EXPERIMENTAL PAPER

Antioxidant activity of *Trachystemon orientalis* (L.) G. Don (Borage) grown and eaten as food in Ordu, Turkey

MELEK COL AYVAZ

Department of Chemistry
Faculty of Arts and Sciences
Ordu University
52200 Ordu, Turkey

phone: +90452 234 50 10 1669, fax: +90452 233 91 49,
e-mail: melekcol@hotmail.com

Summary

Introduction: Instead of synthetic antioxidants, using of natural products with antioxidant activity is demanded. **Objective:** The aim of this study is to evaluate the antioxidant activity and total phenolic and flavonoid contents of the *Trachystemon orientalis* (borage) obtained from Ordu. **Methods:** The water and ethanol extracts of the borage plant were investigated by using several *in vitro* antioxidant activity tests such as total antioxidant capacity, $O_2^{\cdot -}$, $\cdot OH$, DPPH and ABTS radicals scavenging activities, hydrogen peroxide scavenging activity, FRAP, Fe^{2+} chelating and reducing power assays in order to make effective comparisons. **Results:** Water extract of the *T. orientalis* had the more phenolic (90 mg GA/g extract) and flavonoid (56.88 mg CT/g extract) contents than ethanol extract. Furthermore, both of the extracts showed strong antioxidant and radical scavenging activities determined by different *in vitro* models. The highest total antioxidant capacity as ascorbic acid equivalents of 244.50 mg/g extract was also calculated for water extract. Generally, a relationship between total phenolic content and antioxidant activity was established. It is also an important finding for an edible food source that the phenolic and flavonoid content and antioxidative activities for water extract was higher than the ethanol extract. **Conclusion:** *T. orientalis* could be used as a food ingredient instead of synthetic antioxidants and all results will contribute to the recent increase in investigations on using natural products in many areas such as food, pharmacy, alternative medicine and natural therapy.

Key words: *Trachystemon orientalis*, antioxidant activity, phenolic content, flavonoid content, water extract, ethanol extract

INTRODUCTION

Antioxidant supplements, or foods containing antioxidants, can be used to conserve the human body from the oxidative damage [1]. Synthetic antioxidants are the most commonly used antioxidants in food and pharmaceutical industries as food preservatives [2]. However, usage of these substances is limited because of their potential health hazards [3]. Due to these facts, consumers showing the demand for natural products are becoming more aware [4]. Natural products, in the form of pure compounds or extracts with antioxidant activity can overcome all these problems and may help the endogenous defense system of the body [5]. Recently, functional foods or food supplements that can protect humans from oxidative stress and several diseases have attracted worldwide interest [6].

A large percentage of the natural antioxidants in foods are provided by phenolic compounds. Plants are a good source of natural antioxidants containing effective bioactive compounds. Due to these exceptional features, fresh and dried aromatic plants as well as their processed products have been widely used as flavourings since ancient times. However, plants have been subject of studies in terms of their antioxidative capacities for last few years [7].

The aim of this work was to determine phenolic and flavonoid contents and evaluate the *in vitro* antioxidant activity using different contemporary methods of water and ethanol extracts of *Trachystemon orientalis* (borage) which is grown and eaten as food in Ordu.

MATERIAL AND METHODS

Plant material

Trachystemon orientalis (borage) used in this study was provided from local markets in Ordu in April 2012. Edible body parts of the plant material without flowers were dried in the shade at room temperature. After drying, it was powdered. To evaluate the effect of the dependence of antioxidative activity on the extraction solvent, both water and ethanol extracts were prepared. For the preparation of the aqueous extract, 15 g of powdered sample was extracted in 300 ml boiling water by stirring for 30 minutes [8]. Extract was filtrated and lyophilized. To prepare the ethanol extract, the dried sample was incubated with solvent in shaking water bath at ambient conditions (12–16 hours) [8] and filtrated. Filtrates obtained as a result of repetition of this process were combined and solvent was evaporated at 40°C. Lyophilized and evaporated samples were weighed for quantification of extractable compounds from plants and resolved in water and ethanol, respectively.

