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Comparison of Antioxidant Activity of *Rheum ribes* Fruits and Seed Methanolic Extracts against Protein Oxidation and Lipid Peroxidation

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ABSTRACT
This work was performed to comparison antioxidant activity of *Rheum ribes* fruit and seed methanolic extracts against to protein oxidation. Consequently determined that antioxidant capacity with cupric of method, respectively in fruit extract to be 2.44±0.212, in seed to be extract 5.30±0.245 mmol g^{-1}, antioxidant radical scavenging with ABTS* method in fruit extract to be 11-78%, in seed extract 2-15% levels. The $OH^{\cdot}$ power reduction for the *Rheum ribes* fruit extract 34-49, 22±0.04%, while seed extract to be 49-80±0.04%, H_2O_2 removal activity for fruit extract was at 30.31-57.87%, while seed extract was at %33.33-54.57 levels. Also were determined the total phenolic content, respectively in fruit extract to be 2.312±0.0007 in seed extract to be 3.298±0.0007 mg of GA equivalent/g, total flavonoid content in fruit extract to be 2.79±0.0066, in seed extract to be 7.31± mg 0.1787 of Q equivalent/g, total anthocyanin content, respectively in fruit extract to be 0.5 mg of cyanidin-3-glucoside equivalent/L, in seed extract to be 1.26 mg of cyanidin-3-glucoside equivalent/L. were found. Protein oxidation in fruit was 40.64-56.5%, in seed extract it was 37.98-51.46% were determined. Anti-lipid peroxidation inhibition % in fruit extract to be 13.72-27.4%, in seed extract to be 8.459-27.09% was determined.

Key words: *Rheum ribes*, antioxidant activity, protein oxidation, lipid peroxidation

INTRODUCTION

Rheum ribes; Polygonaceae family. It grows between 1000 and 4000 m on donate rocks, among stones and slopes and is distributed in the temperate and subtropical regions of the world, chiefly in Western Asia (Turkey, Syria, Lebanon, Iraq, Iran, Azerbaijan, Armenia) to Afghanistan and Pakistan. It growing yellowish white blooms in May-June, 40-150 cm tall, perennial, herbaceous type. It is a medicinal herb, often used in pharmacological research. On the basis of all these features, its powerful active ingredients contained lines. Especially; when cooked is quite rich in polyphenols amount was observed. Therefore, this study aimed that its determine of total antioxidant capacity, total phenolic content, total flavonoid content and total anthocyanin content of methanol extract.

Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, where as natural antioxidants can be phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), or carotenoids as well as ascorbic acid (Larson, 1988; Hudson, 1990; Hall and Cupped, 1997).

Phenolic compounds are excellent oxygen radical scavengers because the electron reduction potential of the phenolic radical is lower than the electron reduction potential of oxygen radicals (Grace, 2005; Bors *et al*., 1990) and also because phenol radicals are generally less reactive than oxygen

radicals (Bors *et al*., 1994). Therefore, phenolic compounds can scavenge reactive oxygen intermediates without promoting further oxidative reactions (Grace, 2005). It follows that many environmental stresses that cause oxidative stress often induce the synthesis of phenolic metabolites (Grace, 2005; Dixon and Paiva, 1995; Pasqualini *et al*., 2003).

Several investigations determined that many of plants have antioxidant capacity and this activity associated with including in phenolic content (Cook and Sammon, 1996).

Flavonoids, groups of polyphenol compounds with known properties, such as radical scavenging activity, anti-inflammatory effect have been isolated from plants (Omale and Okafor, 2008).

Anthocyanin's are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. They belong to a parent class of molecules called flavonoids synthesized via the phenyl propionic pathway; they are odorless and nearly flavorless, contributing to taste as a moderately astringent sensation. Anthocyanin's occur in all tissues of higher plants, including leaves, stems, roots, flowers and fruits anthocyanin's also act as powerful antioxidants. However, it is not clear whether anthocyanin's can significantly contribute to scavenging of free radicals produced through metabolic processes in leaves, since they are located in the vacuole and thus, spatially separated from metabolic reactive oxygen species. Some studies have shown hydrogen peroxide produced in other organelles can be neutralized by vacuolar anthocyanin.

