

Antioxidant and free radical scavenging effects of Iraqi sumac (*Rhus coriaria* L)

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Abstract:

This study was carried out to evaluate the antioxidant activity of Iraqi sumac seeds (*Rhus coriaria*. L) (*Anacardiaceae*).

Total phenolic compounds and flavonoids were determined in three different sumac seed extracts (SSE) (aqueous, ethanolic and methanolic extract). For extraction Antioxidant activity of SSE were evaluated by various antioxidant assays, including total antioxidant capacity, reducing power, by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, nitric oxide scavenging, Hydroxyl radical scavenging, and metal ion chelating activities. These various antioxidant activities were compared with ascorbic acid as a standard antioxidant.

The results showed that the three(SSE), contained large amounts of phenolic and flavonoids compounds. The results also showed that the three(SSEs) have antioxidant activities , the methanolic (SSEs) contain larger amounts of phenolic and flavonoid compounds and have a higher antioxidant activities than that of aqueous or ethanolic (SSE).

These results indicated that Iraqi aqueous and alcoholic extracts of Iraqi sumac in especial methanolic (SSE) are good scavengers for reactive oxygen species(ROS) and are a potential source of natural antioxidant, that may be used in pharmaceutical or food industry .

Key words: Antioxidant activity, free radical scavenging, Iraqi sumac (*Rhus coriaria* L.), phenolics, flavonoids..

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Introduction:

Oxidative stress, induced by oxygen radicals is believed to be a primary factor in various degenerative diseases such as cancer, atherosclerosis', gastric ulcer, diabetic and others [1, 2, 3]. Antioxidants are group of compounds that facilitate survival in plant and promote the health of human that consume variety of plant foods [4,5]. Although leaves, roots, flowers, whole plants and stems were examined to be useful phytochemicals in many research projects yet, few reports referred to seeds as source for pharmaceuticals [6,7] .Many antioxidant compound naturally occurring in plants , have been identified as a free radical or

active oxygen scavengers. Recently, interest has increased considerably toward naturally occurring antioxidants to replace synthetic antioxidants which are being restricted due to their side effects such as carcinogenicity [8].

Sumac (*Rhus coriaria* L., family *Anacardiaceae*) is one of the most widely consumed fruit in the world .It is well known popular spice and has been utilized extensively for medicinal and other purposes[9]. phytochemical studies reported that its leaves contained phenolic acids and scavenging activity [10].

Another type of sumac plant as Chinese Sumac, (*Rhus typhina* L.) is mainly used for station and gardening

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whereas its fruit can be used to prepare a kind of beverage called (Sumac-ade) used to treat gastrointestinal disorder, [11]. Also another study indicated that Syrian sumac contains higher concentrations of organic acid than Chinese sumac, [12]. Sumac (*Rhus coriaria*) is believed to have atheroprotective effect and has been consumed in some dishes [13, 14].

Previous studies indicated that the tannin (polyphenolic) and its derivatives are strong antioxidants and can inhibit mechanism leading to vascular smooth muscle cell migration (VSMC), [15, 16]. Recent studies found that pure tannin that extracted from sumac reduced (VSMC) migration by 62% [17].

Sumac is an Iranian spice used vastly in Iranian cuisine and was shown to have antimicrobial activity [18]. A study on stability of antimicrobial activity of sumac during autoclaving shows that sumac is heat stable but has interactions with salt and proteins that reduce its activity against gram-positive bacteria [19]. Other study indicated that the antioxidant activity of sumac extract might have resulted from polyphenolic constituent [20]. Sumac is traditionally used as a table spice. It is recommended for adjustment of blood lipid in diabetic patients. It was indicated that its fruit has an *in vitro* antioxidant properties [21, 22]. The aim of this research is to evaluate the antioxidant activities of Iraqi sumac seeds (*Rhus coriaria*) that may be used as a source of natural antioxidants in pharmaceutical or food industry.

Materials and Methods:

Plant Materials: Iraqi Sumac seeds (*Rhus coriaria* L.) purchased from a local market at Mosul, Iraq, identified by Botanist Agriculture College at Mosul University.

Chemicals : Ascorbic acid , 1,1-diphenyl(-2-picrylhydrazil, DPPH),

sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, 2-deoxyribose and quercetin , ferrous sulphate were purchased from sigma chemical compound., USA.-All other chemicals are of analytical reagent grade.

Methods:

Preparation of Sumac seed extract (SSE).

Three different extracts were prepared from sumac seeds using the procedure described by Badavi et al., [23] with some modifications..

Sumac seeds were milled to fine powder by using mortar. (4.2 g) of Sumac powdered seeds were macerated with (100 ml) of one of the following solvent distilled water (D.W), 20% ethanol and 70% methanol , stirred for 24 h at 5°C then filtered with cheese cloth. The resulting filtrates were used as aqueous, ethanolic and methanolic sumac seed extract. Drying each extract using lyophilizer .