Total phenolic and flavonoid contents

The contents of the soluble phenolics and flavonoid of the extracts were assayed according to the Folin-Ciocalteu method modified by Singleton & Rossi [9] and a method described by Sakanaka *et al.* [10], respectively. The former was calculated as gallic acid (mg GA/g extract) and pyrogallol (mg PG/g extract) equivalent. The latter were calculated as catechin (mg CT/g extract) equivalent.

Antioxidant activity assays

Phosphomolybdenum method [11] was used to determine the total antioxidant content of the extracts and expressed as equivalent of ascorbic acid (AA).

Superoxide radicals were produced by a method of Beauchamp & Fridovich [12] for determination of superoxide radical scavenging activity. In this method, the photochemically reduced riboflavins generated $O_2^{\cdot-}$ which reduced nitro blue tetrazolium (NBT) to form blue formazan. The extracts investigated for SOD activities were added to reaction mixture, in which $O_2^{\cdot-}$ radicals were scavenged, thereby inhibiting the NBT reduction at different ratio. The net value of absorbance was calculated as the decrease in $O_2^{\cdot-}$ level.

Deoxyribose method [13] was used in order to determine the $\cdot OH$ scavenging activities of the extracts. GA and BHA were used as standards.

Free radical scavenging activities were calculated as inhibition of DPPH radicals for different extract concentrations. Values of IC_{50} were calculated from the graph of extract concentrations against the %I values. All results were compared with values obtained by using AA as standard antioxidant instead of test sample.

ABTS⁺ radical scavenging activity was determined according to the method of Re *et al.* [14] and the activity was expressed as Trolox equivalent (TEAC), as described for the DPPH method. Furthermore, the same procedure was carried out with BHA and AA as positive control.

Ferric reducing antioxidant power (FRAP) of the extracts was determined according to the method described by Benzie & Strain [15]. The extract concentration that have the ferric-TPTZ reducing ability in an equivalent ratio with 1 mM $FeSO_4 \cdot 7H_2O$ was defined as equivalent concentration.

The ability of extracts for scavenging of H_2O_2 were determined by following the method developed by Ruch *et al.* [16].

Abilities of extracts to compete with ferrozine for iron (II) ions to form chelate in solution were investigated by the method of Dinis *et al.* [17]. EDTA and a sample without EDTA used as a positive and negative control, respectively.

Measurements of reducing power of extracts were carried out according to method developed by Oyaizu [18].

Ethical approval: The conducted research is not related to either human or animal use.

RESULTS AND DISCUSSION

T. orientalis is a fully hardy perennial herbaceous plant with purple flowers in late Winter, early Spring and mid Spring. Its origin is known as Turkey (Northern) and Bulgaria. It is known that *T. orientalis* is grown in abundance and consumed as food in Ordu. The effect of solvent polarity on the studied parameters or which one is more responsible for the antioxidant activities were examined. Apart from this, the plant extracts which are prepared by using different solvents have various kinds of secondary metabolites. Because of an individual component in extract, the plant can employ single properties in a bioactive way. All assays were carried out on the whole extract. Crude extracts obtained from plants are responsible for potent bioactive properties of plants. Therefore, the advantage of a diet rich in fruits and vegetables is thanks to complex mixture of phytochemicals present in whole foods [19]. Firstly, the total phenolic and flavonoid contents of the extracts were evaluated because of the phenolics are considered to be major contributors to the antioxidant capacity of plants.

