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Antioxidant Activity of Different Parts of Isgin (*Rheum ribes* L.)

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Total antioxidant activity of ether, ethanol and water extracts of different parts of isgin (*Rheum ribes*) was investigated using thiocyanate method for antioxidant activity. Significant differences ($p < 0.05$) were found between the control and the samples containing of isgin (*Rheum ribes*) extracts. Butylated hydroxyanisole (BHA), a powerful synthetic antioxidant was used as a positive control. All of the extracts were compared with BHA, but were not statistically significant difference ($p > 0.05$). Among the extracts, ethanol extract of peel has the highest antioxidant activity. In addition, reducing power, total phenolic content, radical scavenging activity and superoxide anion radical scavenging activity of isgin (*Rheum ribes*) extracts were examined, in this study. The phenolic content analyses of extracts were expressed as gallic acid equivalents per 1000 µg extract.

Key Words: *Rheum ribes*, Antioxidant activity, Superoxide, Isgin, Phenolic content.

INTRODUCTION

Free radicals are produced in normal or pathological cell metabolism from xenobiotics or through ionizing radiation. Electron acceptors such as molecular oxygen react easily with free radicals to become radicals themselves. The primary derivatives of oxygen, such as superoxide anion radicals ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$) and the singlet oxygen ($^1O_2$) play an important role in mediating reactive oxygen species (ROS)-related effects. Short-lived reactive species generated *in situ* can react with non-radicals and produce chain reactions[1,2].

Antioxidants can be defined as compounds that inhibit or delay, but do not completely prevent, oxidation. There are two basic categories of antioxidants, namely synthetic antioxidants are phenolic structures with

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varying degrees of alkyl substitutions, while natural antioxidants can be phenolic compounds, quinones or lactones as well as polyphenolics[3].

Many naturally occurring antioxidative compounds from various plant sources have been identified as free radical inhibitors, active oxygen scavengers or as reducing agents *in vitro*[4-10].

Rheum ribes grows in the east Anatolia Mountains especially Agri, Bingol, Elazig, Hakkari, Kars and Van regions. This plant is called locally as isgin. Edible parts of this plant are leaf stems that eaten raw or cooked by the local people[11,12]. The stems of leaves and flowers have a sour taste, stomachic, anti-nauseant, antivomit and constipation effect. In addition it has beneficial effects for haemorrhoids, measles and smallpox diseases[13]. Some species as *Rheum palmatum* have cytotoxic and antiviral effect. The aim of this study was to investigate antioxidant activity of *Rheum ribes* extracts using different methodology such as ferric thiocyanate methods, reducing power, phenolic content, radical scavenging activity and superoxide anion radical scavenging activity.

EXPERIMENTAL

Ammonium thiocyanate, BHA, phosphate buffered saline (PBS) tablet, β-nicotinamide adeninedinucleotide, reduced form (β-NADH), Nitro blue tetrazolium (NBT) and gallic acid were purchased from Sigma Chemical Co., St. Louis, MO, USA. Ferrous chloride and hydrochloric acid were purchased from Carlo Erba, Milano, Italy. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals, Folin-Ciocalteou's phenol reagent (FCR), linoleic acid, Tween 20 (polyoxyethylen-sorbitan-monolaurat) and N-methylphena-zonium methyl sulfate (PMS) were purchased Fluka Buchs, Switzerland. Sodium dihydrogen phosphate and trichloracetic acid (TCA) were purchased from Merck, Darmstadt, Germany. All the other chemicals were of analytical grade.

Preparation of extracts

Stems of flowers and roots of isgin (*Rheum ribes*) were purchased from a salesman in Erzurum. The peels and flesh of stems were separated with a knife. The roots and other parts of *Rheum ribes* were chopped into small parts with a blender and freeze-dried at 10 µm-Hg and -50ºC using Labconco freeze dryer. The freeze dried samples (14 g) were used for extraction. Ether extraction was performed with a Soxhlet apparatus until extraction solvents became colourless. Extraction was followed by filtration and evaporation of the filtrate to dryness by a rotary evaporator at 30ºC. The residue obtained after filtration was left in a dark place at room temperature to become dry. Dried residue, after ether had been removed from it was mixed with ethanol in a screw-capped Erlenmeyer flask and was shaken in a shaker

to obtain ethanol extract. Extraction was continued until extraction solvents became colourless. The obtained extracts were filtered, the filtrate was collected and ethanol was removed by a rotary evaporator at 40ºC to obtain dry extract. The residue obtained after filtration was left in a dark place at room temperature to become dry. Dried residue was mixed with boiling distilled water and then was stirred on a hot plate for 15 min and subsequently was filtered. Finally, the filtrate was freeze-dried in Labconco freeze-dryer at 10 µm-Hg and -50ºC. Dried extracts were stored at 4ºC. Stock solutions were prepared as 1 mg extract in 1 mL solution. Solvents were chosen as water for dried water extract and ethanol for dried ether and ethanol extract.