The total solid percentage of each extract was determined after lyophilization. Each lyophilized extract (100mg) was dissolved in 100 ml of distilled water to determine its antioxidant activity.

Determination of Total Phenolic Content :

Total phenolic contents (TPC) of Iraqi sumac seed extracts (SSEs) were determined spectrophotometrically using mainly Folin-Ciocalteu method as described by Gao et al [24]. Sumac seed extract (100 µl) was mixed with 0.2 ml of Folin-Ciocalteu reagent, 2.0 ml of H₂O and 1.0 ml of 15% Na₂CO₃ solution. A blank sample was prepared by using the same method without addition of (SSE). The color was measured at (765 nm) after 2 hs at room temperature using Cecil 1010 spectrophotometer. A standard curve prepared by the same method using serial concentrations of standard tannic

acid solution (50-800 µg/ ml) was used for evaluating the TPC of each extract.

Determination of Total Flavonoid Content :

Total flavonoid content (TFC) was determined in (SSE) according to method described by Kumaran and Karunakaran [25]. One milliliter of each (SSE) was mixed with 1.0 ml of aluminum trichloride in ethanol (20 mg/ml) and a drop of glacial acetic acid, then diluted with ethanol to 25 ml. The absorption at (415 nm) was read after 40 min. A blank sample was prepared using 1.0 ml of each (SSE) and a drop of glacial acetic acid then diluted to 25 ml with ethanol. following the same method the absorption of standard quercetin solution (0.5 mg/ml ethanol) was determined . The amount of total flavonoids content in each (SSEs) in quercetin equivalents (QE) was calculated by the following equation:

$$X = (A \cdot m_q) / (A_q \cdot m)$$

Where: X is the flavonoids content (mg/g Sumac seed extract in QE), A is the absorption of Sumac seed extract solution, m is the weight of Sumac seed in (SSE). A_q is the absorption of standard quercetin solution, and m_q is the weight of quercetin in the solution (mg).

Determination of Antioxidant Capacity of Sumac Seed Extracts :

Determination of Total Antioxidant Capacity: Total antioxidant capacity of each (SSE) was assayed mainly by the phosphomolybdenum method described by Kumaran and Karunakaran [25]:

Known volumes (0.1 -0. 3 ml) of each (SSE) were added to test tube then completed to a constant volume (0.3 ml) with D.W. Three milliliter of reagent solution (0.6 M sulfuric acid, 28 .0 mM sodium phosphate and 4.0 mM ammonium molybdate) were added to each tube and mixed well then incubated at 95°C for 90 min.

Blank was prepared by the same procedure without (SSE). Ascorbic acid solution (0.03%, w/v) was used as positive control After cooling to room temperature , the absorbance of the solution was measured at (695 nm) against blank. Increased absorbance of the reaction mixture indicated increased total antioxidant capacity.

Determination of Reducing Power:

The reducing power of sumac seed extracts was determined mainly by the method of Mathew and Abraham [26]:

In clean test tubes, a serial known volumes (0.2-1.0 ml) of each sumac seed extract were added. The solutions were completed to 1.0 ml with D.W. (2.5 ml) of phosphate buffer solution (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide solution (1%, w/v) were added to each tube then mixed well. The mixtures were incubated at 50°C for 20 min. After incubation, 2.5 ml of trichloroacetic acid solution (10%, w/v) were added to each mixture then centrifuged at 5,000 rpm for 10 min. (2.5 ml) of each supernatant was taken in another test tube then one ml of D.W, and 0.5 ml of ferric chloride solution (0.1 %. w/v) were added and mixed well .The absorbance was measured at 700 nm. Blank was prepared by the same procedure without (SSE). Ascorbic acid solution (0 .03%, w/v) was used as positive control. Increased absorbance of the reaction mixture indicated increased reducing power.

Assay of DPPH Radical Scavenging Activity:

The antioxidant activity' of each sumac seed extracts was based on its scavenging activity of the stable DPPH free radical. According to the method described by Lee et al. [27]

(50-150 µl) of each (SSE) were individually added to test tubes then completed to (1.0 ml) by D.W., One millilitre (1.0 ml) of DPPH solution (0.2 mM in ethanol) was added to each tube then mixed well and incubated at

room temperature for 30 min. Control was prepared by the same procedure without sumac seed extract. Ascorbic acid solution (0.03%, w/v) was used as a positive control. The absorbance (A) of the solution was measured at (517 nm). Inhibition of DPPH free radical(I) in percent (I%) was calculated according to equation: $I\% = [(Ac - As) / Ac] * 100$

Where **Ac** is absorbance of ascorbic acid, and **As** is absorbance of sumac seed extract.