Because of their ability to donate hydrogen and form stable radical intermediates [20], antioxidant capacity of plants largely depends on phenolics. Furthermore, these phenolic antioxidants have several biological activities. The total amount of phenolic compounds in water and ethanol extracts were 90 and 21.2 mg GA/g extract and 68.9 and 17.5 mg PG/g extract, respectively. Obtained values were comparable with results presented in the literature for other extracts of plant products or different kinds of edible sources [21-23]. In addition, a report is available in literature that *T. orientalis* obtained from local markets in Giresun have total phenolic in the amount of 82.1 mg pyrocatechol/g DW [24]. The results showed that the highest content of total phenolic compounds found in extract obtained with water. A similar conclusion was also reached in a study on *Smilax excelsa* L. leaf extracts [25]. But the examples such that of an investigation on *Cynara cardunculus* extracts [23] indicate this can not be so always, are also available in the literature. Otherwise, it should be known that none of the extraction procedures used is completely suitable for whole phenolics from all materials. In different studies, genetic factors, variations of cultivars, and the extraction procedures caused the variation in phenolic contents [21]. As a result, a comparison of several results from literature is difficult.

One of the most common groups of phytochemicals are flavonoids, having a lot of chemical and biological activities such as radical scavenging properties found in plants. Moreover, flavonoids have many functions in plants. These can be used as defensive compounds against insects and several pathogens and are ideal natural antioxidants because of their active hydroxyl groups [26]. Owing to their flavonoid capacities, plants have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer [27]. The flavonoid contents of water and ethanol extracts were found as 56.88 and 29.4 mg CT/g extract. These results show that the flavonoid contents of the extracts prepared with different solvents

of various polarities were considerably different. The results are comparable with the values obtained for hydromethanolic extracts of some medicinal species from South Algeria [2].

Antioxidant activities

When determining the antioxidant capacity of a plant extract, in order to obtain more accurate results using several methods, of more than one type, is better. It is known that different methods are based on different reaction mechanisms since antioxidants may exhibit their useful effect by three main processes such as hydrogen atom transfer, single electron transfer and metal chelation [28]. Varieties of phytochemicals including and the associated variety in chemical moieties are other reasons of using different assays more than one [27]. So, in the current study, *in vitro* antioxidant activities of extracts were determined using various methods.

In phosphomolybdenum method, Mo^{6+} is reduced to form a green phosphate/ Mo^{5+} complex at acidic pHs in the presence of antioxidant material [28]. So, high absorbance values resulting from more intense green color indicate that the sample possesses significant antioxidant activity. Total antioxidant activity (TAA) of extracts were calculated as 92.50 and 244.50 mg AA/g for ethanol and water extracts, respectively. There was a correlation between the TAAs and both phenolic and flavonoid contents. Observation of a correlation like this is quite important. It had been shown that a high total phenol content increases the antioxidant activity [29]. Özen [24] had reported a result for the TAA of *T. orientalis* provided from Giresun, according to the same method. Due to differences in calculation procedures ($\mu\text{mol } \alpha\text{-tocopherol/g}$ of extract) to make comparison with the current study had not been possible. Based on the results of various studies [30, 31] it can be said that phenolic substances in the context of a plant extract and synergistic interactions may be responsible for its antioxidant activity [2].

As a result of a normal process of energy generation, $\text{O}_2^{\cdot -}$ is formed in the human body. Actually it is quite unreactive compared to the other radicals. But it is a precursor of the more reactive oxygen species such as H_2O_2 , $^{\cdot}\text{OH}$ and singlet oxygen, contributing to tissue damage and various diseases. Therefore it is known to be very harmful to living cell components [32].

Both ethanol and water extracts had a scavenging activity on the SOD radicals in a dose dependent manner. IC_{50} values were calculated as 1800, 74, 59, 37 and $0.8 \mu\text{g/ml}$ for alcohol extract, water extract, AA, BHA and SOD enzyme, respectively. These values show that, especially, the water extract of the *T. orientalis* were strong enough to compared with standard antioxidant substances. Most examples in the literature are similar. In these reports, the investigated samples had lower SOD anion radical scavenging activity than standard antioxidants [26]. The reason for this can be attributed to the presence of relative concentration of

bioactive constituents and mixture of impurities/other nutrients in the extracts. SOD radicals scavenging power of the extracts may depend on their contents such as phenolics [27]. An antioxidative extract can terminate the radical chain reaction by scavenging the superoxide radicals by combining with superoxide radical ions to form stable radicals [28].

