± 0.5 <sup>abB</sup>	20.5 ± 0.5 <sup>aAB</sup>	22.5 ± 0.5 <sup>aA</sup>
<i>B. subtilis</i>	11.5 ± 0.5 <sup>abB</sup>	12.0 ± 0.0 <sup>bcB</sup>	13.0 ± 0.0 <sup>cbB</sup>	17.0 ± 1.0 <sup>bcA</sup>	18.5 ± 0.5 <sup>bA</sup>
<i>S. aureus</i>	9.0 ± 1.0 <sup>bcC</sup>	11.5 ± 0.5 <sup>bcBC</sup>	15.0 ± 1.0 <sup>bbB</sup>	19.5 ± 1.5 <sup>aA</sup>	22.0 ± 1.0 <sup>aA</sup>
<i>L. monocytogenes</i>	13.0 ± 0.0 <sup>abB</sup>	14.0 ± 0.0 <sup>abB</sup>	15.0 ± 0.0 <sup>bbB</sup>	18.5 ± 0.5 <sup>abA</sup>	18.5 ± 1.5 <sup>bA</sup>
<b>Gram-negative bacteria</b>					
<i>E. coli</i>	7.0 ± 0.0 <sup>cdD</sup>	10.0 ± 0.0 <sup>cdC</sup>	11.5 ± 0.5 <sup>cdB</sup>	13.5 ± 0.5 <sup>deA</sup>	14.0 ± 0.0 <sup>ca</sup>
<i>S. typhimurium</i>	7.5 ± 0.5 <sup>bcC</sup>	8.0 ± 1.0 <sup>deBC</sup>	9.5 ± 0.5 <sup>eABC</sup>	10.0 ± 0.0 <sup>fgAB</sup>	10.5 ± 0.5 <sup>da</sup>
<i>H. pylori</i>	9.5 ± 0.5 <sup>bdD</sup>	11.3 ± 0.3 <sup>bcCD</sup>	12.5 ± 0.5 <sup>cc</sup>	15.0 ± 1.0 <sup>cdB</sup>	17.5 ± 0.5 <sup>bA</sup>
<b>Yeasts</b>					
<i>S. cerevisiae</i>	9.0 ± 0.8 <sup>bcAB</sup>	11.0 ± 0.8 <sup>ca</sup>	10.3 ± 1.0 <sup>deA</sup>	7.7 ± 1.6 <sup>hb</sup>	9.3 ± 1.6 <sup>dAB</sup>
<i>P. pastoris</i>	8.3 ± 1.2 <sup>bcC</sup>	10.7 ± 0.9 <sup>cdAB</sup>	9.3 ± 0.4 <sup>eBC</sup>	11.5 ± 0.5 <sup>efA</sup>	9.3 ± 1.6 <sup>dABC</sup>
<i>K. lactis</i>	8.3 ± 0.4 <sup>bcA</sup>	7.0 ± 1.0 <sup>ca</sup>	9.3 ± 1.6 <sup>ea</sup>	8.3 ± 0.4 <sup>ghA</sup>	8.7 ± 1.2 <sup>da</sup>

- Values are expressed as mean ± SD (n = 3). Means in the same row with different capital letters are significantly different ( $P < 0.05$ ). Means in the same column with different small letters are significantly different ( $P < 0.05$ ).

**Table- 2: Minimum inhibitory concentration of fruit extract against some bacteria and yeasts.**

Microorganism	MIC (µg/ml)	
<b>Gram-positive bacteria</b>	<i>B. cereus</i>	500 ± 0.01 <sup>e</sup>
	<i>B. subtilis</i>	1500 ± 0.02 <sup>d</sup>
	<i>S. aureus</i>	1500 ± 0.01 <sup>d</sup>
	<i>L. monocytogenes</i>	1000 ± 0.03 <sup>de</sup>
<b>Gram-negative bacteria</b>	<i>E. coli</i>	3500 ± 0.01 <sup>c</sup>
	<i>S. typhimurium</i>	3000 ± 0.01 <sup>c</sup>
	<i>H. pylori</i>	1000 ± 0.01 <sup>de</sup>
<b>Yeasts</b>	<i>S. cerevisiae</i>	7000 ± 0.02 <sup>a</sup>
	<i>P. pastoris</i>	5200 ± 0.02 <sup>b</sup>
	<i>K. lactis</i>	5700 ± 0.01 <sup>b</sup>

- Values are expressed as mean ± SD (n = 3). Means with different letters are significantly different ( $P < 0.05$ ).

**Table- 3: DPPH radical scavenging activity (%) of fruit extract and ascorbic acid (control).**

Sample concentration (mg/ml)	Syrian sumac fruit extract	Ascorbic acid
0.01	34.53 ± 0.25 <sup>a</sup>	27.90 ± 2.53 <sup>a</sup>
0.02	38.78 ± 0.57 <sup>b</sup>	51.32 ± 2.61 <sup>a</sup>
0.03	46.41 ± 1.19 <sup>b</sup>	80.49 ± 2.34 <sup>a</sup>
0.04	52.62 ± 0.44 <sup>b</sup>	97.05 ± 0.11 <sup>a</sup>
0.05	61.79 ± 0.87 <sup>b</sup>	97.60 ± 0.22 <sup>a</sup>
0.1	73.42 ± 0.28 <sup>b</sup>	97.82 ± 0.01 <sup>a</sup>
0.4	95.42 ± 0.01 <sup>a</sup>	97.82 ± 0.01 <sup>a</sup>
1	95.42 ± 0.01 <sup>a</sup>	97.82 ± 0.01 <sup>a</sup>

- Values expressed as mean ± SD (n=3). Means in same row with different letters are significantly different ( $P < 0.05$ ).

**Table- 4: Reducing power (absorbance at 700nm) of fruit extract and ascorbic acid (control).**

Sample concentration (mg/ml)	Syrian sumac fruit extract	Ascorbic acid
0.05	0.34 ± 0.01 <sup>b</sup>	0.81 ± 0.02 <sup>a</sup>
0.1	0.64 ± 0.01 <sup>b</sup>	1.33 ± 0.12 <sup>a</sup>
0.2	1.03 ± 0.02 <sup>b</sup>	1.66 ± 0.02 <sup>a</sup>
0.4	1.42 ± 0.10 <sup>a</sup>	1.86 ± 0.11 <sup>a</sup>
0.6	1.60 ± 0.02 <sup>b</sup>	2.00 ± 0.07 <sup>a</sup>
0.8	1.70 ± 0.06 <sup>b</sup>	2.02 ± 0.05 <sup>a</sup>
1	1.73 ± 0.06 <sup>b</sup>	2.05 ± 0.04 <sup>a</sup>

- Values are expressed as mean ± SD (n = 3). Means in the same row with different letters are significantly different ( $P < 0.05$ ).