Determination of total phenolic content: Determination of total phenolic content was carried out according to Singleton and co-workers[14] with a slight modification. Extract solution (contains 1000 µg of extracts) was transferred in a volumetric flask and then the final volume was adjusted to 23 mL by the addition of distilled water. Afterward, 0.5 mL of Folin-Ciocalteu reactive was added into this mixture and after 3 min, 1.5 mL of Na_2CO_3 (2 %) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature and then absorbance was measured at 760 nm. Gallic acid was used as the standard for the calibration curve. Total phenolics were calculated as µg gallic acid equivalents (GAEq) using the following linear equation based on the calibration curve:

$$GAEq = \frac{\text{Absorbance (760 nm)} - 0.0180}{0.0023}$$

Total antioxidant activity by ferric thiocyanate method: Total antioxidant activity was determined by thiocyanate method[15]. In order to evaluate the antioxidant activity of extracts, 300 µL stock solutions which previously mentioned were mixed linoleic acid emulsion includes Tween-20 (2.5 mL; 0.02 M; pH 7.4) and mixture was completed to 5 mL final volume with phosphate buffer saline (0.01 M, pH 7.4) in a test tube and placed in darkness at 40ºC to accelerate oxidation. At intervals during incubation, 100 µL sample, 4.7 mL ethanol, 100 µL $FeCl_2$ (20 mM, in 3.5 % HCl solution), 100 µL 30 % NH_4SCN were mixed in a test tube and the amount of peroxide was recorded by reading the absorbance at 500 nm at intervals.

Reducing power: This method was carried out according to Oyaizu method[16]. For this purpose, 300 µL stock solutions were completed to 1 mL volume with distilled water and then phosphate buffer saline (2.5 mL, 0.2 M, pH 6.0) and 2.5 mL of $K_3[Fe(CN)_6]$ (1 %) were added to this solution and then the mixture was incubated at 50ºC for 20 min. After incubation, 2.5 mL TCA (10 %) was added to this reaction mixture. The

upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increasing reducing power.

DPPH radical scavenging activity: Radical scavenging activity was carried out according to methodology described by Blois[17] with slight modification. DPPH radical solution (2 mM) in ethanol was prepared and then 1 mL of this solution was mixed with 3 mL of extract solution (contains 3 mg extract) in ethanol; finally, after 0.5 h, the absorbance was measured at 515 nm. Decreasing absorbance was accepted as increasing radical scavenging activity.

Superoxide scavenging activity: Superoxide radical scavenging activity was performed according to Liu and co-workers[18] with a modification. Superoxide radicals generated in a PMS-NADH system by oxidation of NADH and assayed by the reduction of NBT. 300 µL of sample (contains 300 µg extract), 300 µL NADH (468 mM), 300 µL NBT (300 µM), 300 µL PMS (60 µM) and 1.8 mL of *tris*-HCl buffer solution (16 mM, pH 8.0) were mixed in a test tube. The colour reaction (after 15 min) between superoxide radicals and NBT was detected at 560 nm using a spectrophotometer (Jasco V-530). L-ascorbic acid was used as a control. The per cent inhibition was calculated using the following formula:

$$O_2^{\bullet -} \text{ scavening effect } (\%) = \left(\frac{A - A_1}{A} \right) \times 100$$

where A was the absorbance of the control and A_1 was the absorbance of the sample.

Statistical analysis: Statistical analysis involved use of the SPSS 10.0 software. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple-range tests.

RESULTS AND DISCUSSION

Antioxidant effectiveness of natural products are often mostly due to phenolic compounds[19]. It was reported that phenolic compounds play an important role in inhibiting auto-oxidation of the oils[20,21]. Therefore, it is important that total phenol assays. The results show that extracts of *Rheum ribes* contains very high phenolic content (Fig. 1). Especially ethanol extracts had higher phenolic content than other extracts.