Hydroxyl radical is one of the most harmful free radicals among all the reactive oxygen species, mainly responsible for cell or tissue injury [2, 33]. There is no specific enzyme to defend against hydroxyl radicals in humans. So, it is important to investigate several additives with $\cdot\text{OH}$ scavenging activity of very high degree. Furthermore, it was reported that the $\cdot\text{OH}$ scavenging capacity of extract directly reflects its antioxidant activity [28]. 2.5 $\mu\text{g/ml}$ of BHA scavenged the hydroxyl radicals in the ratio of 33%. However, similar ratio could be provided with greater amounts (350 $\mu\text{g/ml}$) of GA. The scavenging activity on $\cdot\text{OH}$ of the ethanol extract was high enough to compare with the activity of BHA. 73% of the hydroxyl radicals were scavenged by the amount of 20 $\mu\text{g/ml}$ of ethanol extract. However, the activity of water extract was significantly lower than that of BHA. 1.7 mg/ml of the water extract defused the 51% of the hydroxyl radicals. This value was almost consistent with the value obtained for GA. Because, the 1.4 mg/ml of the GA was scavenged the hydroxyl radical in the ratio of 68%. Very similar results have been reported in the literature for *S. excelsa* L. leaf extracts [25].

DPPH is frequently used to investigate radical scavenging activity of natural compounds because of its quite stable radical character. The DPPH $\cdot$ has an unpaired electron caused to a strong absorbance at 517 nm and a purple color. In the presence of an antioxidant, DPPH $\cdot$ is reduced to its non-radical form, a stable diamagnetic molecule and the absorbance decreases. The degree of the reduction in absorbance are proportional to the extent of the free radical scavenging potentials of the sample/antioxidant [34]. DPPH radical scavenging activities of both extracts were concentration-dependent. The IC_{50} values were calculated as 0.41, 2.6 and 0.029 mg/ml for water, ethanol extracts and AA utilizing from the studies on different concentrations. As the IC_{50} value of the extract increases, the DPPH radical scavenging activity decreases. The water extract has higher scavenging activity against DPPH free radical than ethanol extract, but the IC_{50} values for both extract were higher than the value found for AA. It is usually thought that there is a correlation between total phenol contents and antioxidant activity of the plant extracts. In the current study, this kind of correlation was actually observed between total phenol contents and DPPH radical scavenging activities of the extracts. Furthermore it is reported in literature that polar or phenolic compounds cause higher antioxidant activity [5]. Thereby, our results are reasonable. Besides, the found values were much higher than calculated for extracts from the roots of *Dissotis thollonii* Cogn [35]. There is an important difference between these two studies. They had found the lowest activity for water extract as different from ours [35]. On the other hand, there are several studies compatible with ours, such as carried out on crude extracts of *Commiphora myrrha* resin. They calculated

the IC_{50} values for DPPH scavenging activities in the range of 0.07–11.33 mg/ml for different polarities [36]. Özen [24] investigated the *T. orientalis* extract among various wild edible plants and revealed the minimum DPPH scavenging activity for *T. orientalis* (50% inhibition for 100 μ g/ml).

