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Full Length Research Paper

Determination of the antioxidant properties of ethanol and water extracts from different parts of *Teucrium parviflorum* Schreber

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This study was designed to examine *in vitro* antioxidant of extracts of *Teucrium parviflorum* Schreber. The plants were extracted successively in ethanol and water using soxhlet-apparatus. They were screened for possible antioxidant activity using the ABTS$^{\bullet}$ (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)) radical scavenging capacity, DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl) radical scavenging capacity, superoxide anion radical scavenging capacity, hydrogen peroxide scavenging capacity, reducing power and metal chelating activities. These various antioxidant activities were compared with standard antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol. The extracts of *T. parviflorum* showed strong antioxidant activity and effective antioxidant assay. This antioxidant property depends on concentration and increase with increased amount of sample. In addition, total phenolic compounds in the extracts of *T. parviflorum* were determined as pyrocatecol equivalents. The results obtained in the present study indicated that *T. parviflorum* is a potential source of natural antioxidant.

Keywords: *Teucrium parviflorum*, antioxidant activity, medicinal plants, extract, flower, leaves.

INTRODUCTION

Since ancient times, many official herbs have provoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infections and preservation of foods from the toxic effects of oxidants. The preservative effect of many plant species and herbs suggests the presence of antioxidative and antimicrobial constituents (Hirasa and Takemasa, 1998). Many medicinal plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids (Velioglu et al., 1998). Many herb species, especially those belonging to the *Lamiaceae* family, such as sage, oregano and thyme, show strong antioxidant activity (Hirasa and Takemasa, 1998). A number of phenolic compounds with strong antioxidant activity have been identified in these plant extracts (Nakatani, 1997). The potential of the antioxidant constituents of plant materials for the maintenance of health and protec-tion from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move towards functional foods with specific health effects (Loliger, 1991).

The genus *Teucrium* is represented by 27 species in Turkey (Ekim, 1982). Two new species have been mentioned by Duman in the second supplement to the Flora of Turkey (Güner et al., 2000) and the total number has reached 30 species by adding the new records (Dönmez, 2006). *Teucrium* species have been used as a stimulant, tonic, diaphoretic and appetizers, and against stomach pains and diabetes in Turkish folk medicine

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Abbreviations: BHA, Butylated hydroxyanisole; **BHT,** butylated hydroxytoluene; **ABTS$^{\bullet}$,** 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid; **DPPH$^{\bullet}$,** 2,2-diphenyl-1-picrylhydrazyl radical; **ROS,** reactive oxygen species; **RSC,** free radical scavenging capacity; **NADH,** nicotinamide adenine dinucleotide; **TCA,** trichloracetic acid; **NBT,** nitroblue tetrazolium; **PMS**, phenazine methosulphate.

(Baytop, 1999).

The human body has several antioxidant defense systems to protect healthy cell membranes from active oxygen species and free radicals (Halliwell, 1994; Kaur and Kapoor, 2001). The innate defense systems may be supported by antioxidative compounds taken as foods, cosmetics and medicine. Therefore, the antioxidative compounds provided by the diet may enrich the antioxidative status of living cells and thus reduce the damage, particularly in the elderly (Shukla et al., 1997). The most widely used antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been restricted recently because of serious concerns about their carcinogenic potential (Buxiang and Fukuhara, 1997). Therefore, there is great interest in finding new and safe antioxidants from natural sources (Gazzani et al., 1998). Recently, natural plants have received much attention as sources of biologically active substances including antioxidants, antimutagens and anticarcinogens (Dillard and German, 2000). Numerous studies have been carried out on some plants such as rosemary, sage and oregano, which resulted in the development of natural antioxidant formulations for food, cosmetic and other applications. However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still scarce. Therefore, the assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants, functional foods and nutraceuticals (Miliauskas et al., 2004).

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, cartenoids, dietary glutathionine, vitamins and endogenous metabolites and such natural products are rich in antioxidant activities (Kivits et al., 1997). Reactive oxygen species (ROS) including free radicals such as superoxide anion radicals ($O_2^{\bullet-}$), hydroxyl radicals ($OH^{\bullet}$), singlet oxygen ($^1O_2$) and non-free radical species such as hydrogen peroxide (H_2O_2) are various forms of activated oxygen and often generated by oxidation product of biological reactions or exogenous factors (Gulcin et al., 2002b). Electron acceptors such as molecular oxygen, react easily with free radicals to become radicals themselves, also referred to as reactive oxygen species (ROS) (Grisham and McCord, 1986). ROS have aroused significant interest among scientists in the past decade. Their broad range of effects in biological and medicinal systems has drawn the attention of many experimental works (Gulcin et al., 2002a).