This work was performed to determined comparison of antioxidant capacity total phenolic, total flavonoid contents and anthocyanin content of *Rheum ribes* fruit and seed methanol extracts against to protein oxidation and lipid peroxidation.

MATERIALS AND METHODS

Reagent, chemical and instrumentation: *Rheum ribes* were obtained from a bazaar in Elazig. All solvents were of analytical-grade reagents. They were purchased from Merck, Sigma-Aldrich. Analysis was performed with the Jasco V-530 UV spectrophotometer and Shimadzu UV-1601 spectrophotometer. Centrifugation was Nüvefej 615 and Hettic marks. For the cuprac method; neocuproine (Nc), $CuCl_2.2H_2O$ salt, ammonium sulphate, for the phenolic method; Na_2CO_3 (sodium carbonate), Folin reagent, antioxidant total flavonoid content; $NaNO_2$ (sodium nitrite) $AlCl_3$ (aluminum chloride), NaOH (sodium hydrooxide), for the anthocyanin method; HCl (hydrochloric acid), KCl (potassium chloride), CH_3COONa (sodium acetate) were used. For ABTS method trolox, (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), peroxydisulphate, for H_2O_2 method ammonium molybdate, sulfuric acid, potassium iodide, sodium thiosulphate. For the OH^- method; iron sulphate, sodium salicylate, hydrogen peroxide. For protein oxidation; DNPH (dinitrophenylhydrazin), BSA (bovine serum albumin), $FeCl_3$ (ferric chloride), (TCA) trichloroacetic acid, HCl (hydrochloric acid) guanidine hydrochloride.

Preparation extract of solutions: Plant samples 1/10 (g mL^{-1}) ratio was extracted with methanol (Gupta *et al*., 2008).

Preparation of solutions: For determination of antioxidant capacity; a copper (II) chloride solution at a concentration of 10^{-5} M. Copper (II) chloride solution at a concentration of 10^{-5} M was prepared from $CuCl_2 \cdot 2H_2O$. Ammonium sulphate of buffer at pH 7.0 was prepared. Neocuproine (Nc) solution at a concentration of 7.5×10^{-3} M was prepared by dissolving 0.15 g of Nc in 99% Ethanol and diluting to 25 mL water and 75 mL with ethanol.

The preparation of the ABTS* solution used in the experiments was performed by dissolving 6.6 mg of potassium peroxydisulfate and 30 mg of ABTS in 7.8 mL of distilled water. The solution was then incubated at room temperature for 12-16 h. The resulting colored solution was diluted with distilled water until it has an absorbance of 0.700±0.020 absorbance at 734 nm.

For the H_2O_2 method 3% ammonium molybdate solution was prepared by taking 3 g of ammonium molybdate weight 100 mL dissolving, 2 M 100 mL sulfuric acid (H_2SO_4) preparation; 19.616 mL H_2SO_4 was taken and 100 mL dissolution, 1.8 M 100 mL potassium iodide (KI) 22.84 g weight and 100 mL dissolving. Five millimole 100 mL sodium thiosulphate ($Na_2S_2O_3$) solution was prepared by taking 0.12 g weight and dissolving in 100 mL of distilled water.

For the OH. method 1.5 mM iron (II) sulphate solution was prepared to taking 0.0227 g ($FeSO_4$) weight and 100 mL dissolution, 20 mM sodium salicylate solution of 0.32 weight and 100 mL dissolved. Six millimole hydrogen peroxide (H_2O_2) was prepared to taking 0.204 mL and dissolving in 100 mL of distilled water.

Antioxidant phenolic content employed the preparation of 7.5% Na_2CO_3 solution (w/v), 1 M Folin reagent was also prepared.

For antioxidant total flavonoid content; 5% $NaNO_2$ (sodium nitrite) solution (w/v) was prepared and 10% $AlCl_3$ solution (w/v) was also prepared and used.

For Anthocyanin content several buffer solutions were prepared. pH 1.0 buffer solution employed 125 mL 0.2 M KCl +375 mL 0.2 M HCl, pH 4.5 buffer solution used 400 mL 1 M CH_3COONa +240 mL 1 M HCl +360 mL H_2O.