Assay of Nitric Oxide Scavenging Activity:

The scavenging activity of nitric oxide by each (SSE) was determined according to the method described by Kumaran and Karunakaran [25]:

0.5 ml of sodium nitroprusside solution (10 mM in 0.1 M phosphate buffer saline, pH 7.4) was mixed with different volumes of each sumac seed extract (0.1 -0.5 ml) then D.W was added to each tube to complete a known volume, (1. 0 ml). The test tubes were incubated at room temperature for 150 min then 0.5 ml of Griess reagent. (1% sulfanilamide, 2% H_3PO_4 and 0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride) was added to each tube and mixed well. The absorbance was measured at 546 nm. Control was prepared by the same procedure without (SSE). Ascorbic acid solution (A).(0.03%, w/v) was used as positive control. Scavenging activity of nitric oxide by (SSE) was calculated by the following equation:

$$\text{Scavenging activity (\%)} = [(Ac - As) / Ac] * 100$$

Where **Ac** is absorbance of ascorbic acid and **As** is absorbance of (SSE) Assay of Hydroxyl Radical Scavenging Activity:

The scavenging activity of hydroxyl radical by (SSEs) was assayed mainly by deoxyribose method as described by Nagai *et al.* [28].

0.45 ml of sodium phosphate buffer solution (0.2 M. pH 7.0), 0.15

ml of 2-deoxyribose solution (10 mM), 0.15 ml of FeSO₄-EDTA solution(10 mM FeSO₄, 10mM EDTA), 0.15 ml of H₂O₂ solution (10 mM) and sumac seed extract (50-100μl) were mixed and completed to (1.5 ml) with D.W, then incubated at 37°C for 4 h. the reaction was stopped by adding 0.75 ml of trichloroacetic acid solution (2.8%, w/v) and 0.75 ml of thiobarbituric acid solution (1% in 50mM NaOH solution). The solutions were boiled for 10 min and cooled in water. The absorbance (A) of the solution was measured at 520 nm. Control was prepared by the same procedure without (SSE). Ascorbic acid solution (0.03%, w/v) was used as positive control. Inhibition of deoxyribose degradation in (I%) was calculated using the following equation:

$$I\% = [(Ac-As)/Ac]*100$$

Where **Ac** is absorbance of ascorbic acid and **As** is absorbance of (SSE)

Assay of Fe Chelating Activity:

The ability of (SSEs) to chelate ferrous ion (Fe^{+2}) was determined using a modified method of Minotti and Aust [29] as described by Oboh *et al.* [30].

(150μl) of freshly prepared ferrous sulphate solution (500 μM) was added to a reaction mixture (168 μl)of tris-HCl buffer solution (0.1 M. pH 7.4) and (SSE) (10-25μl). The solution was completed by saline solution (0.9% NaCl, w/v) to (561μl). The reaction mixture was incubated for 5 min at room temperature, then 13μl of 1. 10-phenanthroline solution (0.25%, w/v) was added. The absorbance (A) was measured at(510 nm). Control was prepared by the same procedure without (SSE). Ascorbic acid solution (0.03% w/v) was used as positive control. The Fe chelating activity (%) was calculated according to the equation:

$$\text{Ferrous chelating activity (\%)} =$$

$$[(Ac - As)/Ac] * 100$$

Whereas Ac is absorbance of ascorbic acid and As is absorbance of (SSE).

Statistical Analysis:

The results were analyzed by an analysis of variance ($P < 0.05$) and the means separated by Duncan's multiple range test. The results were processed by SPSS 11.5[31]

Results and Discussion:

Sumac Seed Extracts a Source of Antioxidants:

Previous studies indicated that the plant phenolics constituent is one of the major group of compounds acting as primary antioxidants or free radical terminators. Flavonoids, as a group of natural compounds, are likely to be the most important natural phenolics [25]. The antioxidant activity of each sumac seed extracts (aqueous, ethanolic and methanolic) were evaluated using various antioxidant assays, including total antioxidant capacity, reducing power, DPPH radical scavenging, nitric oxide scavenging, hydroxyl radical scavenging and metal ion chelating

activities. The various antioxidant activities of sumac seed extracts were compared with standard antioxidant (ascorbic acid).

Phenolic and Flavonoid Contents of (SSE):

The results showed that (SSEs) contain a significant amount of phenolic and flavonoids compounds (Table 1). It was found that methanolic (SSE) contains a high amount of phenolic and flavonoids compounds (570.20 and 247.33 mg/g sumac seeds, respectively) than that of aqueous and ethanolic (SSEs) (136.66, 222.563 and 8.2, 119 mg/g sumac seeds, respectively). Total solids of lyophilized SSE were 41.6%, 29%, and 28% for methanolic, ethanolic and aqueous respectively. The results indicated that methanolic SSE was the best solvent for the extraction of Phenolic and flavonoid compounds. The result indicated also that phenolic and flavonoid compounds present in methanolic SSE is higher than that of ethanolic and aqueous SSE.