There are increasing suggestions by considerable evidence that the free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, ageing, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans (Halliwell, 1994). Several methods have been developed to measure the free radical scavenging capacity (RSC), regardless of the individual compounds which contribute towards the total capacity of a plant product in scavenging free radicals. The methods are typically based on the inhibition of the accumulation of oxidized products, since the generation of free radical species is inhibited by the addition of antioxidants and this gives rise to a reduction of the end point by scavenging free radicals. The reliable method to determine RSC involves the measurement of the disappearance of free radicals such as 2,2-azino-bis (3-ethylbenzenthiazoline-6-sulphonic) acid radical ($ABTS^{\bullet+}$), the 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet+}$) or other colored radicals, with a spectrophotometer (Sánchez-Moreno et al., 1998). Owing to the increase demand for information about the total RSC of all types of plant extracts, an easy, rapid and reliable method for the determination of RSC of various samples might be useful. The method should not be time-consuming, but sensitive enough to screen differences between plants parts used for herbal medicine, which include the flower, top, aerial and roots (Choi, et al., 2002).

Furthermore, the antioxidant activity and radical scavenging capacity of *Teucrium parviflorum* has not previously been published. In this study, *in vitro* antioxidant, radical scavenging and antimicrobial properties of the methanol, water and chloroform extracts of *T. parviflorum* growing in the eastern part of Turkey were investigated.

MATERIALS AND METHODS

Chemicals

Ferrous chloride, α-tocopherol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), 3-(2-Pyridyl)-5,6-bis (4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), nicotinamide adenine dinucleotide (NADH), butylate-dhydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trichloracetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Plant material and extraction procedure

The herbal parts of *T. parviflorum* Schreber was collected from Karga Mountain, Elazığ region in the Eastern Anatolia of Turkey, during flowering (June 2009). The voucher specimens were deposited at the Herbarium of the Department of Biology, Fırat University, Elazığ-Turkey. The taxonomic identification of plant materials was determined by using flora of Turkey (Davis, 1975). The aerial parts of the plant material were dried in shade at room temperature and then ground to a fine powder in a mechanic grinder. Then the powdered plant materials (10 g) were extracted with 100 ml of ethanol and water in a Soxhlet extractor. After the filtration of the solvent, the organic phases were independently concentrated under vacuum by evaporating to dryness. The residues were dissolved in the same solvent and stored at -20°C until studied.

ABTS$^{\bullet}$ radical scavenging capacity

ABTS also forms a relatively stable free radical, which decolorizes in its non-radical form (Shirwaikar et al., 2006). The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging capacity was determined according to the method of Re et al. (1999). $ABTS^{\bullet+}$ was produced by reacting 2 mM ABTS in H_2O with 2.45 mM potassium persulfate ($K_2S_2O_8$), stored in the dark at room temperature for 12 h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in 0,1 M sodium phosphate buffer (pH 7.4). Then, 1 ml of $ABTS^{\bullet+}$ solution was added to 3 ml of *T. parviflorum* extracts in ethanol at 100 µg/ml concentrations. The absorbance was recorded 30 min after mixing and the percentage of radical scavenging was calculated for each concentration relative to a blank containing no scavenger. The extent of decolorization is calculated as percentage reduction of absorbance. For preparation of a standard curve, different concentrations of $ABTS^{\bullet+}$ were used. The scavenging capability of test compounds was calculated using the following equation:

$$\% \text{ Inhibition} = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of the control, and A_1 is the absorbance in the presence of the sample of *T. parviflorum* extracts or standards.

DPPH$^{\bullet}$ radical scavenging capacity

The free radical scavenging capacity of *T. parviflorum* extracts was measured by 2,2-diphenyl-1-picryl-hydrazil ($DPPH^{\bullet}$) using the method of Shimada et al. (1992). Briefly, 0.1 mM solution of $DPPH^{\bullet}$ in ethanol was prepared and 1 ml of this solution was added to 3 ml of *T. parviflorum* extracts solution in water at different concentrations (50, 100 and 250 µg/ml). 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 $DPPH^{\bullet}$ radical was calculated using the following equation:

$$DPPH^{\bullet} \text{ scavenging effect } \% = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample of *T. parviflorum* extracts.