The data of linoleic acid peroxidation were plotted in Figs. 2 and 3. Highly significant differences ($p < 0.05$) were found between the control and all of the samples that containing isgin (*Rheum ribes*) extracts (RRE). The antioxidant activities of all of the extracts were higher than BHA, a

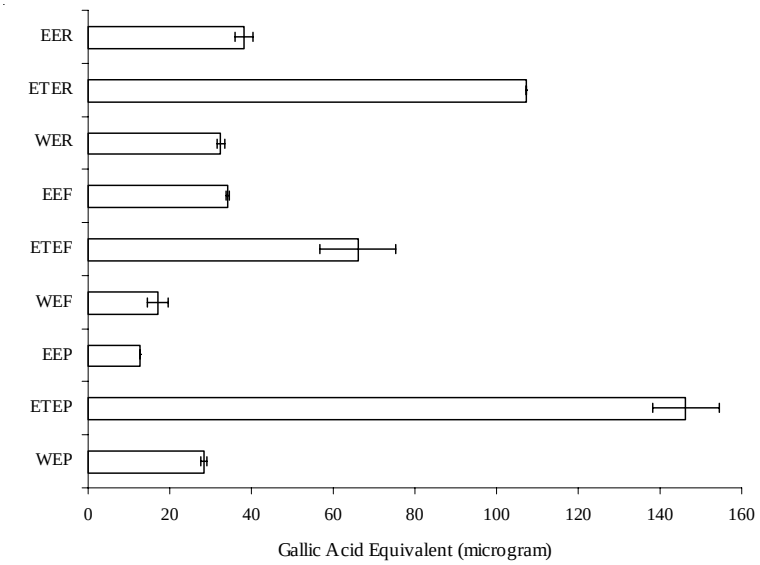

Fig. 1. Amount of total phenols of *Rheum ribes* extracts. All of the samples had 1000 µg extracts (EER: Ether extract of root of *Rheum ribes*, ETER: Ethanol extract of root of *Rheum ribes*, WER: Water extract of root of *Rheum ribes*, EEF: Ether extract of flesh of *Rheum ribes*, ETEF: Ethanol extract of flesh of *Rheum ribes*, WEF: Water extract of flesh of *Rheum ribes*, EEP: Ether extract of peel of *Rheum ribes*, ETEP: Ethanol extract of peel of *Rheum ribes*, WEP: Water extract of peel of *Rheum ribes*, BHA: Butylated hydroxyanisole)

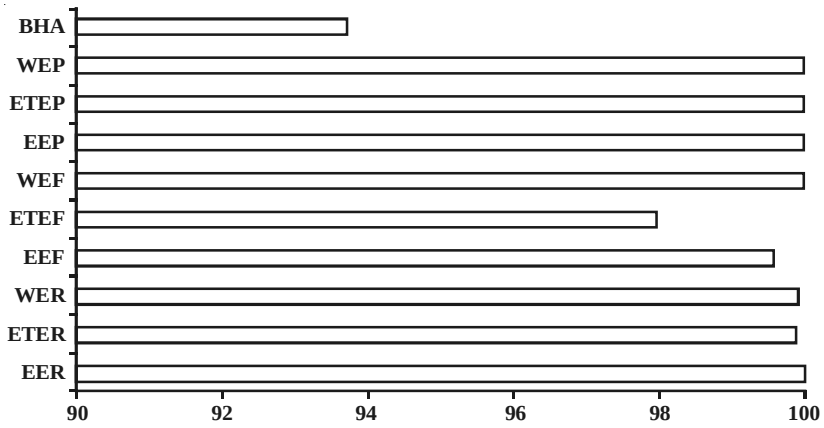

Fig. 2. Antioxidant activity of 300 µg of different extract of *Rheum ribes*) (EER: Ether extract of root of *Rheum ribes*, ETER: Ethanol extract of root of *Rheum ribes*, WER: Water extract of root of *Rheum ribes*, EEF: Ether extract of flesh of *Rheum ribes*, ETEF: Ethanol extract of flesh of *Rheum ribes*, WEF: Water extract of flesh of *Rheum ribes*, EEP: Ether extract of peel of *Rheum ribes*, ETEP: Ethanol extract of peel of *Rheum ribes*, WEP: Water extract of peel of *Rheum ribes*, BHA: Butylated hydroxyanisole)

