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Full Length Research Paper

Screening of the antioxidant potential of the leaves and flowers from *Rosa horrida* Fischer

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***Rosa* species are widely distributed in Turkey. Ethanolic extracts from flowers and leaves of *R. horrida*, one of the 25 species of *Rosa* used as a traditional Turkish medicine as tonic, diuretic, expectorant, stomachic and for the treatment of dyspepsia, diarrhea and nephritis were tested *in vitro* for their ability to scavenge $DPPH^{\cdot}$, $ABTS^{\cdot+}$ and superoxide radicals, reduce Fe (III) to Fe (II) and to inhibit peroxidation of phophatidylcholine liposomes induced with ascorbate/Fe (III) in order to establish a relashionship between their antioxidant potency and the content of phenolic compounds. Flowers and leaves extracts showed high DPPH radical scavenging activity as shown by the lowest value of EC_{50}, indicating the strong hydrogen-donating ability. The extracts showed similar metal chelation ability in Fe^{2+}-ferrozin test, reducing power and ability to scavenge $ABTS^{\cdot+}$ radical cation as shown by the small differences of the EC_{50} values, since the EC_{50} values were found to be highest in TBA test, suggesting that the extracts were less effective in protection of liposomes from lipid peroxidation. The activities correlated well with total phenolic and flavonoid contents, indicating that the extracts may play an important role in chemical protection from oxidative damage by possessing endogenious antioxidants, such as phenolics and flavonoids.**

Key words: *Rosa horrida*, leaves, flowers, polyphenol content, antioxidant potential.

INTRODUCTION

Reactive oxygen species (ROS) from both endogenous and exogenous sources may be involved in the etiologies of such diverse human diseases as atherosclerosis, ischemic injury, cancer and neurodegenerative diseases, as well as in processes like inflammation and ageing (Govindarajan et al., 2005). Minimizing oxidative damage may well be one of the most important approaches to the primary prevention or treatment of these diseases since antioxidants may stop the free radical formation in the first place, or interrupt an oxidizing chain reaction. This has attracted a great deal of research interest in naturally occuring nutrive and non-nutrive antioxidants as an alternative solution to health problems. *Rosa* species are widely distributed in Turkey and some of them are used for various purposes in folk medicine. *Rosa horrida* Fischer (Rosaceae) is one of the 25 species of *Rosa* growing in Turkey (Nilsson, 1972; Kultur, 2004). In the European part of Turkey, the genus *Rosa* is represented by 7 species, one of which is *R. horrida*. *Rosa* species have been used as a traditional Turkish medicine as tonic, diuretic, expectorant, stomachic and for the treatment of dyspepsia, diarrhea and nephritis (Kultur, 1998, 2007).The main objective of this study was to complement our previous studies on *Rosa* species, reporting *R. agrestis* (Bitis et al., 2010) and *R. sempervirens* (Bitis et al., 2008) leaves extracts as

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potential sources of phenolic and flavonoid antioxidant compounds by evaluating antioxidant activities of ethanolic extracts from flowers and leaves of *R. horrida* through several biochemical assays including inhibition of lipid peroxidation in soybean phosphatidylcholine liposomes, induced with Fe^{3+}/ascorbate, scavenging effect on $DPPH^{\bullet}$ and superoxide radicals, Trolox equivalent antioxidant capacity, metal chelation ability in Fe^{2+}-ferrozin test system and reducing power. The antioxidant activities of the extracts were compared to that of quercetin as a typical example of a naturally occuring flavonol and BHA, a well-known synthetic antioxidant.

MATERIALS AND METHODS

Chemicals

Nitroblue tetrazolium (NBT), β-nicotinamide adenine dinucleotide reduced (β-NADH), soybean L-α-phosphatidylcholine Type IV-S, butylated hydroxyanisol (BHA), 3-(2-pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (ferrozine), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), 6-hydroxy-2,5,7,8,-tetramethylchroman-2-carboxylic acid (Trolox), quercetin and catechin were purchased from Fluka Chemical Co. (Buchs, Switzerland). Phenazine methosulphate (PMS), 2,2-diphenyl-1-picryl-hydrazyl ($DPPH^{\bullet}$), ethylenediamine tetraacetic acid (EDTA), gallic acid and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Potassium ferricyanide, trichloroacetic acid (TCA), thiobarbituric acid (TBA), ferrous and ferric chloride were obtained from Merck. All other reagents were of analytical grade.

