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Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant

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Abstract

The antioxidant activity of chloroform and methanol extract of roots and stems of Rhubarb (*Rheum ribes* L.), which are used for medicinal purposes and also its fresh stems and petioles are consumed as vegetable, was studied. The antioxidant potential of both extracts of roots and stems were evaluated using different antioxidant tests, namely total antioxidant (lipid peroxidation inhibition activity), DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging, superoxide anion radical scavenging, ferric reducing power, and cupric reducing power (CUPRAC), and metal chelating activities. Total antioxidant activity was also measured according to the β-carotene bleaching method, and all four extracts exhibited stronger activity than known standards, namely butylated hydroxytoluene (BHT) and α-tocopherol. 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. However, both methanol extracts exhibited higher DPPH radical scavenging activity than the corresponding chloroform extracts, moreover, methanol extract of the stems showed better activity than BHT. In addition, both root extracts showed more potent superoxide anion radical scavenging activity than BHT, and comparable with well known radical scavenger L-ascorbic acid. Except chloroform extract of the roots, the other three extracts exhibited better metal chelating activity than quercetin. Also, total phenolic and flavonoid contents in both extracts of the roots and stems of *R. ribes* were determined as pyrocatechol and quercetin equivalents, respectively.

Keywords: *Rheum ribes*; Rhubarb; Işkın; Antioxidant activity; Edible plant; Medicinal plant

1. Introduction

Rheum species are medicinally important plants due to the presence of anthracene derivatives occurring in the subterranean parts of the plant. *Rheum ribes* L. (Polygonaceae) is the source of one of the most important crude drugs in the Middle East (Kashiwada, Nonaka, Nishioka, & Yamagishi, 1988). Rhubarb roots are used as oriental laxative medicine and an antipsoriatic drug in Iran (Shokravi & Agha Nasiri, 1997). *R. ribes* is locally known as "ışkın, uşgun or, uçgun" and grown mostly in Eastern Turkey, Lebanon and Iran. In Turkey, Polygonaceae family is represented by eight genera

Abbreviations: *L-AA*, L-ascorbic acid; EDTA, ethylene diamine tetra acetic acid; TCA, trichloroacetic acid; FCR, Folin–Ciocalteu's reagent; DPPH, 1,1-diphenyl-2-picrylhydrazyl; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TOC, α-tocopherol; NADH, nicotinamide adenine dinucleotide; NBT, nitrotetrazolium blue chloride; PMS, *N*-methylphenazonium methyl sulfate; PEs, pyrocatechol equivalents; QEs, quercetin equivalents; SD, standard deviation; CARR, chloroform extract of the stems; MARR, methanol extract of the stems; CRRR, chloroform extract of the roots; MRRR, methanol extract of the roots.

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and 70 species. *R. ribes* is the only *Rheum* species growing in Turkey (Cullen, 1966). Young shoots and petioles of *R. ribes* are used against diarrhea as well as stomachic and antiemetic while juice of some parts of the plant is used against hemorrhoids, measles, smallpox and cholagogue (Baytop, 1999). Its fresh stems and petioles are consumed as vegetable, and stems are also eaten fresh, which are used as digestive and appetizer in Bitlis, Eastern Turkey, while the roots are used to treat diabetes (Abu-Irmaileh & Afifi, 2003; Tabata et al., 1994), hypertension (Abu-Irmaileh & Afifi, 2003), obesity (Abu-Irmaileh & Afifi, 2003), ulcer (Tabata et al., 1994), diarrhea (Tabata et al., 1994) and as antihelmintic and expectorant (Tabata et al., 1994). The decoction extract of *R. ribes* roots possess significant blood sugar lowering activity in alloxan-induced diabetic mice, although this extract did not show hypoglycemic action in healthy mice (Özbek, Ceylan, Kara, Özgökce, & Koyuncu, 2004). There are a few chemical studies on *R. ribes* in Turkey; in one study, the roots of the plant collected from Erzincan, chrysophanol, physcion, rhein, aloeemodin, physcion-8-*O*-glucoside, aloeemodin-8-*O*-glucoside, sennoside A and rhaponticin have been isolated (Meriçli & Tuzlacı, 1990; Tuzlaci & Meriçli, 1992). In another study, the aerial parts of *R. ribes*, collected from Hakkari, have also been studied, and chrysophanol, physcion, emodin, quercetin, 5-desoxyquercetin, quercetin 3-*O*-rhamnoside, quercetin 3-*O*-galactoside and quercetin 3-*O*-rutinoside have been found (Tosun & Akyüz-Kızılay, 2003).