Superoxide anion scavenging capacity

Measurement of superoxide anion scavenging capacity of *T. parviflorum* extracts was based on the method described by Liu et al. (1997) with slight modification. One millilitre (1 ml) of nitroblue tetrazolium (NBT) solution (156 mmol/l NBT in 100 mmol/l phosphate buffer, pH 7.4), 1 ml NADH solution (468 mmol/l in 100 mmol/l phosphate buffer (pH 7.4) and 100 µl of sample solution of *T. parviflorum* extracts in water were mixed. The reaction started by adding 100 µl of phenazine methosulphate (PMS) solution (60 mmol/l PMS in 100 mmol/l phosphate buffer, pH 7.4) 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 indicated increased superoxide anion scavenging capacity. The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of the control and A_1 is the absorbance of *T. parviflorum* extracts or standards (Ye et al., 2000).

Hydrogen peroxide scavenging capacity

The ability of the *T. parviflorum* extracts to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Hydrogen peroxide concentration was determined spectrophotometrically measuring absorption with extinction coefficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Extracts (50, 100 and 250 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40 mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 min later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both *T. parviflorum* extracts and standard compounds was calculated:

$$\% \text{ Scavanged } H_2O_2 = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of *T. parviflorum* extracts or standards.

Reducing power

The reducing power of *T. parviflorum* extracts was determined by the method of Oyaizu (1986). Different concentrations of *T. parviflorum* extracts (50, 100 and 250 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) were added to the mixture, which was then centrifugated for 10 min at 3000 rpm (Universal 320R 2005, UK). 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 the absorbance was measured at 700 nm in a spectrophotometer. Increased absorbance of the reaction mixture indicates increased reducing power.

Metal chelating activity

The chelating of ferrous ions by the *T. parviflorum* extracts and standards was estimated by the method of Dinis et al. (1994). Briefly, extracts (50, 100 and 250 µg/ml) were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left to stand at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. All test and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula:

$$\%\text{Inhibition} = [(A_o - A_1)/A_o] \times 100$$

where A_o is the absorbance of the control and A_1 is the absorbance in the presence of the sample of *T. parviflorum* extracts or standards. The control does not contain $FeCl_2$ and ferrozine complex formation molecules.

Determination of total phenolic compounds

Total soluble phenolic compounds in the *T. parviflorum* extracts were determined with Folin–Ciocalteu reagent according to the method of Slinkard and Singleton, (1977) using pyrocatechol as a standard phenolic compound. Briefly, 1 ml of the *T. parviflorum* extracts

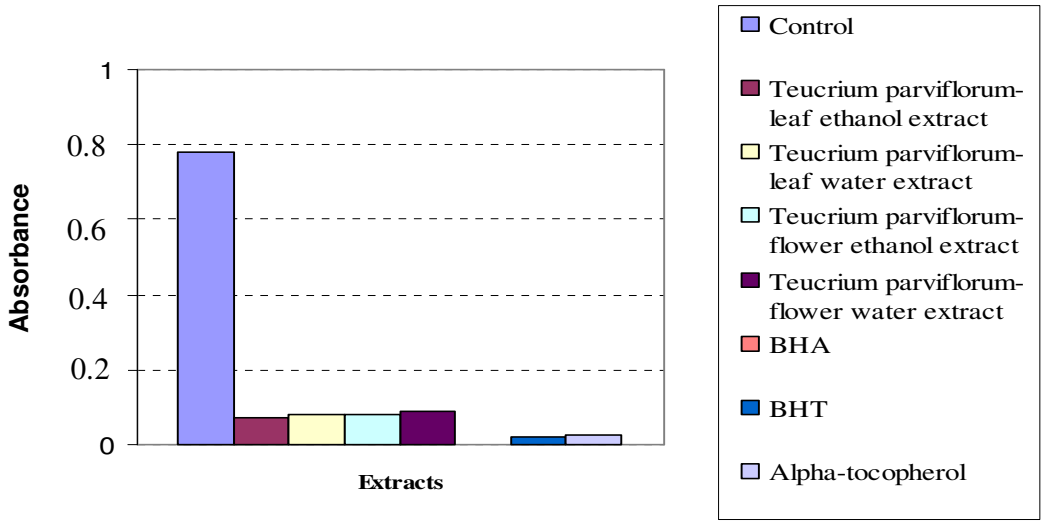

Figure 1. $ABTS^{\bullet+}$ radical-scavenging capacity of water and ethanol extracts of *T. parviflorum* leaves and flowers, BHA, BHT and α -tocopherol.

Table 1. Percentage of $ABTS^{\bullet}$ radical-scavenging capacity of water and ethanol extracts of *T. parviflorum* leaves and flowers, BHA, BHT and α-tocopherol.