Table (1): Total Solid Phenolic and Flavonoid contents of SSEs

Sample (SSE)	Total solids (%) of lyophilized (SSE)	Phenolic content of (SSE) (mg/g sumac seed)	Flavonoids content of (SSE) (mg/g sumac seed)
Aqueous	28 ^a ±2.645	136.667 ^a ±12.583	8.2 ^a ±3.364
Ethanolic	29 ^a ±5.291	222.563 ^b ±23.788	119 ^b ±8.544
Methanolic	41.6 ^b ±5.197	570.2067 ^c ±82.194	247.33 ^c ±119
(L.S.D)	0.797	2.31	1.66

-Values are means of three replicates $M \pm S.E.$ Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$

- L.S.D Least Significant Difference

Total Antioxidant Capacity:

In the present study the total antioxidant capacities of the three SSEs and standard (ascorbic acid) were assayed (Table 2). It was found that the antioxidant activity of the three

SSEs were increased with increasing concentration of the SSEs. It was also found that methanolic SSEs showed a higher antioxidant capacity than aqueous and ethanolic SSEs. The antioxidant capacities of sumac seed extracts and the standard (ascorbic

acid)were (MSSE at 90.9 ppm > ascorbic acid < ESSE at 60.6 ppm > WSSE at 90.9 ppm > WSSE at 60.6

ppm < ESSE at 30.3 ppm > WSSE at 30.3 ppm).

Table (2): Total antioxidant capacity of(aqueous, ethanolic, Methanolic) SSEs and Ascorbic acid

Sample SSE	Conc.(ppm)	Total antioxidant capacity (O.D _{695nm})
Aqueous	30.3	1.143 ^a ±0.0152
	60.6	1.174 ^a ±0.095
	90.9	1.279 ^{ab} ±0.234
Ethanolic	30.3	1.386 ^b ±0.0321
	60.6	1.545 ^c ±0.043
	90.9	1.72 ^d ±0.062
Methanolic	30.3	1.175 ^a ±0.131
	60.6	1.65 ^{cd} ±0.166
	90.9	1.941 ^e ±0.137
Ascorbic acid(standard)	27.3	1.713 ^d ±0.0321
L.S.D	-	0.0113

- Values are means of three replicates $M \pm SE$. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$

- L.S.D Least Significant Difference.

Reducing Power of SSE :

Data (Table 3) show the reducing power(reductive capability) of sumac seed extracts compared to the standard(ascorbic acid). Reductive ability was determined by the ability of SSEs to reduce ferric ion to ferrous ion. As in the antioxidant activity, the reducing power of SSEs increased with increasing concentration. Earlier studies by Tanaka *et al.* [32] and Duh [33] have revealed a direct correlation between antioxidant activity and reducing power of certain plant extracts. The reducing properties are generally associated with the presence

of reductive compounds which have been shown to exert antioxidant action by donating a hydrogen atom [34].Reductive compounds are also reported to react with certain precursors of peroxide, thus preventing peroxide formation. However, the antioxidant activity of antioxidants has been attributed by various mechanisms, in addition to prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity' and radical scavenging [35].

Table (3): Total Reduction power of (aqueouse , ethanolilc , Methanolic) SSEs and Ascorbic acid

Samples SSE	Conc.(ppm)	Reducing power (O.D _{700nm})
Aqueous	23.52	0.789 ^a ±0.092
	70.58	1.25 ^{bc} ±0.109
	117.64	1.751 ^d ±0.075
Ethanollic	23.52	1.032 ^{ab} ±0.0072
	70.58	1.418 ^{cd} ±0.028
	117.64	1.657 ^d ±0.173
Methanolic	23.52	1.276 ^{bc} ±0.367
	70.58	1.519 ^{cd} ±0.055
	117.64	2.103 ^e ±0.397
Ascorbic acid (standard)	35.3	1.498 ^{cd} ±0.048
L.S.D	-	0.021

- Values are means of three replicates $M \pm S.E$ Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$
- L.S.D Least Significant Difference.

DPPH Radical Scavenging Activity:

1,1-diphenyl(-2-picrylhydrazil, (DPPH), is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The radical scavenging activity of DPPH radical is determined by the decrease in absorbance at 517 nm induced by antioxidants. Data indicated that the SSEs are able to reduce the stable radical DPPH to the yellow-colored DPPH (Table 4).