ABTS free radical scavenging assay is an antiradical activity test different from DPPH method and widely used to evaluate antioxidant activity in foods and biological systems [33] for both the polar and non-polar samples [14]. The ABTS radical is generated by the reaction of ABTS and potassium persulphate, and when an antioxidant is added, $ABTS^{\bullet+}$ is converted to a non-radical form [33]. This transformation is followed by reduction in absorbance at 734 nm. The $ABTS^{\bullet+}$ scavenging abilities of water and ethanol extracts were determined as 1725 and 240 mmol trolox/kg dry weight, respectively. Calculated values for both extracts were significantly different and the values obtained for BHA (11617 mmol trolox/kg dry matter), and AA (8298 mmol trolox/kg dry weight). $ABTS^{\bullet+}$ scavenging activities for asparagus and broccoli extracts had been previously determined to be 26.2 mmol Trolox equivalent/kg dry weight as average [37]. So it can be said that the borage plants have the higher antioxidant character than asparagus and broccoli according to ABTS method.

FRAP method is a significant indicator of potential antioxidant activity of the sample. In this method, the reducing power of the antioxidant sample of ferrous ion (Fe^{3+}) to ferric ion (Fe^{2+}) is evaluated [37]. FRAP of the extracts were calculated in terms of mmol Fe^{2+} /kg and obtained values were compared with the values found for BHA and trolox. Determined FRAP value for alcohol extract (3236 mmol Fe^{2+} /kg) was considerably higher than that found for BHA (1498 mmol Fe^{2+} /kg). While the highest activity (6994 mmol Fe^{2+} /kg) was found in the presence of trolox, the smallest (741 mmol Fe^{2+} /kg) value was calculated for the water extract. Values found in current study were higher than the FRAP activity calculated for different kinds of the *Cynara cardunculus* extracts [23].

It is stated that reactivity of the H_2O_2 is actually low and it is not able to trigger the lipid peroxidation. Although, it has a potential to create the reactive hydroxyl radical through Fenton reactions. So, H_2O_2 can be toxic to cells [25]. Consequently, the removal of the H_2O_2 by antioxidant defense system in food and cells is essential. H_2O_2 scavenging abilities of the *T. orientalis* extracts were not effective compared to the standards such as BHA, AA, GA. Because 50 μ g/mL of BHA, AA and GA could scavenge the H_2O_2 in the ratio of 66, 35 and 33%, respectively. However, 250 μ g/ml of water and ethanol extracts could just scavenge 30 and 6.3% of H_2O_2 . In another study, the H_2O_2 scavenging activity of extracts of *S. excelsa* leaf had been found as in average higher than 90% at a concentration of 5 mg/ml. However, this values was also lower than standard ones [25].

Iron is essential for oxygen transport, respiration, and enzyme activity [38]. At the same time, Fe^{2+} triggers lipid peroxidation by breaking down hydrogen through Fenton reaction [32]. Then it gets a harmful component of the cell and causes the oxidative damage in living tissues and cells [38]. Extracts or

compounds which can stabilize transition metals with their chelating activity are supposed to prevent lipid peroxidation [28]. Due to this fact, investigation of the extracts in terms of chelating activity was also important. For this purpose, *T. orientalis* extracts were tested according to their ability to compete with ferrozine for Fe^{2+} ions in the reaction mixture. Both extracts and positive control (EDTA) had an ability to chelate iron (II) ions in proportion to the increase in concentration. Although, among these three, EDTA appeared to be a better chelator of Fe^{2+} ions than both extracts. IC_{50} value of EDTA (0.00856 mg/ml) was dramatically higher than that of water (0.27 mg/ml) and ethanol (1.42 mg/ml) extracts. There was a correlation between the obtained IC_{50} values for chelation activity and the amount of total polyphenols and flavonoids. The chelating activity of the methanolic extract of *Juniperus drupacea* Labill. berries from Turkey had been reported as IC_{50} (2.26 mg/ml) [22]. Based on this information it can be said that borage plant has higher chelating activity than the samphire. However, in all studies encountered in the literature it was also reported that the EDTA had better chelating activity than the examined samples [5, 7]. Furthermore, Özen [24] had found the chelating ability of the 100 $\mu\text{g/ml}$ of the hydroalcoholic extract of the *T. orientalis* from Giresun as lower than 40%. This finding is also consistent with what we found.