Plant

Flowers and leaves of *R. horrida* Fischer were collected in May, 2008 from Silivri-Istanbul (Turkey) and identified by Dr. Sukran Kultur. Voucher specimens have been deposited in the Herbarium of the Faculy of Pharmacy, Istanbul University (ISTE 86065).

Preparation of extracts

The dried flowers (10 g) and leaves (10 g) of *R. horrida* were separately extracted with ethanol (95°) in a Soxhlet apparatus. The ethanolic extracts were evaporated to dryness under reduced pressure and controlled temperature (40 to 50°C) in a rotary evaporator. All the extracts were kept at -20°C and were dissolved in ethanol before use.

Determination of total phenolic compounds

Phenolic compounds in the ethanolic extracts from flowers and leaves of *R. horrida* were estimated by a colorimetric assay, based on procedure described by Slinkard and Singleton (1977). The results were expressed as gallic acid equivalents (GAE)/g of extract.

Determination of the total flavonoid contents

Total flavonoid contents of the extracts were determined by using a colorimetric method described by Sakanaka et al. (2005). The results were expressed as means ± standard deviation (SD) mg of (+)-catechin equivalents per g of extract.

Antioxidant activity on liposome peroxidation

Lipid peroxidation assay was based on the method described by Duh et al. (1999). The formation of lipid peroxidation products was assayed by the measurement of TBARS levels on the basis that malondialdehyde (MDA) reacted with thiobarbituric acid at 532 nm according to Buege and Aust (1978). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the sample with those of controls not treated with the extract using the following equation:

Inhibition effect (%) = (1 - Absorbance of sample at 532 nm/Absorbance of control at 532 nm) × 100

DPPH radical scavenging activity

The DPPH free radical scavenging activity of the ethanolic extracts from flowers and leaves of *R. horrida* was measured according to the procedure described by Brand-Williams et al. (1995). The ability to scavenge DPPH radical was calculated by the following equation:

DPPH radical-scavenging activity (%) = (1 - Absorbance of sample at 517 nm/Absorbance of control at 517 nm) × 100

The ABTS radical scavenging activity

The ABTS radical scavenging activity of the extracts were measured using the Trolox equivalent antioxidant coefficient (TEAC) assay as described by Re et al. (1999). The ability to scavenge $ABTS^{\bullet+}$ radical was calculated by the following equation:

$ABTS^{\bullet+}$ radical scavenging activity (%) = (1 **-** Absorbance of sample at 734 nm/Absorbance of control at 734 nm) × 100

The total antioxidant capacity value in a sample was assessed as TEAC; a value of 1 TEAC in a sample was defined as a concentration that is equivalent to 1 mM of Trolox.

Superoxide radical scavenging activity

The effects of the ethanolic extracts from flowers and leaves of *R. horrida* on generation of superoxide radicals were determined by the nitroblue tetrazolium reduction method (Nishikimi et al., 1972). The abilities to scavenge the superoxide radical were calculated using the following equation:

Superoxide radical scavenging activity (%) = (1 **-** Absorbance of sample at 560 nm/Absorbance of control at 560 nm) × 100

Chelating activity on Fe^{2+}

The extracts were assessed for their ability to compete with

ferrozine for iron (II) ions in free solution. The chelating ability of ferrous ions by the ethanolic extracts from flowers and leaves of *R. horrida* was estimated by the method of Dinis et al. (1994). Thepercentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:

Chelating activity (%) = (1 - Absorbance of sample at 562 nm/Absorbance of control at 562 nm) × 100

Reducing power

The reducing powers of the ethanolic extracts from flowers and leaves of *R. horrida* were determined according to the method described by Chung et al. (2005).