The radical scavenging effect of each sumac seed extract at high concentration (75.0 ppm) and ascorbic acid standard solution with the DPPH

radical was: Methanolic, Ethanollic, aqueous, and ascorbic acid (87.66 %, 72.66% 58.33% and 34.33%. Data also revealed that the- scavenging activity of each SSEs was increased with increasing the concentration of each extract(Table 4). It has been found that cysteine, glutathione, ascorbic acid, tocopherol, flavonoids, tannins and aromatic amines (p-phenylene diamine, p-aminophenol, etc.), reduce and decolorize DPPH by their hydrogen donating ability' [25], Phenolic compounds of SSEs are probably involved in antiradical activity.

Table (4): Scavenging activity of (aqueous, ethanollic, Methanolic)SSEs and Ascorbic acid against DPPH radical

Sample SSE	Conc.(ppm)	Scavenging activity (%)
Aqueous	25	40.66 ^{ab} ±2.081
	50	48.33 ^{bc} ±9.712
	75	58.33 ^{cd} ±7.571
Ethanollic	25	60.00 ^{cd} ±5.000
	50	69.00 ^{de} ±3.605
	75	72.66 ^e ±2.081
Methanolic	25	74.33 ^e ±2.081
	50	81.00 ^{ef} ±7.937
	75	87.66 ^f ±12.013
Ascorbic acid (standard)	23	34.33 ^a ±4.509
L.S.D	-	0.032

- Values are means of three replicates $M \pm S.E$ Numbers in the same column followed by the same letter are not significantly different at $p < 0.05$
- L.S.D Least Significant Difference.

Nitric Oxide Scavenging Activity:

Nitric oxide (reactive nitrogen species), formed during their reaction with oxygen or with superoxide, such as NO_2 , N_2O_4 , N_3O_4 , NO_3 . and NO_2 are very reactive. These compounds are responsible for altering the structural and functional behavior of many cellular components. Nitric oxide is also implicated in inflammation, cancer and other pathological conditions [36]. Incubation of sodium nitroprusside resulted in linear time-dependent nitrite production. Data as shown in (Table 5) revealed that each of the three SSEs has the nitric oxide scavenging activity. It was found that the ability of Methanolic SSE on nitric

oxide scavenging activity was also higher than that of Ethanolic, aqueous. There was a positive correlation between the concentration of sumac seed extract, and scavenging activity against nitric oxide. Methanolic (333.33 ppm) possesses a higher nitric oxide scavenging activity. Accordingly, sumac seed extracts may have the property to counteract the effect of NO formation and may be of considerable interest in preventing the illness effects of excessive NO generation in the human body. Moreover; the scavenging activity may also help to arrest the chain of reactions initiated by excess generation of NO that are detrimental to the human health.

Table (5): Nitric oxide scavenging activity of (Aqueous, ethanolic, Methanolic) seed extract and Ascorbic acid

Sample SSE	Conc.(ppm)	Scavenging activity (%)
Aqueous	66.66	27.00 ^a ±6.082
	200	43.33 ^{bc} ±9.712
	333.33	71.1 ^e ±8.702
Ethanolic	66.66	34.66 ^{ab} ±1.527
	200	51.00 ^{cd} ±6.557
	333.33	80.667 ^e ±6.658
Methanolic	66.66	41.00 ^{bc} ±6.557
	200	56.667 ^d ±2.081
	333.33	91.00 ^f ±6.557
Ascorbic acid(standard)	10.0	56.66 ^d ±1.527
L.S.D	-	0.002

- Values are means of three replicates $M \pm S.E.$ Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$

- L.S.D Least Significant Difference

Hydroxyl Radical Scavenging Activity:

Free radicals and other reactive species are constantly generated *in vivo* by both accidents of chemistry and specific metabolic reaction. The most important reactions of free radicals in aerobic cells involve molecular oxygen and its radical derivatives (superoxide anion and hydroxyl radicals), peroxides and transition metals. Reactive species are thought to play an important role in aging and in the pathogenesis of numerous degenerative or chronic diseases, such as cancer, cardiovascular diseases, diabetes and

atherosclerosis [36]. The scavenging abilities of ascorbic acid and sumac seed extracts on hydroxyl radical inhibition were shown in (Table 6). Hydroxyl radical scavenging activity was seen with each of the three sumac seed extract, It was found that methanolic SSE was more effective than both ethanolic and aqueous SSE. The scavenging activity was found to be increased with increasing concentration of SSE (Table 6). The three sumac seed extracts at concentration 333.33 ppm could be arranged according to their hydroxyl radical scavenging activities : Methanolic SSE (87.3%), Ethanolic (73.66%), aqueous

SSE 34.66%) . As previously shown, methanolic was more effective than

Ethanolic and aqueous in hydroxyl radical scavenging activity.