Extracts (100 µg/ml)	ABTS assay (%)
Control	0
T. parviflorum-leaf ethanol extract	90.7
T. parviflorum-leaf water extract	89.7
T. parviflorum-flower ethanol extract	89.6
T. parviflorum-flower water extract	88.4
BHA	99.9
BHT	97.3
α-Tocopherol	96.9

solution (contains 1000 µg extract) in a volumetric flask was diluted with distilled water (46 ml). One milliliter (1ml) of Folin–Ciocalteu reagent was added and the content of the flask was mixed thoroughly. After 3 min, 3 ml of Na_2CO_3 (2%) was added and was then allowed to stand for 2 h with intermittent shaking. The absorbance was measured at 760 nm in a spectrophotometer. The total concentration of phenolic compounds in the *T. parviflorum* extracts was determined as microgram of pyrocatechol equivalent by using an equation that was obtained from standard pyrocatechol graph (Gulcin, 2002b):

Absorbance = 0.001 × total phenols [pyrocatechol equivalent (µg)] – 0.0033

RESULTS AND DISCUSSION

ABTS radical-scavenging capacity

All the tested compounds exhibited effective radical cation scavenging activity (Figure 1.). The scavenging effect of *T. parviflorum* and standards on $ABTS^{\bullet+}$ decreased in the order: BHA > BHT > α-tocopherol > ethanol extract of *T. parviflorum* leaves > water extract of *T. parviflorum* leaves > ethanol extract of *T. parviflorum* flowers > water extract of *T. parviflorum* flowers (99.9, 97.3, 96.9, 90.7, 89.7, 89.6 and 88.4%, respectively) at the concentration of 100 µg/ml (Table 1). No significant differences in $ABTS^{\bullet+}$ scavenging potential were found among *T. parviflorum* extracts, BHA, BHT and α-tocopherol.

$DPPH^{\bullet}$ radical scavenging capacity

$DPPH^{\bullet}$ is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares et al., 1997). The reduction capability of $DPPH^{\bullet}$ radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, $DPPH^{\bullet}$ is often used as a substrate to evaluate antioxidative activity of antioxidants (Duh et al., 1999) (Figure 2). We used α-tocopherol as standards (Figure 3). The scavenging effect of water and ethanol extracts of *T. parviflorum* leaves and flowers and standards on the $DPPH^{\bullet}$ radical decreased in the order: α –tocopherol > ethanol extract of *T. parviflorum* leaves > ethanol extract of *T. parviflorum* flowers > water extract of *T. parviflorum* leaves > water extract of *T. parviflorum* flowers (Figure 3). 100 µg of water and ethanol extracts of *T. parviflorum* leaves and flowers exhibited 71, 76.3, 66 and 74.2% $DPPH^{\bullet}$ scavenging capacity, respectively. On the other hand, at the same dose, α-tocopherol exhibited 95% $DPPH^{\bullet}$ scavenging capacity. These results indicates that both *T. parviflorum* leaves and flowers extracts have a noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

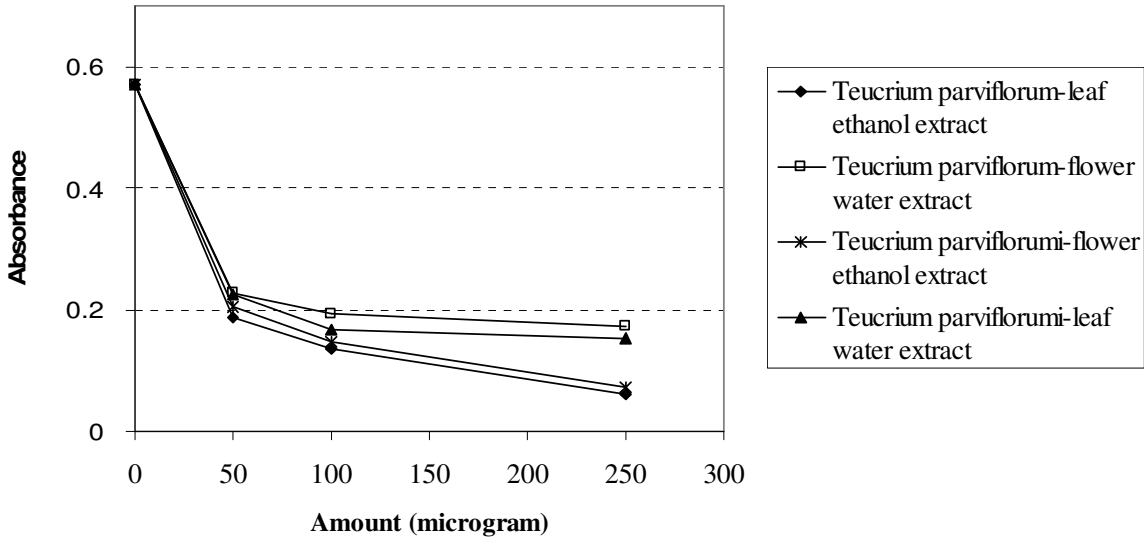

Figure 2. DPPH• radical scavenging capacity of water and ethanol extracts of *T. parviflorum* leaves and flowers.