The roots, stalk and leaves of extracts of *R. ribes*, grown in Iran, were investigated for their antimicrobial activity against gram negative pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus* spp., *Pseudomonas aeruginosa* and *Neisseria gonorrhoeae*. The roots and leaves extracts have demonstrated significant antimicrobial activities (Bazzas, Khajehkaramadin, & Shokooheizadeh, 2005). In another antimicrobial activity study against *Bordetella bronchiseptica*, *Micrococcus luteus*, *K. pneumoniae*, *Serratia marcescens* and three isolates of *Staphylococcus aureus*, the extract of roots of *R. ribes* exhibited an effective antibacterial activity on *M. luteus*, *K. pneumoniae* and *S. aureus* (Bonjar, 2004a; Bonjar, 2004b). Antiviral activities of *R. ribes* were also evaluated against Herpes simplex virus and Sindbis virus and its extracts showed high anti-Herpes simplex virus activity (Hudson, Lee, Sener, & Erdemoglu, 2000).

Some studies on *Rheum* species rather than *R. ribes* were carried out; *Rheum emodi* (Krenn et al., 2003), *Rheum officinale* (Cai, Sun, Xing, & Corke, 2004; Leonard et al., 2006), *Rheum maximowiczii* (Kogure et al., 2004), *Rheum tangiticum*, *Rheum palmatum*, *Rheum coreanum* and *Rheum undulatum* (Matsuda et al., 2001) have been studied for their antioxidant activity. From *R. undulatum*, four antraquinone glucosides, a naphthalene glucoside and ten stilbenes were isolated, and their radical scavenging activities ($DPPH^{\cdot}$ and $O_2^{\cdot-}$) were investigated. The stilbenes and the naphthalene glucosides have been shown to be active; however anthraquinones and sennosides were not active. In addition, most stilbenes isolated from *R. undulatum* inhibited lipid peroxidation on erythrocyte membrane ghost system method (Matsuda et al., 2001).

Food such as fruits, vegetables and grains are reported to contain a wide variety of antioxidant components, including phenolic compounds. These compounds are found to be well correlated with antioxidant potential (Katalinić, Miloš, Modun, Music, & Boban, 2004).

Considering the phenolic constituent profile of *R. ribes*, particularly their flavonoids, stilbenes and anthraquinones, they appear to provide a potential source of antioxidants. Synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ) have been widely used in the food industry to prevent oxidative deterioration, but BHA and BHT are suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Wichi, 1986). Some chemicals that occur naturally in plants have begun to receive much attention as safe antioxidants, as they have been consumed by people and animals for years (Namiki, 1990). Therefore, the development and utilization of more effective antioxidants of natural origin are desired. Regarding to the consumption of Rhubarb in rural area of eastern Turkey as well as in the Middle-East, and some other Asian countries we aimed to investigate antioxidant activities of *R. ribes* through different antioxidant activity tests by comparing with standards such as α-tocopherol, quercetin, BHT, BHA and L-ascorbic acid in the present study.

2. Materials and methods

2.1. Chemicals and spectral measurements

Potassium ferricyanide, ferrous chloride, ferric chloride, chloroform, methanol, pyrocatechol, L-ascorbic acid (L-AA), quercetin, copper (II) chloride, ethylenediaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from E. Merck (Darmstadt, Germany). Folin-Ciocalteu's reagent (FCR), β-carotene, linoleic acid, polyoxyethylene sorbitan monopalmitate (Tween-40), 1, 1-di-phenyl-2-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and α-tocopherol (TOC), 3-(2-pyridyl)-5,6-bis(4-phenyl-sulphonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), neocuproine and ammonium acetate were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Sternheim, Germany). Nitrotetrazolium blue chloride (NBT) and *N*-methylphenazonium methyl sulphate (PMS) were obtained from Fluka Chemie (Fluka Chemie GmbH, Sigma-Aldrich, Sternheim, Germany). All other chemicals and solvents were of analytical grade. All UV–Vis measurements were recorded on a Shimadzu UV–1601 (Kyoto, Japan).