Table(6): Hydroxyl radical scavenging activity of (aqueous, ethanolic and Methanolic) SSEs and Ascorbic acid

Sample SSE	Conc.(ppm)	Scavenging activity (%)
Aqueous	16.66	22.00 ^a ±2.645
	25	28.33 ^{bc} ±2.081
	33.33	34.66 ^b ±3.055
Ethanolic	16.66	51.33 ^c ±3.214
	25	62.33 ^d ±5.859
	33.33	73.66 ^e ±4.041
Methanolic	16.66	67.00 ^d ±2.000
	25	79.33 ^e ±4.041
	33.33	87.30 ^f ±2.516
Ascorbic acid(standard)	10.0	30.33 ^b ±5.686
L.S.D	-	0.0312

- Values are means of three replicates $M \pm S.E$ Numbers in the same column followed by the same letter are not significantly different $P < 0.05$

- L.S.D Least Significant Difference

Metal Chelating Activity :

The metal chelating activity is based on chelating of ferrous ions by the reagent 1,10-phenanthroline, which is a quantitative formation of a complex with ferrous ions [37]. The formation of a complex is probably disturbed by other chelating reagents, which results in the reduction of the formation of red- colored complex. Measurement of the rate of reduction of the color, therefore, allows estimation of the chelating activity of the coexisting chelator [37].It was found that the three(SSEs) in this assay interfered with the formation of ferrous complex with the reagent 1,10-phenanthroline, indicating that they have chelating activities and captures the ferrous ion before 1,10-phenanthroline. The percentages of metal scavenging capacity of tested sumac seed extracts were shown in(Table 7). The metal scavenging effect of the three SSEs at concentration 43.55 ppm were found in the following order: Methanolic, Ethanolic, Aqueous.The data also showed that the metal chelating activity of each SSEs was increased with increasing

concentration of the extract. It was reported that some chelating agents, are effective as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion [34]. The obtained data revealed that SSEs demonstrated an effective capacity for iron binding, suggesting that its action as antioxidant can be related to its iron-binding capacity.

In general, it was demonstrated that the three SSEs are good scavengers of active oxygen species, including superoxide anion radical, hydroxyl radical and nitric oxide radical. The antioxidant activity of SSEs is attributed to their contents of antioxidant agents, including mainly phenolics and flavonoids compound.

The higher antioxidant activity of methanolic SSE in comparison with Ethanolic,aquouse SSE was associated with its content of total phenolics and flavonoids (Table 1). Phenolic compounds have been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compounds is mainly due to their redox properties,

which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [38]. It was suggested that

polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans [30]. Another part of this study is concerning black grape seeds (*Vitis Vinifera* L)

Table (7): Metal chelating activity of Aqueous , ethanolic and Methanolic)seed extract and Ascorbic acid

Samples SSEs	Conc.(ppm)	Fe Chelation(%)
Aqueous	17.42	7.366 ^a ±1.026
	26.13	16.33 ^b ±1.527
	43.55	34.66 ^{cd} ±2.516
Ethanolic	17.42	16.33 ^b ±6.110
	26.13	17.33 ^{bc} ±2.110
	43.55	23.33 ^d ±2.516
Methanolic	17.42	18.33 ^{bcd} ±1.527
	26.13	39.66 ^e ±3.055
	43.55	49.33 ^f ±5.131
Ascorbic acid(standard)	13.1	22.00 ^{bcd} ±2.000
L.S.D	-	0.041

- Values are means of three replicates $M \pm S.E$. Numbers in the same column followed by the same letter are not significantly different at $P < 0.05$

- L.S.D Least Significant Difference

References:

1. Muratmatsu, H.K., Kogawa, M., Tanaka, K.O., Nishihori, K.K., Tandnitus. 1995. superoxid dismutase in SAS human tongue carcinoma cell line is a factor defining invasiveness and cell motility, *Cancer res.* 55(24):6210-6214.
2. .Smith, M.A.G, perry, L.M. V.E, And reson, M.F. and N. Kowall. 1996. Oxidative damage in Alzheimer's. *Nutr.* 382(6587):120-121.
3. Mohammadi S., Montasser. K. S., and Monavar. F. A. 2010. Antidiabetic properties of the ethanolic extract of *Rhus coriaria* fruits in rats, *Daru.* 18(4): 270-270.
4. connor A, Luby j.j, Tong C.B.S, fince, H.I.F. 2002. Genotypic and environmental Variation in antioxidant activity total phenolic content and anthocyanin content among blueberry cultivars. *J. Am. soc. Hortic .sci.* 127(1):89-97.
5. Lampart-Szezapa E., Korczak, J., Nogala-kaluck M., Zawirska-Wojtasiak R. 2003. antioxidant properties of lupin seed products . *Food chem.* 83(2):279-285.
6. Cai y., Luo Q, Sun M, Cork H. 2004. antioxidant activity and phenolic compound of 112 traditional Chinese medicinal plant associated with anticancer. *Life Sci.* 74(17):2157-2184.
7. Joyk B., Donald L, Wyse C. C, Sheaffer K. L, kauppi R, Gray F, nancy J.E, , David D.B and Russell F. B .2009. Antioxidant and Antimicrobial activity of seed from plant of the mississippi river basin. *J med plants Res.* 3(10):707-718.
8. Ito N.S, Fkushima A, Hasgera M.S and Ogiso T. 1983. carcinogenicity of butylated hydroxyl anisole in F 344 rats *J national cancer institute.* 70:343-347.
9. Rayne S, Mazz G. 2007. Biological activities of extract from sumac (*Rhus SPP*) a review; *plant foods Hum. Nutr.* 62(25):165-175
10. Mallyanov M, Lslmbekov S.L and Al, Kamav F.G. 1995. Chemistry of Natural compound . 31:268.
11. Rima K, Hao Z, Wei C .2011. Antimicrobil and antioxidant activities of Chinese sumac (*Rhus Typhina* L) fruit extract. *Food cont.* 22(1):128-132.