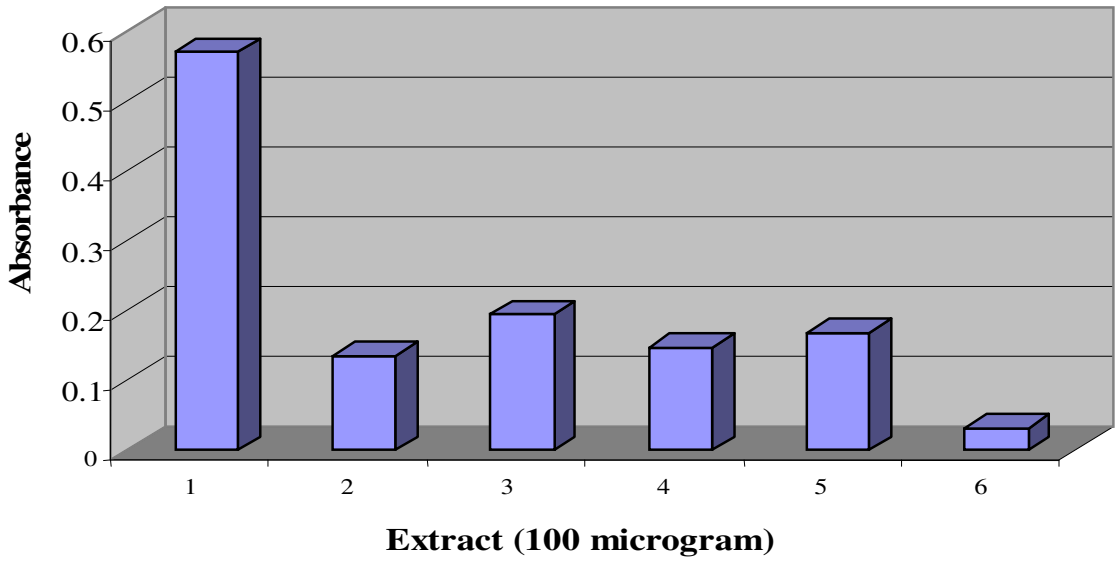

Figure 3. DPPH• radical scavenging capacity of water and ethanol extracts of *T. parviflorum* leaves and flowers (100 µg), α-tocopherol. 1. Control 2. Ethanol extract of *T. parviflorum* leaves 3. Water extract of *T. parviflorum* flowers 4. Ethanol extract of *T. parviflorum* flowers 5. Water extract of *T. parviflorum* leaves 6. α-Tocopherol.

Superoxide anion scavenging capacity

In the PMS/NADH-NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture (Figure 4). Table 2 shows the percentage inhibition of superoxide radical generation of 100 µg/ml of water and ethanol extracts of *T. parviflorum* leaves and flowers and comparison with same doses of BHA, BHT and α-tocopherol. Both extracts of *T. parviflorum* leaves and flowers have strong superoxide radical scavenging activity and exhibited higher superoxide radical scavenging activity than BHT and α -tocopherol. The percentage inhibition of superoxide generation by 100 mg doses of BHA, BHT, α-tocopherol, water and ethanol extracts of *T. parviflorum* leaves and flowers was found to be 99, 88, 81, 97.9, 60.9, 97.9 and 59.4%, respectively. Superoxide radical scavenging activity of these samples followed the order: BHA > water extract of *T. parviflorum* leaves > water extract of *T. Parviflorum* flowers > BHT > α-tocopherol >