2.2. Plant material and preparation of extracts

The stems and the roots of *R. ribes* were obtained from a local market in Istanbul, but were collected from Bitlis,

Turkey. The roots (250 g) and stems (250 g) were extracted separately with 2.5 L chloroform for four times (24 h × 4) at room temperature (25 °C), filtered and evaporated to dryness *in vacuo* to leave 10.55 g chloroform extract of the stems (CARR) and 13.05 g chloroform extract of the roots (CRRR). The residue plant materials were extracted with 2.5 L aqueous methanol (1:1, v/v) for four times (24 h × 4) at room temperature (25 °C), filtered and evaporated to dryness *in vacuo* to leave residue extracts. Methanol extract (12.51 g) of the stems (MARR) and methanol extract (13.15 g) of the roots (MRRR) were obtained.

2.3. Determination of the antioxidant activity with the β-carotene bleaching method

The antioxidant activity of chloroform and methanol extracts of the roots and stems of *R. ribes* was evaluated using β-carotene–linoleic acid model system (Miller, 1971). β-carotene (0.5 mg) in 1 mL of chloroform was added to 25 μL of linoleic acid, and 200 mg of Tween 40 emulsifier mixture. After evaporation of chloroform under vacuum, 100 mL of distilled water saturated with oxygen, were added by vigorous shaking. Four thousand microlitres of this mixture were transferred into different test tubes containing different concentrations of the sample. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. The emulsion system was incubated for 2 h at 50 °C. A blank, devoid of β-carotene, was prepared for background subtraction. Quercetin, BHT and α-tocopherol were used as standards.

2.4. Free radical scavenging activity

The free radical scavenging activity of chloroform and methanol extracts of the roots and stems of *R. ribes* was determined by the DPPH assay described by Blois (1958). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 4 mL of this solution was added to 1 mL of sample solutions in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation (Gülçin, Oktay, Kireçci, & Küfrevioğlu, 2003):

$$\text{DPPH Scavenging Effect } (\%) = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

2.5. Superoxide anion scavenging activity

Measurement of superoxide anion scavenging activity of chloroform and methanol extracts of the roots and stems of *R. ribes* was based on the method described by Liu, Ooi, and Chang (1997) with slight modification. Superoxide radicals are generated in PMS–NADH systems by oxidation of NADH and assayed by the reduction of NBT. In this experiment, superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0) containing 1 mL of NBT (50 μM) solution, 1 mL NADH (78 μM) solution and sample solutions. The reaction started by adding 1 mL of PMS solution (10 μM) to the mixture. The reaction mixture was incubated at 25 °C for 5 min, and the absorbance at 560 nm was measured against blank samples. Decreased absorbance of the reaction mixture indicates increased superoxide anion scavenging activity. The percentage inhibition of superoxide anion radical generation of three parallel measurements was calculated using the following formula:

$$\text{Inhibition } (\%) = [(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}] \times 100$$

where A_{control} is the absorbance of control and A_{sample} is the absorbance in the presence of the extracts or standards. (Gülçin et al., 2003).

2.6. Reducing power

The reducing power of chloroform and methanol extracts of the roots and stems of *R. ribes* was determined according to the method of Oyaizu (1986). Sample solutions at different amounts were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%). After the mixture was incubated at 50 °C for 20 min, 2.5 mL of TCA (10%) were added and the mixture was centrifuged at 1000*g* (MSE Mistral 2000, London, UK) for 10 min. Supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and 0.5 mL of ferric chloride (0.1%), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

2.7. Cupric reducing antioxidant capacity (CUPRAC)

The cupric reducing antioxidant capacity of the extracts of roots and stems of *R. ribes* was determined according to the method of Apak, Güçlü, Özyürek, and Karademir (2004). To a test tube 1 mL each of 10 mM Cu (II), 7.5 mM neocuprine, and NH_4Ac buffer (1 M, pH 7.0) solutions were added. Extracts at different concentrations were added to the initial mixture so as to make the final volume 4.1 mL. The tubes were stoppered, and after 1 h, the absorbance at 450 nm was recorded against a reagent blank.

2.8. Metal chelating activity

The chelating activity of *R. ribes* extracts on Fe^{2+} was measured as reported by Decker and Welch (1990). The extracts were added to a solution of 2 mM $FeCl_2$ (0.1 mL). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). The mixture was shaken vigorously and left standing at room temperature for 10 min.