For protein oxidation, 20 mM phosphate-buffered solution was used with 100 mL capacity. Fifty micromole $FeCl_3$ was prepared in 100 mL of volume. One micromole ascorbic acid was prepared and the solution volume was 100 mL. The 6 M guanidine hydrochloride was prepared by dissolving 57.92 g of the compound in 100 mL of distilled water.

Anti-lipid peroxidation, 50 µL linoleic acid, 412 µL twenty-20, 0.005 M phosphate buffer (pH:7.4), 55% ethanol, 30% ammonium thiocyanate were used.

Antioxidant capacity was determined by cuprac method: A sample 10 mL was taken from the extracts. A series of solutions were prepared with concentrations of 50, 100, 250 and 500 ppm for determination of the antioxidant capacity. To a test tube were added 1 mL each of Cu (II), Nc and $(NH_4)_2SO_4$ buffer solutions. Antioxidant sample (or standard) solution (x mL) and H_2O (1.1-x) mL were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered and after half hour, the absorbance at 450 nm (A450) was recorded against a reagent blank. The standard calibration curve of each antioxidant compound was constructed in this manner as absorbance versus concentration and the molar absorptivity of the CUPRAC method for each antioxidant was found from the slope of the calibration line concerned (Apak *et al*., 2004).

The scheme for normal measurement is summarized as follows:

The 1 mL of Cu (II)+1 mL of Nc +1 mL of buffer +x mL of antioxidant solution+(1.1-x) mL of H_2O; total volume 4.1 mL, measure A450 against a reagent blank after half hour of reagent addition.

Assess of antioxidant capacity by ABTS* method: ABTS radical scavenging activity was determined by a method described in literature (Yen and Chien (2000). Relative antioxidant capacity was measured using ABTS radical. This method is used to determine the activity of free radical chain breaker of antioxidant made. Briefly; from the 100 mg mL^{-1} extract solution, aliquots were taken to obtain different concentrations of extracts (10, 20, 30, 40 and 50 mg mL^{-1}) into test tubes. To these solutions, added 2.3 mL ABTS radical solution and allowed the tubes to stand at room temperature for 30 min, then the absorbance was measured at 734 nm against blank. Blind methanol (solvent extracts) were used. Antioxidant Activity (AA) is calculated by the following formula:

$$AA = 1-[AE_{30}/AK_{30}]* 100$$

where, AA: Antioxidant activity, AE: Extract absorbance and AK: Control (blank) absorbance.

Assess of H_2O_2 removal activity: H_2O_2 removal activity measurement was determined according to the method of literature (Zhao *et al*., 2006). The 0.1 mM H_2O_2 solution with 1 mL capacity was prepared by mixing with various concentrations (1.3 and 5 mg mL^{-1}) of solution to make the final volume 1 mL. To this solution was added 100 µL of 3% ammonium molybdate. Ten milliliter of 2 M H_2SO_4 and 1.8 M KI 7.0 mL was added. The solution obtained was vortexes thoroughly. The solution; while 5 mM Na_2SO_3 is stirring until the yellow color disappeared. Removal activity (%) was calculated according to the following formula:

$$\text{Removal activity (\%)} = (V0-V1)/V0*100$$

where, Vo: Example absence, for control spent the amount of H_2O_2.

Asses of OH^- power reduction: OH^- power reduction was determined according to the method literature (Smirnoff and Cumbes, 1989). For a total of 3 mL solution, 1 mL $FeSO_4$ (1.5 mM), 0.7 mL H_2O_2 (6 mM), 0.3 mL (20 mM) sodium salicylate were used. This solution was added to the extract to make the following concentration (1.3 and 5 mg mL^{-1}). At 37°C the solution incubated for 60 min. Then 532 nm absorbance readied against blank. Ascorbic acid was used as standard. The % OH^- power reduction was calculated according to the following formula:

$$OH^- \text{ power reduction (\%)} = [1-(A_1-A_2)/A_0]*100$$

where, A_0: Control absorbance, A_1 was the absorbance in the presence of the sample; A_2 was the absorbance without sodium salicylate.