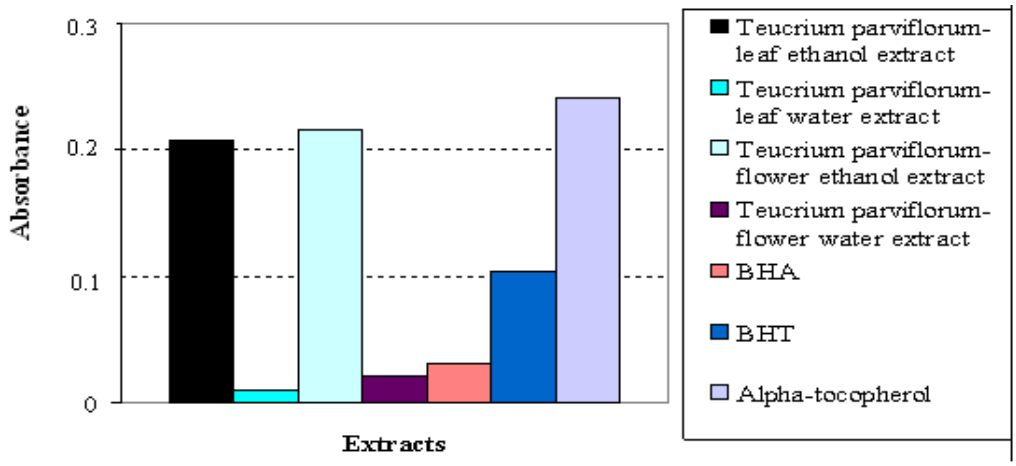

Figure 4. Superoxide anion radical scavenging activity of water and ethanol extracts of *T. parviflorum* leaves and flowers, BHA, BHT and α-tocopherol by the PMS–NADH–NBT method

Table 2. Percentage of superoxide anion scavenging activity of extracts of *T. parviflorum*, BHA, BHT and α-tocopherol.

Extracts	% Superoxide anion scavenging activity (100 µg)
T. parviflorum-leaf ethanol extract	60.9
T. parviflorum-leaf water extract	97.9
T. parviflorum-flower ethanol extract	59.4
T. parviflorum-flower water extract	96
BHA	99
BHT	88
α-Tocopherol	81

ethanol extract of *T. parviflorum* leaves > ethanol extract of *T. parviflorum* flowers.

Hydrogen peroxide scavenging activity

The ability of both extracts of *T. parviflorum* leaves and flowers to scavenge hydrogen peroxide was determined according to the method of Ruch et al. (1989). The scavenging ability of water and ethanol extracts of *T. parviflorum* leaves and flowers on hydrogen peroxide is shown in Figure 5 and compared with BHA, BHT and α-tocopherol as standards. These results showed that both *T. parviflorum* leaves and flowers extracts had stronger hydrogen peroxide scavenging activity. These values are close to BHA, but lower than that BHT and α -tocopherol. The hydrogen peroxide scavenging effect of 250 µg of both extracts of *T. parviflorum* leaves and flowers and standards decreased in the order of BHT > ethanol extract of *T. parviflorum* flowers > α-tocopherol > ethanol extract of *T. parviflorum* leaves > water extract of *T. parviflorum* leaves > BHA > water extract of *T. parviflorum* flowers. Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Halliwell, 1991). Thus, the removing of H_2O_2 is very important for antioxidant defence in cell or food systems. (Turkoglu, 2010).

Reducing power

Figure 6 shows the reductive capabilities of samples *T. parviflorum* leaves and flowers extracts when compared to BHA, BHT and α-tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}–Fe^{2+} transformation in the presence of *T. parviflorum* leaves and flowers extracts samples using the method of Oyaizu (1986). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir et al., 1995). However the antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim et al., 2000). Like the antioxidant activity, the reducing power of both *T. Parviflorum* leaves