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of triplicate analysis. Statistical comparisons were performed with Student's *t*-test. Differences were considered significant at $p < 0.05$. The correlation coefficient (r^2) between the parameters tested was established by regression analysis.

RESULTS AND DISCUSSION

Extract yield (amount of total extractable compounds), total phenolics and flavonoids contents

Table 1 presents extraction yields (amount of total extractable compounds), polyphenol and flavonoid contents, obtained from the 10 g of leaves and flowers extracts of *R. horrida*. Flowers and leaves extracts of *R. horrida* gave a yield of 0.180 and 0.144 g extractable compounds (EC) per gram of dry weight (DW), respectively. These amounts were comparable with results described by Ozkan et al. (2004) who reported that the yields of extracts of *R. damascena* fresh and spent flowers ranged from 10.70 ± 1.93 to 22.27 ± 1.98%. Although the extractable compounds of the extract from flowers (18%) were significantly higher than those of leaves (14.4%), the part of total phenolic compounds in the total extractable compounds found for the leaves extract was comparable with the part of total phenolic compounds found for the flower extract. The ratio of total phenolic compounds to the total extractable compounds was 15% for both the extracts.

Estimation of total phenolics (PC) using the Folin-Ciocalteu reagent and gallic acid as a standard revealed that both extracts are rich sources of polyphenols. Contents of phenols and flavonoids expressed as mg/g extract were similar for these two extract (Table 1). This indicated that the type of phenolic compounds and flavonoids in flowers and leaves did not vary markedly. It was observed that flowers and leaves contain 159.3 ± 1.76 and 152.0 ± 0.69 mg GAE/g extract or 28.7 ± 0.12 and 21.9 ± 0.58 mg/g DW, respectively. These values were lower than that reported for fresh (276.02 ± 2.93 mg GAE/g) and spent (248.97 ± 2.96 mg GAE/g) flowers of *R. damascena* (Ozkan et al., 2004) and for leaves (489.76 ± 22.33 mg/g extract) of *R. agrestis* (Bitis et al., 2008). Our results revealed that *R. horrida* flowers and leaves extracts contain 68.25 ± 0.93 and 67.64 ± 0.78 mg/g extract or 12.1 ± 0.14 and 9.73 ± 0.13 mg/DW of flavonoids, respectively, expressed in catechin equivalents (CE).

Antioxidant activity on lipid peroxidation

Antioxidant activities of leaves and flowers ethanolic extracts from *R. horrida* were studied on the level of lipid peroxidation in liposomes, induced by Fe^{3+}/ascorbate system and measured spectrophotometrically by the Thiobarbituric acid (TBA) test. The inhibitory effect of the extracts increased with increase in the concentration. There was no significant difference in inhibition ($p > 0.05$) between flowers (91.32 ± 0.68%) and leaves (91.15 ± 0.45%) extracts at a concentration of 10 mg/ml. Inhibitory effects were comparable to that of quercetin (95.26 ± 1.35%) and BHA (96.06 ± 1.27%) at a dose of 0.08 and 0.04 mg/ml, respectively.

Based on the EC50 values (effective concentration at which the antioxidant activity was 50%) shown in Table 2, the antioxidant activity of the leaves (2.99 ± 0.050 mg/ml) extract was significantly ($p < 0.05$) better that that of the flowers (3.51 ± 0.14 mg/ml) extract. It can be explained with the diversity of the phytochemical composition. Both extracts were significantly less effective ($p < 0.05$) than the reference antioxidants, quercetin (0.035 ± 0.0025 mg/ml) and BHA (0.016 ± 0.0017 mg/ml). It was concluded that both extracts inhibited phospholipid peroxidation, showing a powerful effect. The correlation between antioxidant activity and phenolics (r^2 = 0.9861 and 0.9999 for flowers and leaves extracts, respectively) and flavonoid (r^2 = 0.9932 and 0.9981 for flowers and leaves extracts, respectively) contents was significant, indicating that the extracts may play an important role in chemical protection from oxidative damage by possessing endogenious antioxidants, such as phenolics and flavonoids. Similarly, *R. agrestis* leaves extracts (Nowak and Gawlik-Dziki, 2007; Bitis et al., 2008) have been reported to have a strong antioxidant activity against lipid peroxidation. Mavi et al. (2004) also reported that aqueous extract of *R. pimpinellifolia* have relatively high peroxidation inhibition ability, with an EC_{50} value of 23 mg/l.