After the mixture reached equilibrium, the absorbance was determined at 562 nm, results were given as percentage inhibition.

2.9. Determination of total phenolic concentration

The concentrations of phenolic content in all extracts were expressed as microgram of pyrocatechol equivalents (PEs), determined with FCR according to the method of Slinkard and Singleton (1977). One millilitre of the solution (contains 1 mg) of the extracts in methanol was added to 46 mL of distilled water and 1 mL of FCR, and mixed thoroughly. After 3 min, 3 mL of sodium carbonate (2%) were added to the mixture and shaken intermittently for 2 h at room temperature. The absorbance was read at 760 nm. The concentration of phenolic compounds was calculated according to the following equation that was obtained from standard pyrocatechol graph:

$$\text{Absorbance} = 0.08237\ \text{pyrocatechol}\ (\mu g) + 0.00058 \quad (R^2 : 0.9985)$$

2.10. Determination of total flavonoid concentration

Measurement of flavonoid concentration of the extracts was based on the method described by Moreno, Isla, Sampietro, and Vattuone (2000) with a slight modification and results were expressed as quercetin equivalents. An aliquot of 1 mL of the solution (contains 1 mg) extracts in methanol were added to test tubes containing 0.1 mL of 10% aluminium nitrate, 0.1 mL of 1 M potassium acetate and 3.8 mL of methanol. After 40 min at room temperature, the absorbance was determined at 415 nm. Quercetin was used as a standard (Park, Koo, Ikegaki, & Contado, 1997). The concentrations of flavonoid compounds were calculated according to following equation that was obtained from the standard quercetin graph:

$$\text{Absorbance} = 0.06648\ \text{quercetin}\ (\mu g) - 0.01586 \quad (R^2 : 0.9972)$$

2.11. Statistical analysis

All data on all antioxidant activity tests are the average of triplicate analyses. The data were recorded as mean ± SD. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by student's-t test, p-values < 0.05 were regarded as significant, p-values < 0.01 were regarded as very significant.

3. Results and discussion

There are several methods for determination of antioxidant activities. The chemical complexity of extracts, often a mixture of dozens of compounds with different functional groups, polarity and chemical behaviour, could lead to scattered results, depending on the test employed. Therefore, an approach with multiple assays for evaluating the antioxidant potential of extracts would be more informative and even necessary. In this study, mainly six methods, β-carotene bleaching method, DPPH radical scavenging activity, superoxide anion radical scavenging activity, metal chelating activity, ferric reducing power, and cupric reducing power were used. The concentrations of total phenolic and flavonoids were also calculated for the extracts (Table 1).

Fig. 1 shows the total antioxidant activity of the extracts of stems and roots of *R. ribes*, compared with TOC, BHT and quercetin, which were determined by the β-carotene bleaching method. Total antioxidant activity increased with increasing amount of the extracts. All the tested extracts showed greater antioxidant activity than BHT and TOC, and almost the same activity to that of quercetin. Even chloroform extracts of the roots at 50 and 100 μg concentrations (91.09 ± 0.80% and 93.14 ± 1.17%, respectively) were more active than the same concentrations of quercetin (86.11 ± 1.09% and 86.21 ± 1.10%, respectively).

Free radicals have a significant effect on oxidation of unsaturated lipids (Kaur & Perkins, 1991); DPPH radical was used as a stable free radical to determine antioxidant activity of natural compounds (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The method is based on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical DPPH–H. Fig. 2 shows the activity of free radical scavenging of different amounts of extracts of *R. ribes*. The difference between the extracts and control was statistically significant ($p < 0.05$). Radical scavenging activity increases with increasing the amount of the

Table 1
Total phenolic and flavonoid contents of various extracts of steams and roots of *Rheum ribes*[a]

Sample	Phenolic contents (μg PEs/mg extract)[b]	Flavonoid contents (μg QEs/mg extract)[c]
Chloroform extract of steams (CARR)	22.68 ± 1.10	20.10 ± 0.12
Methanol extract of steams (MARR)	35.71 ± 1.23	13.66 ± 0.75
Chloroform extract of roots (CRRR)	48.66 ± 1.23	145.59 ± 0.22
Methanol extract of roots (MRRR)	25.91 ± 1.09	16.23 ± 0.47

[a] Values expressed are means ± SD of three parallel measurements ($p < 0.05$).
[b] PEs, pyrocatechol equivalents.
[c] QEs, quercetin equivalents.