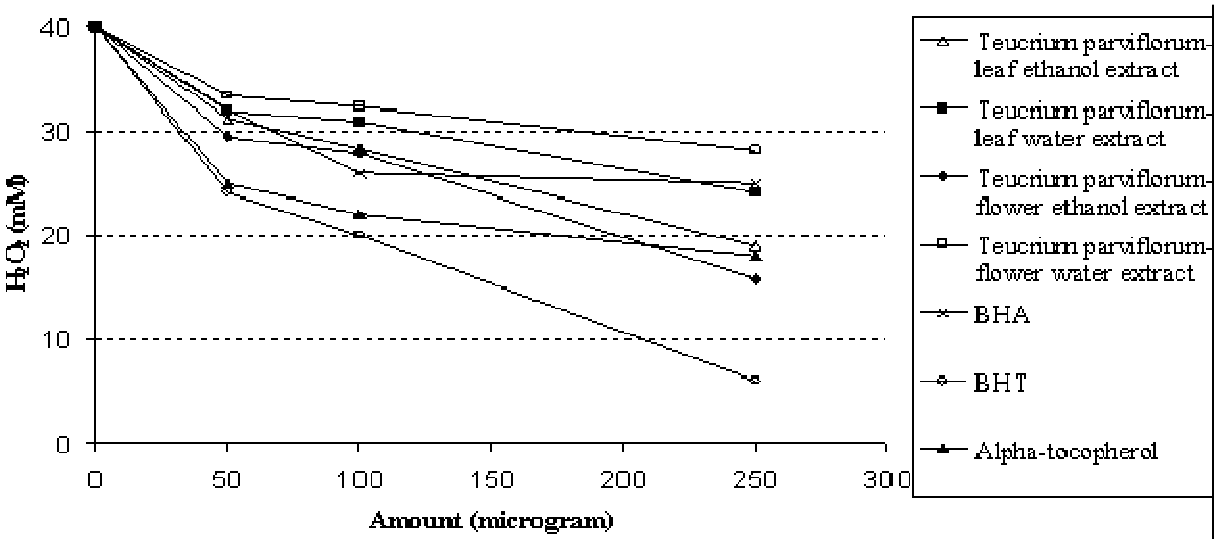

Figure 5. Hydrogen peroxide scavenging activities of water and ethanol extracts of *T. parviflorum* leaves and flowers, BHA, BHT and α-tocopherol.

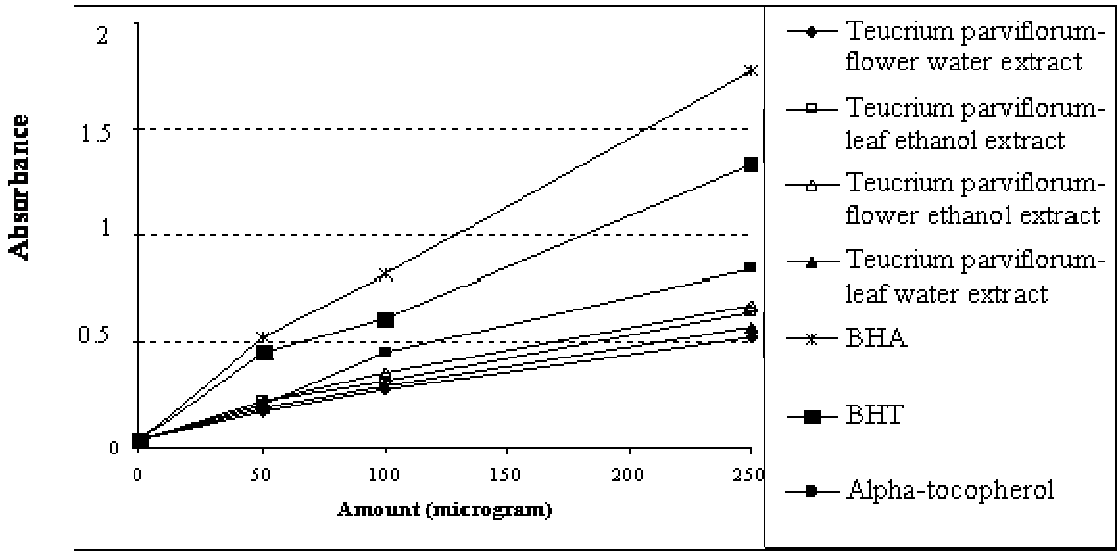

Figure 6. Reducing power of water and ethanol extracts of *T. parviflorum* leaves and flowers, BHA, BHT and α-tocopherol (spectrophotometric detection of the Fe^{+3}–Fe^{+2} transformation).

and flowers extracts increased with increasing amount of sample. Reducing power of water and ethanol extracts of *T. parviflorum* leaves and flowers and standard compounds followed the order: BHA > BHT > α-tocopherol > ethanol extract of *T. parviflorum* flowers > ethanol extract of *T. parviflorum* leaves > water extract of *T. parviflorum* leaves > water extract of *T. parviflorum* flowers.

Metal chelating activity

The chelating of ferrous ions by the extracts of *T. parviflorum* was estimated by the method of Dinis et al. (1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in the red colour of the complex. Measurement of colour reduction therefore allows estimation of the metal chelating activity of the coexisting chelator (Yamaguchi et al., 2000). In this assay, extracts of *T. parviflorum* and standard compounds interfered with the formation of ferrous and ferrozine complex, suggesting that they have chelating activity and are able to capture ferrous ion before ferrozine.