DPPH radical scavenging activity

Extracts showed a high hydrogen-donating ability in the

Table 1. Total extractable compounds (EC), total phenolic compounds (PC) (as gallic acid equivalents) and total flavonoids (as catechin equivalents) in the ethanolic extracts from flowers and leaves of *R. horrida* Fischer.

Extract	EC (g/g DW)	PC (mg/g extract)	Flavonoid (mg/g extract)	PC/EC (%)
Flowers	0.180	159.3±1.76[a]	68.25±0.93[a]	15.9
Leaves	0.144	152.0±0.69[a]	67.64±0.78[a]	15.2

Values are the means of three replicates ± standard deviation (SD), values with different letters in the same column were significantly different ($p < 0.05$). DW: Dry weight.

Table 2. EC_{50} values of ethanolic extracts from flowers and leaves of *R. horrida* Fischer.

Ethanolic extracts	EC_{50} (mg/ml)	
	Flowers	Leaves
Anti-LPO	3.51±0.140[a]	2.99±0.050[b]
DPPH$^{\bullet}$	0.61±0.035[a]	0.62±0.031[a]
$ABTS^{+\bullet}$	1.44±0.110[a]	1.47±0.102[a]
Superoxide anion	1.46±0.058[a]	2.54±0.112[b]
Chelation activity	0.99±0.036[a]	1.04±0.018[a]
Reducing power	1.13±0.043[a]	1.16±0.026[a]

Values are the means of three replicates ± standard deviation (SD), values with different letters in the same row were significantly different ($p < 0.05$).

presence of DPPH stable radical. At 1.25 mg/ml the flowers (93.01 ± 0.18 %) and leaves (92.80 ± 0.80%) extracts did not differ in their DPPH radical scavenging abilities ($p > 0.05$), which were comparabale to that of quercetin (94.14 ± 0.67%) and BHA (93.61 ± 0.22%) at 0.16 mg/ml. The DPPH radical scavenging activities, expressed in the term of EC_{50} (the effective concentration at which the DPPH radicals were scavenged by 50%) were similar ($p > 0.05$) for flowers (0.608 ± 0.035 mg/ml) and leaves (0.620 ± 0.031 mg/ml) extracts but significantly different ($p < 0.05$) from the EC_{50} values obtained for the reference antioxidants, quercetin (0.092 ± 0.0086 mg/ml) and BHA (0.046 ± 0.0080 mg/ml).

The obtained results were in agreement with the phenolics ($r^2 = 0.8499$ and 0.8614 for flowers and leaves extracts, respectively) and flavonoid ($r^2 = 0.7804$ and 0.8067 for flowers and leaves extracts, respectively) contents determined for each extract. These results indicated that phenolic compounds and flavonoids are likely to contribute to the radical scavenging activity of these plant extracts. It is well known that phenolics and flavonoids possess many of the properties of an efficient free radical scavenger, and their hydrogen donor capacity probably accounts in large part for the antioxidant activities of these extracts. Our results were consistent with the previous observation that *R. agrestis* (Bitis et al., 2008) contain radical-scavenging agents that could directly react with and quench stable DPPH radicals. Flavonoids are the most important constituents in the leaves of *Rosa* species.

The antioxidant properties of *R. agrestis* leaves extract are mainly connected with the presence of phenolic compounds including flavonoids like diosmetin, kaempherol, quercetin, kaempherol 3-glucoside (astragalin), quercetin 3-rhamnoside (quercitrin), quercetin 3-xyloside, quercetin 3-galactoside (hyperoside) (Bitis et al., 2008). The contents of ellagic acid, quercetin and kaempferol in the extracts from leaves of seventeen *Rosa* species, determined using Solid phase extraction reverse phase high performance liquid chromatography (SPE-RP-HPLC) methods were also reported (Nowak and Gawlik-Dziki, 2007).