Determination of antioxidant total phenolic content: Antioxidant total phenolic content of the methanol extract of herbs was determinate with the Folin-Ciocalteaus assay (Marinova *et al*., 2005). Briefly, 500 µL extract was mixed with 2.25 mL distilled water and then 1 M 250 µL of Folin-Ciocalteaus reagent was added. The mixture was vortexes. For 10 min and was allowed to react for 5 min. Then, 2.5 mL of Na_2CO_3 solution (7.5%) were added. After incubation at room temperature for 1 h, the absorbance of each mixture was measured at 760 nm. The same procedure was also used to the standard solution of Gallic acid (50, 100, 250, 500 ppm) and a standard curve was obtained. Total phenolic contents were expressed as mg of Gallic acid equivalent per 1 g.

Determination of antioxidant total flavonoid content: Antioxidant total flavonoid content of the methanol extract of herbs was determinate with the aluminum chloride colorimetric assay (Marinova *et al*., 2005). The 1 mL extract or standard solution of quercetin (50, 100, 200, 400, 800 mg L^{-1}) was added to tube, then 4 mL distilled water added and the addition was finished with 0.3 mL 5% $NaNO_2$. After 5 min 0.3 mL 10% $AlCl_3$ was added. At the sixth time minute; 2 mL 1 M NaOH was added and the total volume was made up 10 mL with pure water. The solution was mixed. And after 60 min at room temperature incubated. Then

the absorbance against the prepared reagent blank was determined at 510 nm with an UV-spectrometer. The total flavonoid content of extract was expressed as milligrams of quercetin acid equivalents/g.

Determination of total anthocyanin content: Total anthocyanin content of the methanol extract of herbs was determined with by pH different methods (Wrolstad *et al*., 2005a, b). The total anthocyanin content was calculated to be equivalent to cyanidin-3-glucoside.

Total Anthocyanin content (mg L^{-1}) = A× MW×DF×10^3/ε ×L.

A = (A510-A700 nm) pH1.0-(A510-A700 nm) pH4.5.

The MW is the molecular weight of anthocyanin (449.2), ε is Cyaniding 3-glucoside molar absorbance (26900), L is cell path length (1 cm), DF is a dilution factor.

Protein oxidation: Protein oxidation assessment was done with spectrophotometric DNPH method (Draper and Hadley, 1990). Previously prepared stock solution of the extract, 1 mL and 100 mL, were diluted to obtain 100, 250, 500, 750, 1000 µg mL^{-1} concentration solution. This newly obtained solution were added to 800 µL (20 mM) phosphate-buffered (salt potassium) pH 7.4 and 400 µL BSA (4 mg mL^{-1}). Then, to this mix added to 400 µL of $FeCl_3$ (50 µM) and 400 µL (1 mM) ascorbic acid solution and at 37°C for the solution with incubated with 15 min Extraction was performed to determine the carbonyl compound; to do this, the extract was added to 2 mL of a solution of 2,4 dinitrophenyl hydrazine (DNPH) (10 mM) prepared in 2 M HCl. This mix was incubated 15 min at room temperature. After this period; the diluted proteins are precipitated with cold trichloroacetic acid (TCA, 10% concentration). The mixture was incubated for 15 min in an ice bath. After this period; centrifuged at 3000 rpm for 10 min. The supernatants are discarded; the protein pellets are washed three times with 2 mL portions of ethanol/ethyl acetate (1:1, v/v) to remove any free DNPH. Samples are then re-suspended in 6 M guanidine hydrochloride (GdmCl, dissolved in 20 mM phosphate buffer, pH 2.3), Carbonyl contents are determined from the absorbance at 370 nm of each sample against its appropriate blank. The results were expressed as % inhibition. The % inhibition was calculated according to the following formula. As a control BHT used:

$$\text{Inhibition}\,(\%) = \frac{\text{A control-A sample}}{\text{A control}} \times 100$$