As shown in Figure 7, the formation of the Fe^{2+}– ferrozine

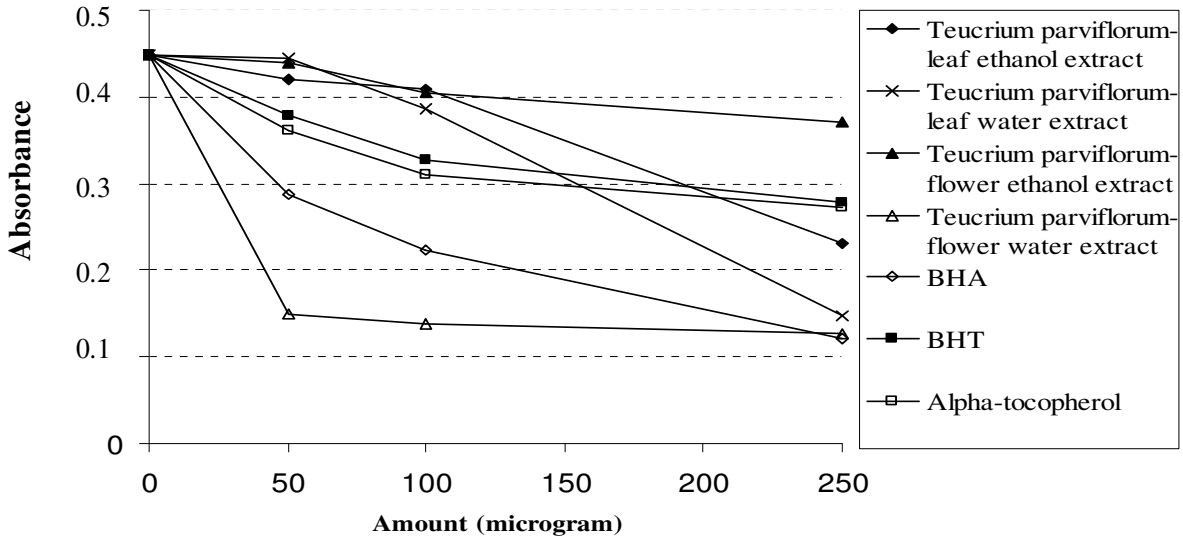

Figure 7. Metal chelating effect of different amount of water and ethanol extracts of *T. parviflorum*, BHA, BHT and α-tocopherol.

complex is not complete in the presence of water and ethanol extracts of *T. parviflorum* leaves and flowers, indicating that both extracts of *T. parviflorum* leaves and flowers chelate with the iron. The absorbance of Fe^{2+}–ferrozine complex was linearly decreased dose dependently (from 50 to 250 µg/ml). The percentages of metal scavenging capacity of 250 µg concentration of water and ethanol extracts of *T. parviflorum* leaves and flowers, α-tocopherol, BHA and BHT were found to be, 66.9, 48.4, 71.8, 17.1, 39.2, 72.9 and 38%, respectively. The metal scavenging effect of both extracts of *T. parviflorum* leaves and flowers and standards decreased in the order of BHA > water extract of *T. parviflorum* flowers > water extract of *T. parviflorum* leaves > ethanol extract of *T. parviflorum* leaves > α-tocopherol > BHT > ethanol extract of *T. parviflorum* flowers.

Metal chelating capacity was significant, since it reduced the concentration of the catalysing transition metal in lipid peroxidation (Duh et al.,1999). It was reported that chelating agents, which form S-bonds with a metal, are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon,1990). The data obtained from Figure 7 reveal that extracts of *T. parviflorum* demonstrated a marked capacity for iron binding, suggesting that their action as peroxidation pro-tector may be related to its iron binding capacity.

The total phenolic compounds

Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups (Hatano et al., 1989). According to the recent reports, a highly positive relationship between total phenols and antioxidant activity was found in many plant species (Velioglu et al., 1998, Gulcin et al., 2002b). 20, 36, 20 and 39 µg pyrocatechol equivalent of phenols was detected in 1 mg of water and ethanol extracts of *T. parviflorum* leaves and flowers.

The phenolic compounds may contribute directly to the antioxidative action (Duh et al., 1999). It is suggested that polyphenolic compounds may have inhibitory effects on mutagenesis and carcinogenesis in humans, when up to 1.0 g daily are ingested from a diet rich in fruits and vegetables (Liu et al., 1997). In addition, it was reported that phenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation (Yen et al., 1993).

Conclusion

The water and ethanol extracts of *T. parviflorum* leaves and flowers showed strong antioxidant activity, reducing power, $DPPH^{\bullet}$ radical, superoxide anion scavenging, hydrogen peroxide scavenging and metal chelating activities when compared to standards such as BHA, BHT and α-tocopherol. The results of this study shows that the water and ethanol extract of *T. parviflorum* leaves and flowers can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. It can be used in stabilising food against oxidative deterioration. However, the polyphenolic compounds or other components responsible for the antioxidant activity of water and ethanol extracts of *T. parviflorum* leaves and flowers are already unknown. Therefore, it is suggested that further work be performed on the isolation and identification of the antioxidant components in *T. parviflorum* leaves and flowers.

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