The ABTS radical scavenging activity

The ABTS radical scavenging activity of the flowers and leaves extracts increased with increasing concentration, reaching similar ($p > 0.05$) radical scavenging activity of 93.77 ± 1.31% and 93.00 ± 1.58%, respectively, at a concentration of 2.5 mg/ml, and values were comparable to those of referance antioxidants, quercetin (91.84 ± 0.20%) and BHA (91.20 ± 1.59%) at a concentration of 0.31 mg/ml. These results confirm the radical scavenging activity found in the stable free radical DPPH assay for flowers and leaves extracts.

We have found that flowers and leaves extracts did not show significant difference ($p > 0.05$) in ABTS radical cation scavenging activities expressed in the term of EC_{50} value (the effective concentration at which the ABTS radicals were scavenged by 50%), however when compared to reference antioxidants, quercetin (0.154 ± 0.0032 mg/m) and BHA (0.198 ± 0.0042 mg/ml), the both tested extracts showed significantly lower ($p < 0.05$) ABTS radical scavenging activity. The TEAC value is a quantification of the effective antioxidant activity of the extracts. The antioxidant capacity of flowers and leaves extracts from *R. horrida* amounted to 8.85 and 8.78 mM TEAC/g extract, respectively, and was directly correlated

with the total phenolics (r^2 = 0.9997 and 0.9105 for flowers and leaves extracts, respectively) and flavonoid (r^2 = 0.9978 and 0.8906 for flowers and leaves extracts, respectively) contents.

Our results, showing a high ABTS cation radical scavenging activity of 2.21 ± 0.03 and 2.19 ± 0.036 mM/L Trolox equivalents at 2.5 mg/ml for *R. horrida* flowers and leaves extracts, are supported by our previous finding of 2.30 ± 0.016 mM TEAC/L at 0.5 mg/ml for *R. agrestis* ethanolic extract (Bitis et al., 2008). These results confirm the ones obtained by DPPH method (r^2 = 0.8813 and 0.8516 for flowers and leaves extracts, respectively) and indicate that extracts are potent antioxidant and free radical scavenging agents.

Superoxide radical scavenging activity

At a concentration of 5 mg/ml, the superoxide-radical scavenging activities were 93.25 ± 1.61 and 85.87 ± 1.80% for the flowers and leaves extracts, respectively, which were comparable to that of quercetin (94.81 ± 1.11%) at 1.25 mg/ml. BHA showed no detectable superoxide radical scavenging effect. Based on the EC_{50} values (the effective concentration at which the superoxide radicals were scavenged by 50%), the results of our study showed that the flowers extract (1.46 ± 0.058 mg/ml) have higher ($p < 0.05$) superoxide radical-scavenging ability than the leaves (2.54 ± 0.112 mg/ml) extract. The diversity of phenolic compounds and their different distribution in the plant may explain the lower activity obtained for the leaves extract. When compared to the flavonoid, quercetin (0.513 ± 0.013 mg/ml), the scavenging activity of both the extracts was found to be significantly ($p < 0.05$) lower. Superoxide radical scavenging activity correlated well with total phenolics (r^2 = 0.9575 and 0.9577 for flowers and leaves extracts, respectively) and flavonoid (r^2 = 0.9506 and 0.9434 for flowers and leaves extracts, respectively) contents.

Chelating activity on Fe^{2+}

Iron can stimulate lipid peroxidation by the Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals that can themselves abstract hydrogen and initiate the chain reaction of lipid peroxidation (Halliwell and Gutteridge, 1984). The chelating ability of both the extracts on ferrous ions were similar ($p > 0.05$). Flowers and leaves extracts chelated ferrous ions by 92.90 ± 1.57 and 92.48 ± 2.22%, respectively, at 5 mg/ml, whereas EDTA, used as a positive control, showed an excelent chelating ability of 99.8 ± 0.51% at a lower concentration of 0.31 mg/ml. The chelating activities of the flowers (0.993 ± 0.036 mg/ml) and leaves extracts (1.041 ± 0.018 mg/ml) from *R. horrida*, expressed in the term of EC_{50} (the effective concentration at which ferrous ions were chelated by 50%) were significantly lower ($p < 0.05$) than that of EDTA (0.160 ± 0.003 mg/ml).