The reducing power of an extract which is related to its electron transfer ability means that it has a potential antioxidant activity [33]. The compounds having reducing power causes a reduction in the Fe^{3+} /ferricyanide complex to Fe^{2+} and the formation of Fe^{2+} is monitored by measuring the absorbance at 700 nm [24]. Greater absorbance indicates greater reducing power. Donating a hydrogen atom and reacting with the peroxide precursor by such compounds cause to break the free-radical chain and inhibit peroxide formation [39]. The order of the reductive potential was $\text{AA} > \text{GA} > \text{BHA} > \text{water extract} > \text{ethanol extract}$. As in table 1, at a very low concentrations the investigated samples did not cause an increase in the absorbance because of their low reducing power, whereas at very high concentrations, at which the samples had just reducing power, the standard antioxidants caused an immeasurably high absorbance because of their very high reducing power. Therefore, the assay was carried out at different concentrations for each of these. According to these, the reducing power of the 0.28 mg/ml of the water extract and 0.7 mg/ml ethanol extract were equivalent to the reducing power of 0.07 mg/ml BHA and 0.03 mg/ml BHA or 0.025 mg/ml AA, respectively. The reducing power capacities of the extracts could be attributed to the presence of the natural antioxidants such as phenolic compounds in the plant material [2]. The obtained values were already consistent with the findings for total phenolics. It was reported that the water extract of the *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis* had higher reducing power than the other kinds of extracts and BHA or BHT [7]. Other than that, in many studies the reducing power had been found to be lower than the standards [2, 25, 26]. The borage plant had more reducing power than the *S. excelsa* l. leaf [25].

Table 1.

Total phenolic and flavonoid contents and antioxidant activities of the ethanol and water extracts of *T. orientalis* determined using various methods

Samples	Total phenolics [mg GA/g] – [mg PG/g]	Total flavonoids [mg CT/g]	Total antioxidant capacity [mg AA/g]	ABTS [mmol TX/kg]	FRAP [mmol Fe ²⁺ /kg]	IC ₅₀		
						SOD [μg/ml]	DPPH [mg/ml]	Fe ²⁺ chelating activity [mg/ml]
Water extract	90-68.9	56.88	244.50	1725	741	74	0.41	0.27
Ethanol Extract	21.2-17.5	29.4	92.50	240	3236	1800	2.6	1.42
BHA	–	–	–	11617	1498	37	–	–
Ascorbic acid (AA)	–	–	–	8298	–	59	0.029	–
Trolox (TX)	–	–	–	–	6994	–	–	–
SOD	–	–	–	–	–	0.8	–	–
EDTA	–	–	–	–	–	–	–	0.00856

CONCLUSIONS

The results obtained for total phenolic and flavonoid contents, total antioxidant capacities and O₂^{•−}, DPPH and ABTS radicals scavenging activities, FRAP and Fe²⁺ chelating assays are given in table 1 and a relationships between total phenolic content and antioxidant activity were observed. However, the hydroxyl radical sacevenging activity and the FRAP values of the extracts were not conformed with this relationship. A diversity like this was supposed because of the differentiation of the potential chemical composition of the water and ethanol extracts. While ethanol extract may consist of many phenolic compounds, water extract may contain some polysaccharide and water-soluble phenolic compounds [33]. Since tested plant is edible, it is a highly valuable finding that the highest activities were found in the presence of water extract. *T. orientalis* could be used as a food ingredient instead of synthetic antioxidants and all results will contribute to the recent increase in investigations on using natural products in many areas such as food, pharmacy, alternative medicine and natural therapy. It is thought that the observed activities were derived from the synergistic effect of various components contained in extracts. Therefore, further phytochemical studies can be carried out on the extracts to isolate their active compounds responsible for these activities. Further laboratory assays will be necessary to ascertain new and beneficial activities of this plant.