Anti-lipid peroxidation: This work used linoleic acid method for anti-lipid peroxidation. Linoleic acid emulsion was prepared with linoleic acid (50 µL, 0.04 g) and Tween (412 µL, 0.04 g) in phosphate buffer (50 mL, 0.05 M, pH 7.4). A reaction solution, containing extracts (0.2 mL, 1.0, 3.0 5.0 mg mL^{-1}), linoleic acid emulsion (2.5 mL) and phosphate buffer (2.3 mL, 0.2 M, pH 7.0) were mixed with a homogenizer. The reaction mixture was incubated at 37°C in the dark and the degree of oxidation was measured according to the thiocyanate method (Mitsuda *et al*., 1996) by sequentially adding ethanol (4.7 mL, 55%), ammonium thiocyanate (0.1 mL, 30%) and sample solution (0.1 mL). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm and the inhibition percent of linoleic acid peroxidation was calculated according to the following formula. As a control, no extract linoleic acid emulsion and standard BHT and a-tocopherol:

$$\text{Inhibition}\,(\%) = \frac{\text{1\_absorbance of sample at 500 nm}}{\text{Absorbance of control at 500 nm}} \times 100$$

RESULTS AND DISCUSSION

An antioxidant is a molecule that inhibits the oxidation of other molecules. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols (Sies, 1997).

In a study of the antioxidant activity of chloroform and methanol extract of roots and stems of rhubarb (*Rheum ribes*) has been studied. The antioxidant potential of both extracts of roots and stems were evaluated using different antioxidant. Particularly, higher activity was exhibited by roots with 93.1 and 84.1% inhibitions of chloroform and methanol extracts, while 82.2 and 82.0% inhibitions by stem extracts, respectively. Also; methanol extracts exhibited higher DPPH radical scavenging activity than the corresponding chloroform extracts. In addition, both roots extracts showed more potent superoxide anion radical scavenging activity. Total antioxidant activity was also measured according to the β-carotene bleaching method and all four extracts exhibited stronger activity than known standards, namely butyrate hydroxytoluene (BHT) and α-tocopherol (Ozturk *et al*., 2007).

In this study, the strong antioxidant capacity of *Rheum ribes* in seed and fruits was determined. For *Rheum ribes* fruits' antioxidant capacity was determined to be 2.44±0.212 mmol g^{-1} Gallic acid equivalent antioxidant capacity, while *Rheum ribes* seed's antioxidant capacity was founded to be 5.30±0.245 mmol g^{-1}. Gallic acid equivalent antioxidant capacity. All results presented Fig. 1.

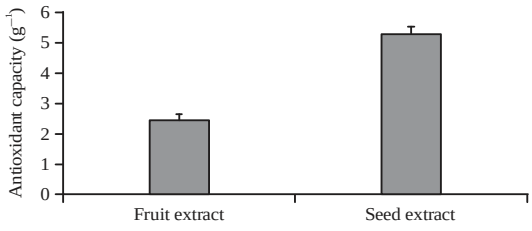

Fig. 1: Antioxidant capacity mmol Gallic acid equivalent/g

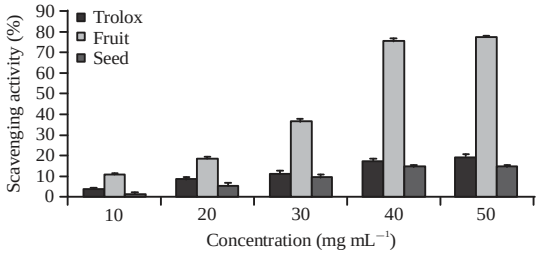

Fig. 2: ABTS radical scavenging activity (%)

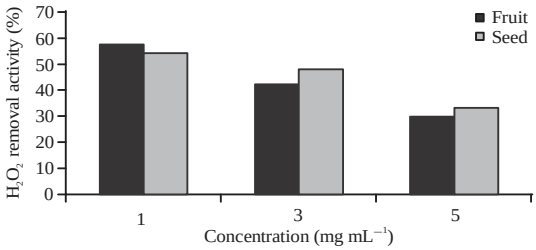

Fig. 3: H_2O_2 removal activity (%)

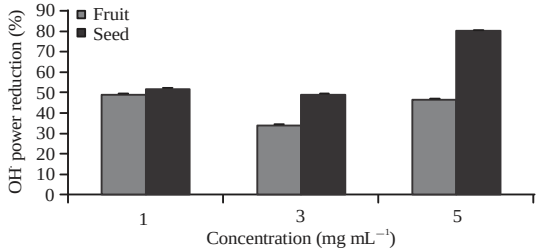

Fig. 4: OH• power reduction (%)

In this study, we have determined that radical scavenging for the fruit extract was at 11-78% levels, while seed extract 2-15% levels. Trolox activity was at the lowest level. When the Concentration increases, the radical scavenging % increase was observed. The highest radical scavenging % was determined at 50 mg mL^{-1} fruit extracts. All date presented in Fig. 2.