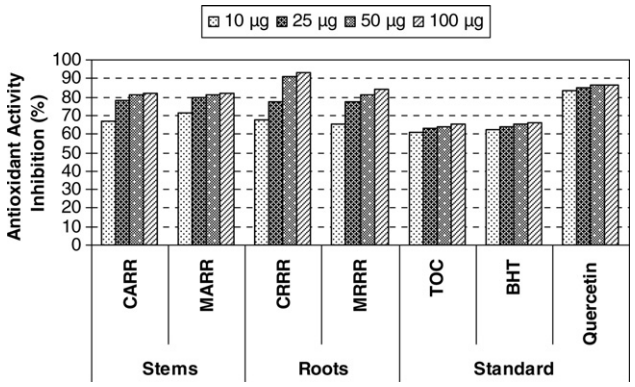

Fig. 1. Inhibition (%) of lipid peroxidation of extracts of *R. ribes*, BHT, TOC and quercetin, by the β-carotene bleaching method (CARR, chloroform extract of the stems; MARR, methanol extract of stems; CRRR, chloroform extract of roots; MRRR, methanol extract of roots).

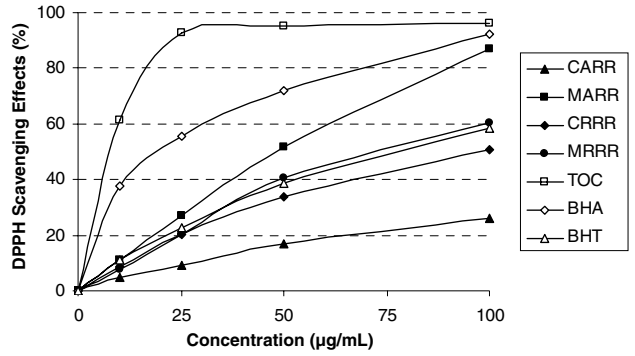

Fig. 2. Free radical scavenging activity of the extracts of *R. ribes* (CARR, MARR, CRRR and MRRR), BHT and TOC by DPPH.

extracts. Both methanol extracts of the stems and roots exhibited higher activity than BHT at $\geqslant 50$ µg/mL concentrations. Methanol extract of the stems ($87.07 \pm 0.54\%$) showed the highest DPPH radical scavenging activity among all the tested extracts, followed by methanol extract of the roots ($60.60 \pm 0.86\%$) and chloroform extract of the roots ($50.87 \pm 0.30\%$) at 100 µg/mL concentration.

Among the transition metals, iron is known as lipid oxidation pro-oxidant. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactive free radicals via the Fenton Reaction,

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{-}OH + {}^{\cdot}OH$$

Ferric ion also produces radical from peroxides although the rate is 10-fold less than that of Fe^{2+} (Halliwell & Gutteridge, 1984; Miller, 1996). Ferrous ion chelating activities of the extracts, EDTA and quercetin are shown in Fig. 3. The difference between the extracts and control was statistically significant ($p < 0.05$). Metal chelating activity increased with increasing concentration of the extracts. Methanol extract of the stems ($93.71 \pm 0.80\%$) showed the highest metal chelating activity among all the extracts studied, which has comparable results with that of EDTA. However, quercetin showed less metal chelating activity

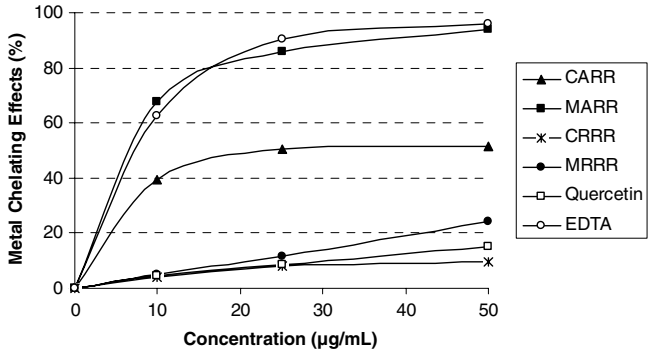

Fig. 3. Metal chelation effect of the extracts of *R. ribes* (CARR, MARR, CRRR and MRRR), EDTA and quercetin on ferrous ions.

than all extracts, except for chloroform extract of the roots with an inhibition of $15.18 \pm 0.88\%$.