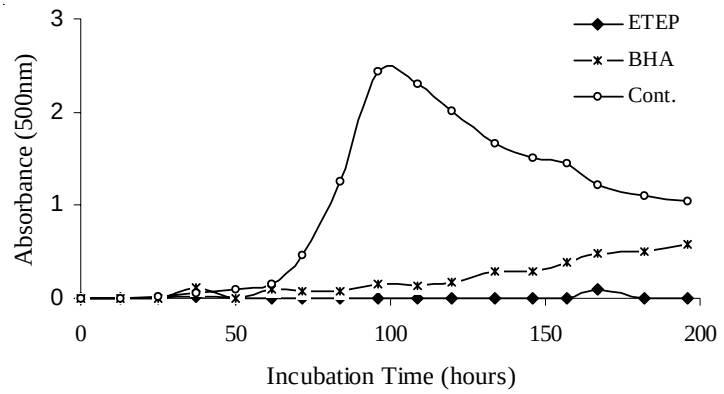

Fig. 3. Reducing power of *Rheum ribes* extracts (contains 300 µg extract) on lipid peroxidation by thiocyanate method (EER: Ether extract of root of *Rheum ribes*, ETER: Ethanol extract of root of *Rheum ribes*, WER: Water extract of root of *Rheum ribes*, EEF: Ether extract of flesh of *Rheum ribes*, ETEF: Ethanol extract of flesh of *Rheum ribes*, WEF: Water extract of flesh of *Rheum ribes*, EEP: Ether extract of peel of *Rheum ribes*, ETEP: Ethanol extract of peel of *Rheum ribes*, WEP: Water extract of peel of *Rheum ribes*, BHA: Butylated hydroxyanisole)

known synthetic antioxidant, but the differences were not statistically significant ($p > 0.05$) (Fig. 3). The ethanol extracts of peels of isgin (*Rheum ribes*) when, compared with other parts of this plants had higher antioxidant activity but no significant difference ($p > 0.05$). Among of the peel extracts, ethanol extract of peel showed higher antioxidant activity. It was reported that reducing power was associated with antioxidant activity[22]. Pitotti and co-workers[23] noted that the antioxidative effects of Maillard reaction products were shown to be concomitant with the development of reducing power. Okuda and co-workers[24] elucidated that tannin in medicinal plants and drugs are effective against liver injury by inhibiting the formation of lipid peroxide owing to their reducing effect on coexisting substances or by preventing their oxidation. Reducing power of extracts was shown in Fig. 5. When it was compared with BHA, reducing power of samples was less but all of the extracts showed effective reducing ability.

As shown from data (Fig. 5.) all of the extracts of *Rheum ribes* showed radical scavenging activity. This method was carried out according to Gülçin and co-workers[25]. The DPPH assay measures hydrogen atom (or one electron) donating activity and hence provides a measure of free radical scavenging antioxidant activity. DPPH is a purple-coloured stable free radical. It becomes reduced to yellow coloured stable free radical diphenylpicryl hydrazine[26].

Superoxide scavenging effect of *Rheum ribes* extracts was shown in Table-1. As shown from results all the extracts showed superoxide