In this study, we have determined that both in fruit extracts and seed extracts, when the concentration increases, in H_2O_2 radical removal activity was decreased. H_2O_2 removal activity for fruit extract was at 57.87-30.31%, while seed extract was at 54.57-33.33% levels. All results presented Fig. 3.

OH• Power reduction was high levels in the seed, low levels in fruit extract. The OH• power reduction for the *Rheum ribes* fruit extract 34.0-49 and 22±0.04%, while seed extract to be 49-80±0.04%, all results presented in Fig. 4.

According to a study published in 2003, the authors investigated the only native *Rheum* species growing in Turkey and they found the anthraquinones chrysophanol, physcion and emodin, the flavonoids quercetin, 5-desoxyquercetin, quercetin 3-rhamnoside, quercetin 3-galactoside and quercetin 3-0-rutinoside were isolated (Tosun and Kizilay, 2003).

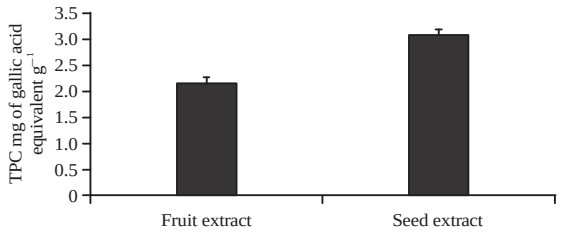

Fig. 5: Antioxidant total phenolic content (TPC) mg Gallic acid equivalent/g

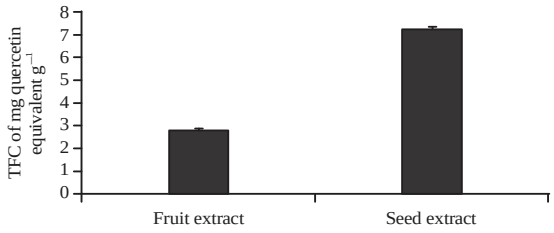

Fig. 6: Antioxidant total flavonoid (TFC) mg of quercetin equivalent/g

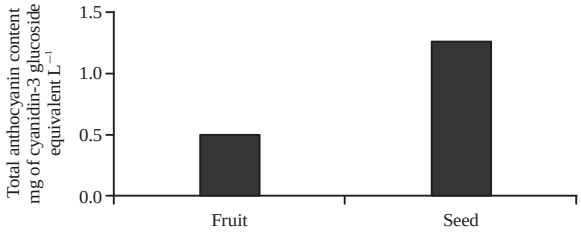

Fig. 7: Anthocyanin content mg of cyaniding-3glucoside/L

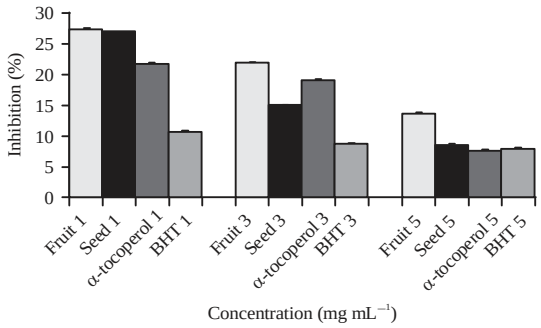

Fig. 8: Anti-lipid peroxidation inhibition (%) (N:3)

In this study, antioxidant total phenolic, antioxidant total flavonoid and total anthocyanin contents determined high seed extract. the total phenolic content, respectively in fruit extract to be 2.312±0.0007 in seed extract to be 3.298±0.0007 mg of GA equivalent/g, total flavonoid content in fruit extract to be 2.79±0.0066 in seed extract to be 7.31±0.1787 mg of Q equivalent/g, total anthocyanin content, respectively in fruit extract to be 0.5 mg of cyanidin-3-glucoside equivalent/L in seed extract to be 1.26 mg of cyanidin-3-glucoside equivalent/L were found. All results presented in Fig. 5-7.