Results, based on chelating ability of both extracts showed that ethanolic extracts from flowers and leaves of *R. horrida* were not good chelating agents compared to EDTA. Although, the chelation effects of extracts on ferrous ions at lower concentrations (below 0.31 mg/ml) were slight, they could minimize the amount of iron in the Fenton reaction or in the lipid peroxidation. In this study, *R. horrida* ethanolic extracts showed relatively higher chelating ability compared to 50% aqueous methanol extracts of *R. foetida* Herrm. (62.54 ± 4.2%), *R. hemisphaerica* J. Herrm. (60.21 ± 2.8%), *R. pulverulenta* M. Bieb. (59.52 ± 2.5%) and *R. canina* L. (61.14 ± 4.2%) (Serteser et al., 2008). Chelating activity correlated well with total phenolics (r^2 = 0.9550 and 0.9563 for flowers and leaves extracts, respectively) and flavonoid (r^2 = 0.9428 and 0.9364 for flowers and leaves extracts, respectively) contents.

Reducing power

At a concentration of 5 mg/ml, ethanolic extracts from flowers and leaves of *R. horrida* ehxibited similar ($p > 0.05$) reducing power of 1.399 ± 0.015 and 1.400 ± 0.038, respectively in terms of absorbance values at 700 nm, which were comparable to that of quercetin (1.394 ±0.023) at 0.625 mg/ml and BHA (1.396 ± 0.011) at 1.25 mg/ml, suggesting that both extracts had strong electron-donating capacity. EC_{50} values (the effective concentration at which the absorbance was 0.5) were comparable for ethanolic extracts from flowers (1.13 ± 0.043 mg/ml) and leaves (1.16 ± 0.026 mg/ml) of *R. horrida*, however significantly lower ($p < 0.05$) than the EC_{50} values obtained for quercetin (0.145 ± 0.0020 mg/ml) and BHA (0.227 ± 0.0094 mg/ml).

The correlation coefficient (r^2) between reducing power and phenolic compounds was 0.9767 and 0.9898 for flowers and leaves extracts, respectively, an indication that the high reducing power of the extracts may be attributed to their phenolic content. A high correlation existed between antioxidant activity of the extracts, measured as inhibition of lipid peroxidation in phosphatidylcholine liposomes and reducing power (r^2 = 0.9987 and 0.9905 for flowers and leaves extracts, respectively), revealing that the reducing ability of the extracts is in a part, contributor to antioxidant activity. Our results were in accordance with other investigators who have also reported that some *Rosa* species as *R. agrestis* (Bitis et al., 2008) and *R. pimpinellifolia* (Mavi et al., 2004) had the high ability to reduce Fe (III) to Fe (II). As can be seen from the EC_{50} values in Table 2, the

extracts tested in the present study showed a higher hydrogen donating ability, scavenging effect on ABTS and superoxide radicals, reducing power and chelating activity when compared to their capability to inhibit lipid peroxidation.

Conclusion

To find new natural sources of active compounds, we have studied the flowers and leaves extracts of *R. horrida* as potential sources of phenolic and flavonoid antioxidant compounds.

Both extracts showed the similar antioxidant profile and content of phenols and flavonoids, indicating that the type of phenolic compounds and flavonoids in flowers and leaves did not vary markedly. A screening of the antioxidant potential of ethanolic extracts from the leaves and flowers of *R. horrida* indicated that both of them have a high ability to scavenge $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and superoxide radicals, chelate ferrous ions, reduce Fe (III) to Fe (II), as well as inhibit lipid peroxidation, and these abilities coincides with a high total phenolic and flavonoid contents. Our results further support the view that some traditionally used medicinal plants are promising sources of potential antioxidants.