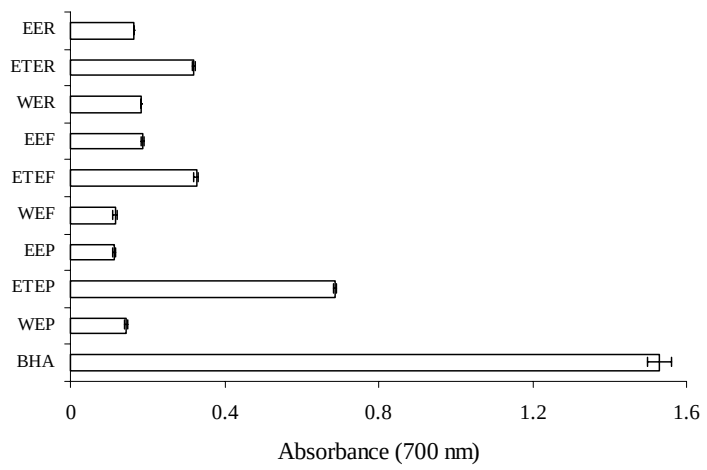

Fig. 4. The inhibition effects of *Rheum ribes* extracts (contains 300 µg extract) on lipid peroxidation by thiocyanate method (EER: Ethanol extract of root of *Rheum ribes*, ETER: Ether extract of root of *Rheum ribes*, WER: Ethanol extract of root of *Rheum ribes*, EEF: Ethanol extract of flesh of *Rheum ribes*, ETEF: Ether extract of flesh of *Rheum ribes*, WEF: Ethanol extract of flesh of *Rheum ribes*, EEP: Ethanol extract of flesh of *Rheum ribes*, ETEP: Ether extract of flesh of *Rheum ribes*, WEP: Ethanol extract of flesh of *Rheum ribes*)

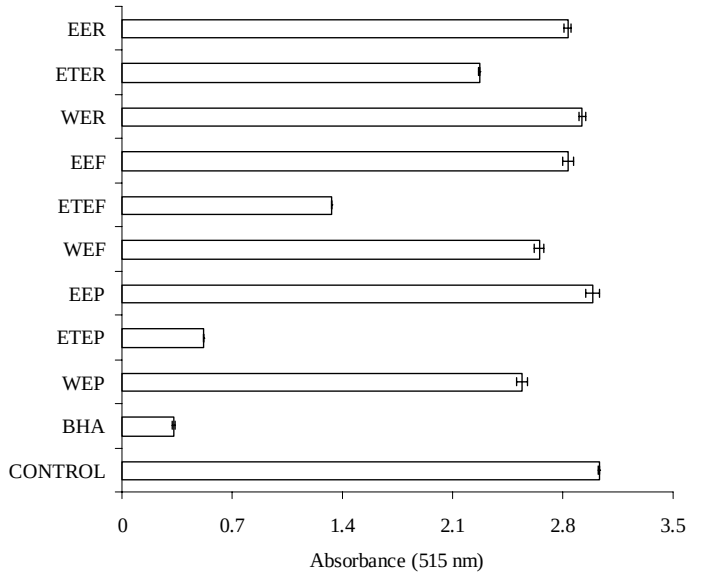

Fig. 5. Free radical scavenging activity of *Rheum ribes* extracts (contains 300 µg extract) measured using the DPPH assay. Lower absorbance indicates higher reactivity with DPPH radicals (n: 3; mean ± SD, (EER: Ether extract of root of *Rheum ribes*, ETER: Ethanol extract of root of *Rheum ribes*, WER: Water extract of root of *Rheum ribes*, EEF: Ether extract of flesh of *Rheum ribes*, ETEF: Ethanol extract of flesh of *Rheum ribes*, WEF: Water extract of flesh of *Rheum ribes*, EEP: Ether extract of peel of *Rheum ribes*, ETEP: Ethanol extract of peel of *Rheum ribes*, WEP: Water extract of peel of *Rheum ribes*, BHA: Butylated hydroxyanisole)

scavenging activity. Ethanol and water extracts had higher effect than ether extracts.

TABLE-1
SUPEROXIDE ANION SCAVENGING ACTIVITY OF ROOT FLESH AND PEEL OF *Rheum ribes* EXTRACTS. ALL OF THE SAMPLES HAD 300 μg EXTRACTS. ASCORBIC ACID WAS USED AS CONTROL

	Ethanol	Ether	Water
Root	58.25 ± 0.36	87.01 ± 0.21	86.69 ± 0.10
Flesh	65.65 ± 0.33	80.58 ± 0.29	80.88 ± 0.41
Peel	38.96 ± 0.25	87.07 ± 0.08	85.41 ± 0.20

Isgin (*Rheum ribes*) includes organic acids, vitamin C, phenolic acids, tannins, flavonoids and anthraquinones and some species of Rheum are used for therapy of cancer[27]. Anthraquinones has lower antioxidant activity from BHA[28], but in present study data shows that isgin (*Rheum ribes*) extracts includes anthraquinones have higher antioxidant activity than BHA ($p > 0.05$). Probably, other contents of this plant have synergist effect for antioxidant activity of anthraquinones.

In conclusion, the results obtained from present study indicated that isgin (*Rheum ribes*) may be accepted a powerful antioxidant. Therefore, it is suggested that further work could be done to elucidate the chemistry of *Rheum ribes*.

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