12. Kossah R, Nsabimana C, Zhao J, Chen H, Tian F, Zhang H and Chen W. 2009. Comparative study on the chemical composition of Syrian sumac (*Rhus coriaria* L.) and Chinese sumac (*Rhus typhina* L.) fruits. *Pak J Nutr* 8(10):1570-1574.
13. Candan F, Sokmer A. 2004. Effect of *Rhus Coriaria* L. (Anacardiaceae) on lipid peroxidation and Free radical Scavenging activity. *Phytother Res* 18(1):84-86.
14. Ozcan M. (2003) antioxidant activities of rosemary, sage and sumac and their combination on stability of natural peanut oil. *J Med food* 6(3):267-270.
15. Harimaya K, Tanaka K, Matsumoto Y, Sato H, Matsuda S, and Iwamoto Y. 2000. antioxidants inhibit TNF alpha-induced motility and invasion of human osteosarcoma cell: possible involvement of NFkappaB activation. *Clin Exp Metastasis* 18(2):121-129.
16. Yasunari K, Kohno M, Kano H, Yokoawa K, Minami M, Yoshikawa J. 1999. antioxidants improve impaired insulin-mediated glucose uptake and prevent migration proliferation of cultured rabbit coronary smooth muscle cells induced by high glucose. *Circulation* 99(10):1370-1378.
17. Hanieh Z and Rami Z. 2008. Tannin extracted from sumac inhibits vascular smooth muscle cell migration. *MC Gill J med* 11:119-123.
18. Fazli MR, Amin Gh, Ahmadian Attari, MM, Ashtiani H, Jamalifar N, and Samadi N. 2007. Anti microbial activities of Iranian sumac and avishan-e shirazi (*Zataria multiflora*) against some food-borne bacteria. *Food Cont* 18(6):646-649.
19. Amin Gh, Ahmadian-Attari MM, Fazeli MR, Jamalifar H, Ashtaini H, Ghobadi A, Shakiba R, and Khanlarbeik M. 2008. The effects of Autoclaving, salt, and protein on antimicrobial activities of Iranian sumac. *J med plants* 7 (4):49-53.
20. Musa O. 2003. effect of sumac (*Rhus Corairai* L.) extracts on the oxidative stability of peanut oil. *J med food* 6(1):63-66.
21. Kosar M, Bozan Temellifi F and Baser K. 2006. antioxidant (*Rhus-Corairai* L.) extract. *Food Chem* 103:956-959.
22. Giancarlo S, Rosalm. N, F, and Franceco M. 2006. Hypoglycemic activity of two spices extract. (*Rhus Corairai* L.) and *Bunium persicim* Boiss. *Natur Prod reas* 20(9):882-886.
23. Badavi, M., Mehrgerdi F, Z, Sarkaki A, Naseri M. K. G and Dianant M. 2008. Effect of grape seed extract on lead induced hypertension and heart rate in rat. *Pak J Biol. Sci.*, 11(6): 882-887.
24. Gao, X. M, Ohlander N, Jeppssoa I, and Trajkovski V. 1999. Phytonutrients and their antioxidant effects in fruits of seabuckthorn (*Hippophae rhamnoides*). *Proceedings of International Workshop on Seabuckthorn*, Beijing, China.
25. Kumaran, A. and Karunakaran R. J. 2007. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LVVT- Food Sci. Technol.* 40(2): 344-352.
26. Mathew, S. and Abraham T. E. , 2006. Studies on the antioxidant activities of cinnamon (*Cinnamomum verum*) bark extracts, through various in vitro models. *Food Chem* 94 (4):520-528.
27. Lee, J Y, Hwang W. I and Lim S T. 2004. Antioxidant and anticancer activities of organic extracts from (*Platycodon grandiflorum*) A. De Candolle roots. *J. Ethnopharmac* 93: 409-415.
28. Nagai, T., R. Inoue, N. Suzuki, T. Myoda and T. Nagashuu. 2005. Antioxidative ability in a linoleic acid oxidation system and scavenging abilities against active oxygen species of enzymatic