ABBREVATIONS

ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; **BHA,** butylated hydroxyanisol; **CE,** catechin equivalents; **DPPH,** 2,2-diphenyl-1-picryl-hydrazyl; **DW,** dry weight; **EC,** extractable compounds; **EC_{50},** the effective concentration at which the antioxidant activity is 50%; **EDTA,** ethylenediamine tetraacetic acid; **GAE,** gallic acid equivalents; **MDA,** malondialdehyde; **PC,** total phenolics; **ROS,** reactive oxygen species; **SD,** standart deviation; **TBARS,** thiobarbituric acid reactive substances; **TEAC,** trolox equivalent antioxidant coefficient; **TRAP,** total radical antioxidant potentials.

REFERENCES

Bitis L, Kultur S, Melikoglu G, Ozsoy N, Can A (2008). Rosa sempervirens L. To study the antioxidant activity of the leaves. 18. Herbal Pharmaceutical Raw Materials Meeting (BİHAT 2008), Fitome Turkey, BİHAT 2008 Special Issue 2, 64, P-55, Istanbul, Turkey.

Bitis L, Melikoglu G, Kultur S, Ozsoy N, Can A (2010). Flavonoids and antioxidant activity of Rosa agrestis leaves. Nat. Prod. Res. 24:580-589.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol.-LWT. *2*8:25–30.

Buege JA, Aust SD (1978). Microsomal lipid peroxidation. Method. Enzymol. 52:302–310,.

Chung Y-C, Chen S-J, Hsu C-K, Chang C-T, Chou S-T (2005). Studies on the antioxidative activity of *Graptopetalum paraguayense* E. Walther. Food Chem. 91:419–424.

Dinis TCP, Madeira VMC, Almeida LM (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. Arch. Biochem. Biophys. 315:161–169.

Duh PD, Tu YY, Yen GC (1999). Antioxidant activity of water extract of Harng Jyur (*Chrysanthemum morifolium* Ramat). Food Sci. Technol.-LWT. 32:269–277.

Govindarajan R, Vijayakumar M, Pushpangadan P (2005). Antioxidant approach to disease menagement and the role of 'Rasayana' herbs of Ayurveda. J. Ethnopharmacol. 99:165–178.

Halliwell B, Gutteridge JMC (1984). Review article: Oxygen toxicity, oxygen radicals, transition metals and disease. Biochem. J. 219:1–14.

Kultur S (1998). A pharmaceutical botanical investigation on wild Rosa species grown in North-West of Turkey. PhD thesis, Istanbul University, Health Institute, Istanbul, Turkey.

Kultur S (2004). A new Rosa species for European flora. J. Fac. Pharm. Istanbul 37:75–83.

Kultur S (2007). Medicinal plants used in Kirklareli Province (Turkey). J. Ethnopharmacol. 111:341–364.

Mavi A, Terzi Z, Ozgen U, Yildirim A, Coskun M (2004). Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *Cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* (Urticaceae). Biol. Pharm. Bull. 27:702–705.

Nilsson O (1972). Rosa L. In P.H. Davis (Ed) Flora of Turkey and the East Aegean Islands, IV, Edinburgh University Press, Edinburgh, pp 106–128.

Nishikimi M, Rao NA, Yagi K (1972). The occurence of superoxide anion in the reaction of reduced phenazine methosulphate and molecular oxygen. Biochem. Biophys. Res. Commun. 46:849–854.

Nowak R, Gawlik-Dziki U (2007). Polyphenols of Rosa L. leaves extracts and their radical scavenging activity. Z. Naturforsch. 62:32–38.

Ozkan G, Sagdic O, Baydar NG, Baydar H (2004). Antioxidant and antibacterial activities of Rosa damascena flower extracts. Food Sci. Technol. Int. 10:277–281.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26:1231–1237.

Sakanaka S, Tachibana Y, Okada Y (2005). Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). Food Chem. 89:569–575.

Serteser A, Kargioglu M, Gok V, Bagci Y, Ozcan MM, Arslan D (2008). Determination of antioxidant effects of some plant species wild growing in Turkey. Int. J. Food Sci. Nutr. 59:643–651.

Slinkard K, Singleton VL (1977). Total phenol analysis: automation and comparison with manual methods. Am. J. Enol. Vitic. 28:49–55.