Superoxide anion, derived from dissolved oxygen by PMS–NADH coupling reaction, reduces NBT. The decrease of absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion. Fig. 4 shows percentage inhibition of superoxide anion radical generation of different amounts of extracts of *R. ribes*, in comparison with the same concentration of L-ascorbic acid, BHT and quercetin. None of the tested extracts exhibited higher superoxide radical scavenging activity than well known standards, namely L-ascorbic acid and quercetin. However, both chloroform ($57.39 \pm 3.33\%$) and methanol ($61.65 \pm 1.21\%$) extracts of the roots have a competition with BHT ($46.99 \pm 0.54\%$) at 100 µg/mL concentration. Results were found to be statistically significant ($p < 0.05$) when compared to control. Superoxide anion radical scavenging activity depends on the redox potential on the actual state of oxidation of the quinones, and oxidized quinones possessed superoxide anion radical scavenging activity (Murakami & Zs.-Nagy, 1990). Fig. 4 indicates that both chloroform and methanol extracts of the roots rendered higher activity than the extracts of the stems. Anhraquinones derivatives are naturally occurring in the subterranean parts of *Rheum* species which were also isolated from *R. ribes* (Meriçli & Tuzlacı, 1990) and their higher

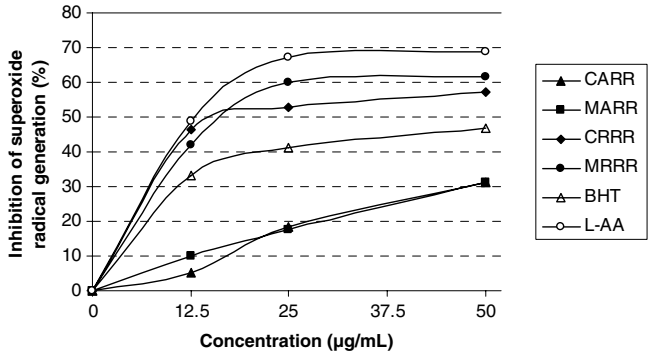

Fig. 4. Superoxide anion radical scavenging activity of the extracts of *R. ribes* (CARR, MARR, CRRR and MRRR), BHT, quercetin and L-ascorbic acid by the PMS–NADH–NBT method.

activity can be related to the presence of anthraquinone derivatives in the plant. This relationship verified that oxidized quinones could show superoxide anion radical scavenging activity as mentioned by Murakami and Zs.-Nagy (1990).

The presence of reductants such as antioxidant substances causes the reduction of the Fe^{3+}-ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Fig. 5 shows the reducing power of the extracts of *R. ribes* and standards such as α-tocopherol and BHT using the potassium ferricyanide reduction method. The reducing power of the extracts increased with increasing concentration. All extracts at all concentrations exhibited higher activities than the control; the differences were significant ($p < 0.01$). Chloroform extracts of the roots and stems showed stronger reducing power than the methanol extracts. Cupric reducing power estimated by the method described by Apak et al. (2004). This method is based on the measurement of absorbance at 450 nm by the formation of a stable complex between neocuproine and copper (I), the latter is formed by the reduction of copper (II) in the presence of neocuproine. This method was correlated with ferric reducing power used in this study (Fig. 6).

Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi & Wanasundara, 1992). Phenolic compounds are very important constituents of plants and their radical scavenging ability is due to their hydroxyl groups (Hatano, Edamatsu, Mori, Fujita, & Yasuhara, 1989). The phenolic compounds may contribute directly to antioxidative action (Duh, Tu, & Yen, 1999). It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily ingested from a diet rich in stems and vegetables (Tanaka, Kuei, Nagashima, & Taguchi, 1998). The concentration of phenolics in the extracts expressed as μg of pyrocatechol per mg of the extract is shown in Table 1. Chloroform extract of the roots (48.66 ± 1.23) had a higher phenolic content than others, while the least phenolics containing one was chloroform of the stems (22.68 ± 1.10).