- hydrolysates from pollen (*Cistus ladaniferus*). *Intl. J Mol Med.* 15(2): 259-63.
29. Mimotti, G. and Aust S.D., 1987. An investigation into the mechanism of citrate-Ferrous-dependent lipid per oxidation. *Free Radical. Biol and Med*, .3(2): 379-387.
 30. Oboh G., Puntel R L. and Rocha J.B.T., 2007. Hot pepper (*Capsicum annum*, *Tepin* and *Capsicum Chinese*, *Habanero*) prevents Ferrous induced lipid per oxidation in brain in vitro. *Food Chem.* 102(1):178-185.
 31. بشير، سعد زغلول، 2003، دليلك الى البرنامج الإحصائي SPSS، المعهد العربي للتدريب والبحوث الإحصائية، الإصدار العاشر، العراق، 249 صفحة
 32. Tanaka, M..C.W, Kuie J.B.T., Nagashima Y. and Taguchi T., 1988, Applications of antioxidative Millard reaction products from histidine and glucose to sardine products. *Nippon Siusan, Gakkoishi.* 54: 1409-1414.
 33. Duh, PD. 1998. Antioxidant activity of burdock (*Arctium lappa* L): Its scavenging effect on free radical and active oxygen. *J. Am Oil Chem Soc.*75(4): 455-461.
 34. Gordon, M.H. 1990. The mechanism of antioxidant action in vitro. In *Food Antioxidants*, Ed., Hudson, B.J.F. Elsevier Applied Science, London. 1-18.
 35. Diplock. A T. 1997. Will the good fairies please prove us that vitamin E lessens human degenerative disease. *Free Rad Res.* 27: 511-532.
 36. Moncada A., Palmer R.M.J and Higgs E.A. 1991 Nitric oxide: physiology, path physiology and pharmacology. *Pharmacological Reviews.* 43(2): 109-142.
 37. Ames, B.N, Shigenaga M.K and Hagen T.M. 1993, Oxidants, antioxidants and the degenerative disease of aging. *Proceedings of the National Academy of Sciences of the United States of America*, 90: 7915-7922.
 38. Dints, T C.PMadeira., V.M.C. and Almeida L.M. 1994. Action of phenolic derivatives acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid per oxidation and as peroxy radical scavengers. *Arch Biochem and Biophy.* 315(1):161-169

تأثير بذور نبات السماق العراقي كمضاد أكسدة وكاسح للجذور الحرة

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الخلاصة:

أجريت هذه الدراسة لتقييم مضادات الأكسدة في بذور نبات السماق العراقي وقد تم تقدير المواد الفينولية والفلافينويدية في بذور نبات السماق باستخدام مستخلصات مائية وكحولية (ايثانولية وميثانولية) كما تم تقدير الفعالية المضادة للأكسدة لبذور نبات السماق باستخدام الطرق التحليلية اللازمة والتي شملت قياس (القدرة الكلية لمضادات الأكسدة، القوة الاختزالية ، وكاسحات الجذور الحرة باستخدام (1، 1- ثنائي فنيل-2- بركيل هدراريل) (DPPH) وكاسحات اوكسيد النتريك وجذر الهيدروكسيل والفعالية المخلبية للأيونات الفلزية . قورنت هذه الفعاليات المختلفة لمضادات الأكسدة مع حامض الاسكوربيك باعتبارها مضاد أكسدة قياسي. وأظهرت النتائج بان مستخلصات بذور السماق العراقي الثلاث تحوي على كميات عالية من مركبات الفينول والفلافينويد. كما أظهرت النتائج بان هذه المستخلصات الثلاث تمتلك فعالية مضادات الأكسدة. وقد وجد أن المستخلص الميثانولي لبذور السماق يحتوي مركبات فينولية وفلافينويدية اكبر كما يمتلك بنفس الوقت فعالية مضادات أكسدة أعلى من تلك في المستخلص المائي أو الايثانولي , وبالنتيجة فان هذا يشير إلى أن المستخلصات المائية والكحولية وخاصة المستخلص الميثانولي لبذور السماق العراقي تعتبر كاسحات جيدة للأصناف الاوكسجينية الفعالة ومصدرًا ممتازاً كمضادات أكسدة طبيعية والتي يمكن استخدامها في الصناعات الصيدلانية والغذائية.