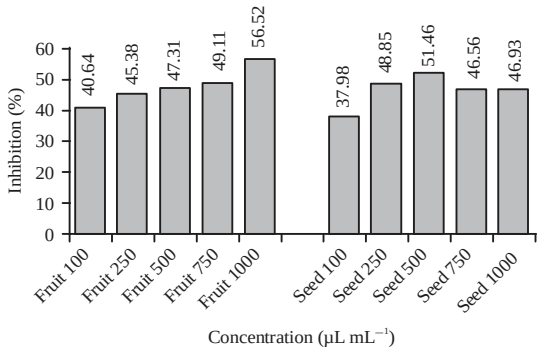

Fig. 9: Protein oxidation inhibition (%) (N:2)

Table 1: Fatty acid composition % (N:1)

Fatty acids	*Rheum ribes* fruits	*Rheum ribes* seeds
Palmitic acid (C16:0)	13.577	34.235
Palmiteolic acid (C16:1)	-	2.546
Steraic acid (C18:0)	-	15.839
Oleic acid (C18:1 w9)	47.749	36.911
Linoleic acid (C18:2 w6)	16.749	3.541
Linolenic (C18:3 w3)	0.741	6.342
Eruric acid (C22:1 w9)	18.431	0.586

Reported that phenolics compounds content of *R. ribes* roots ethanol and aqueous extracts. This component name determined following as Aloe-emodin, respectively in Aqueous extract to be 323.5 μg mL^{-1}, in Ethanol extract 1036 μg mL^{-1}, Emodin, respectively in Aqueous extract to be 1196 μg mL^{-1} in Ethanol extarct 2020 μg mL^{-1}, Chrysophanol, respectively in Aqueous extract to be 636.79 μg mL^{-1} in Ethanol extarct 2243 μg mL^{-1}, Physcion, respectively in Aqueous extract to be 1609 μg mL^{-1}, in Ethanol extarct 1096 μg mL^{-1}, Chlorogenic acid, respectively in Aqueous extract to be 18.8 μg mL^{-1}, in Ethanol extarct 533 μg mL^{-1}, Gallic acid, respectively, in Aqueous extract to be 131 μg mL^{-1}, in Ethanol extarct 1234 μg mL^{-1}, Kaempferol, respectively in Aqueous extract to be 108.64 μg mL^{-1}, in Ethanol extarct 117.6 μg mL^{-1}, Tannic acid, respectively in Aqueous extract to be 40 μg mL^{-1}, in Ethanol extarct 100.4 μg mL^{-1}, Rutin, respectively, in Aqueous extract to be 172.67 μg mL^{-1}, in Ethanol extarct 218.26 μg mL^{-1}. Also same work DPPH activity were determined. The IC_{50} level in Aqueous extract was 25.62 μg mL^{-1}, while, IC_{50} level in Ethanol extract 4.7362 μg mL^{-1} (Abdulla *et al*., 2014).

Another a work determined that the antioxidant activity of *R. ribes* shoot (ASE) and root (ARE) ethyl acetate extract. Consequently, identified that the extracts were revealed to be a potential scavenger of DPPH radicals (IC_{50} value of 206.28 μg mL^{-1} for shoot and 10.92 μg mL^{-1} for root) and ASE phenolic content was 21.11±1.11 mg GAE g^{-1} dried extract, while ARE phenolic content was 207.22±6.96.72 and 50.49±2.03 mg GAE g^{-1} dried extract. Also were determined, respectively in ASE extract to be 2.29±1.04 mg of CAE g^{-1} dried extract, in ARE extract to be 50.49±2.03 mg of CAE g^{-1} dried extract (Uyar *et al*., 2014).

A published work in the year 2000; stated that the authors in the young shoots of *Rheum ribes* L ranges of levels of A vitamins 0.255±0.019-0.363±0.015 μg g^{-1}, E vitamins 0.614±0.014-0.765±0.021 μg g^{-1}, C (Karatas and Gur, 2000).