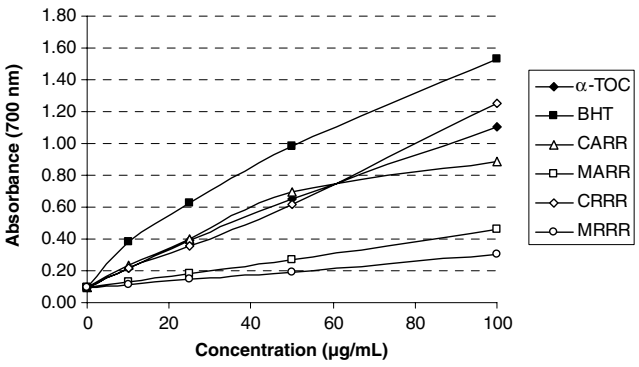

Fig. 5. Reductive potential extracts of *R. ribes* (CARR, MARR, CRRR and MRRR), BHT and α-tocopherol using spectrophotometric detection of the Fe^{3+}–Fe^{2+} transformations.

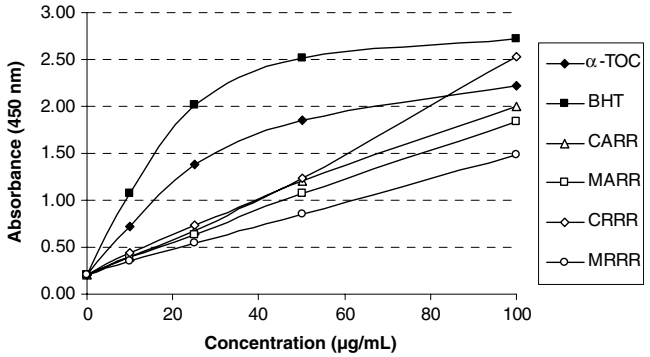

Fig. 6. Cupric reducing antioxidant capacity (CUPRAC) extracts of *R. ribes* (CARR, MARR, CRRR and MRRR), BHT and α-tocopherol using spectrophotometric detection of the Cu^{2+}–Cu^{1+} transformation.

Flavonoids are natural phenolic compounds and well known antioxidants. In various studies, antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids (Cakir et al., 2003). The concentration of flavonoids in the extracts was expressed as μg of quercetin equivalents per mg of the extract, as shown in Table 1. The most flavonoid rich extract was found to be chloroform extract of the roots (145.59 ± 0.22), while methanol extract of the stems (13.66 ± 0.75) was the poorest.

4. Conclusions

The results presented in this study are the first information on the antioxidant activities of *R. ribes*. Among the tested six methods, the highest activity was observed for inhibition of lipid peroxidation in β-carotene–linoleic acid system by all extracts of *R. ribes*. Particularly, chloroform extract of the roots was found to be the most active one, showing better activity than that of the standard quercetin, this finding should be related to the highest flavonoid content of the extract, phenolics, as well.

Both methanol extracts of stems and roots showed also high DPPH scavenging activity, even higher activity than that of the standard BHT. As known, there is a significant linear correlation between phenolic concentration and free radical scavenging activity, especially on DPPH radical. Although, in the present study, the chloroform root extract was found to be richest in phenolic content, both methanol extracts, especially, methanol extract of the stems exhibited highest activity which is rather close to that of BHA and α-tocopherol at 100 μg/mL concentration. This should be depended on structures of phenolics, probably concentration (%) of stilbenes regarding previous studies. Because, it was not found big phenolic amount differences for the extracts. Likewise, both root extracts exhibited more or less the same superoxide anion radical scavenging activity, being better than that of BHT and close to those of other two standards ascorbic acid and quercetin. It should be noted that methanol extract of the stems showed very high metal chelating ability on ferrous ion overlapping with that of EDTA. On the other hand, cupric reducing power of the

extracts was well correlated with ferric reducing power, and chloroform extracts of the roots and stems showed stronger reducing power in both assays.

In conclusion, the results showed the antioxidant importance of *R. ribes*, one of commonly used edible and medicinal plants in Anatolia and Middle East with delicious taste. Thus, *R. ribes* (Anatolian and Middle-Eastern rhubarb) may have been helped people to protect against lipid peroxidation and free radical damage, and its extracts will probably use for the development of safe food products and additives. However, further studies, especially antioxidant activity tests on aqueous extracts of *R. ribes* and isolated constituents are needed.

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