Membrane lipids are particularly susceptible to Lipid Peroxidation (LP). LP can be defined as oxidative deterioration of lipid contains a number of carbon- carbon double bonds (Rice-Evans and Burdon, 1993). A large number of toxic byproducts are formed during LP.

In this study; anti-lipid peroxidation results, while high in fruits, in seeds were lower. All results presented Table 1 and Fig. 8. This situation; Unsaturated fatty acid content of seed can associate with low (Linolenic acid (C18: 3, w3) outside). The greater the degree of unsaturation in a fatty acid the more defenseless it is to lipid peroxidation. Because Oxidation primarily occurs with unsaturated fats due to the more double bonds in the fatty acid. Fatty acid information has been verified our previously work (Yildirım *et al*., 2014). Also, fatty acid information has been presented in this study as table.

Protein carbonylation is the most commonly used measure of oxidative modification of proteins. Protein carbonylation is the introduction of a carbonyl group (aldehydes or ketones) into a protein through oxidation of arginine, lysine, threonine, or proline residues through a multi-step series of reactions thought to involve site-directed formation of oxidative species (Stadtman, 1990).

In this work determined that *Rheum ribes* fruit extract's protein oxidation was 40.64-56.5% and of the seed extract 37.98-51.46%. All results presented Table 1 and Fig. 9.

In a work the antioxidant activty, methanolic and aqueous extracts of *Rheum emodi* Wall was investigated. Consequently, the aqueous extract, though inferior to methanolic extract in its antioxidant potential exhibited efficiency in DNA protection, while the methanolic extract failed to protect the DNA. The amount of total polyphenolics in the extracts was measured by spectrophotometric method. The methanolic extract contained higher polyphenolic contents than aqueous extract (Rajkumar *et al*., 2011).

According to a study published in the 2009 years the antioxidant activity of *Rheum palmatum* extracts was determined in one-, two- and three-year-old roots of rhubarb fertilized with nitrogen at the rates of 50, 100 and 200 kg N ha^{-1}. Consequently, at the highest values were determined in one-year-old plants, antioxidant activity ranged the level of 112-203 μM Trolox/g and total phenolic compounds average content was 22 mg GAE g^{-1} FW. Two-year-old roots were characterized by 3-11% and three-year-old ones by 15-23% lower antioxidant activity and phenolic compounds content. When nitrogen dose increased, polyphenols content, DPPH and FRAP values increased as well, although, ABTS showed a different tendency (Sokol-Letowska *et al*., 2009).

Takeoka and co-worker identified in their work that Antioxidant activity (ABTS assay), total phenolics and total

anthocyanins were determined in the petioles of twenty-nine rhubarb (*Rheum* spp.) varieties In result identified that antioxidant activity ranged from 463±50 (*Rheum officinale*) to 1242±2 μmol Trolox/g, the phenolic content varied from 673±41 (Loher Blut) to 4173±23 mg GAE/100 g. The percentages of two main anthocyanins in rhubarb, cyanidin 3-glucoside and cyanidin 3-rutinoside varied from 66.07:33.93, respectively, in Valentine to 9.36:90.64, respectively in *R. officinale* (Takeoka *et al*., 2013).

Other a work researchers, the phenolic constituents in the roots of *Rheum officinale* and *Rubia cordifolia* were identified with the aid of high-performance liquid chromatography and liquid chromatography-mass spectrometry and by comparison with authentic standards. A total of 17 hydroxyanthraquinones, gallic acid and tannins were isolated and fourteen of them were identified, being the main phenolic component. Hydroxy anthraquinones were the predominant antioxidant phenolic constituents in the roots of *R. cordifolia* and tannins and gallic acid were the predominant antioxidant phenolic constituents in the roots of *R. officinale*.

CONCLUSION

Since, the synthetic antioxidants being used today possess adverse effects, it is of increasing importance to find out and derive natural antioxidants from herbal sources, to reveal their effects and to use them. Our work and the information in the relevant literatures confirmed that, a *Rheum ribes* strong antioxidant and it can prevent oxidative damage. Thus, a scientific foundation for the use of this plant in medicine can be established to improve the healthcare of local users.

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