ACKNOWLEDGEMENTS

I want to thank Ordu University Scientific Researches Project Coordination Department (Project No. AR-1208) for financial support and Assist. Prof. Dr. Arzu

Ozel and Assist. Prof. Dr. Gülin Renda (Karadeniz Technical University, Faculty of Pharmacy, Trabzon, Turkey) for preparing the extracts.

Conflict of interest: Authors declare no conflict of interest.

REFERENCES

1. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. *Food Chem* 2002; 77(2):229-35.
2. Chaouche TM, Haddouchi F, Ksouri R, Atik-Bekkara F. Evaluation of antioxidant activity of hydromethanolic extracts of some medicinal species from South Algeria. *J Chin Med Assoc* 2014; 77(6):302-7.
3. Políteo O, Jukic M, Milos M. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. *Food Chem* 2007; 101(1):379-85.
4. Lachman J, Orsák M, Hejtmánková A, Kovářová E. Evaluation of antioxidant activity and total phenolics of selected Czech honeys. *LWT - Food Sci Technol* 2010; 43(1):52-8.
5. Orhan I, Üstün O. Determination of total phenol content, antioxidant activity and acetylcholinesterase inhibition in selected mushrooms from Turkey. *J Food Compos Anal* 2011; 24(3):386-90.
6. Losso JN. Targeting excessive angiogenesis with functional foods and nutraceuticals. *Trends Food Sci Tech* 2003; 14(11):455-68.
7. Sarikurkcü C, Ozer S, Eskici M, Tepe B, Can Ş et al. Essential oil composition and antioxidant activity of *Thymus longicaulis* C. Presl subsp. *longicaulis* var. *longicaulis*. *Food Chem Toxicol* 2010; 48(7):1801-5.
8. Selen İşbilir Ş. Investigation of antioxidant activities of some plant leaves used as salad-spices. PhD Thesis, Trakya University, Edirne, Turkey. 2008.
9. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vit* 1965; 16(3):144-58.
10. Sakanaka S, Tachibana Y, Okada Y. Preparation and antioxidant properties of extracts of Japanese persimmon leaf tea (kakinoha-cha). *Food Chem* 2005; 89(4):569-75.
11. Prieto P, Pineda M, Aquilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 1999; 269(2):337-41.
12. Beauchamp C, Fridovich I. Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 1971; 44(1):276-87.
13. Aruoma OI. Deoxyribose assay for detecting hydroxyl radicals. *Method Enzymol* 1994; 233:57-66.
14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med* 1999; 26(9-10):1231-7.
15. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 1996; 239(1):70-6.
16. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 1989; 10(6):1003-8.
17. Dinis TCP, Madeira VMC, Almeida, LM. Action of phenolic derivatives (acetoaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch Biochem Biophys* 1994; 315(1):161-9.
18. Oyaizu M. Studies on products of browning reactions: Antioxidant activities of products of browning reaction prepared from glucosamine. *J Nutr* 1986; 44(6): 307-15.
19. Liu RH. Health benefits of fruits and vegetables are from additive and synergistic combination of phytochemicals. *Am J Clin Nutr* 2003; 78(3):517S-20S.
20. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L. Dietary polyphenols and the prevention of diseases. *Crit Rev Food Sci Nutr* 2005; 45(4):287-306.
21. Zhao Y, Du SK, Wang H, Cai M. *In vitro* antioxidant activity of extracts from common legumes. *Food Chem* 2014; 152:462-6.
22. Miceli N, Trovato A, Marino A, Bellinghieri V, Melchini A et al. Phenolic composition and biological activities of *Juniperus drupacea* Labill. berries from Turkey. *Food Chem Toxicol* 2011; 49(10): 2600-8.

23. Kukić J, Popović V, Petrović S, Mucaji P, Ćirić A et al. Antioxidant and antimicrobial activity of *Cynara cardunculus* extracts. Food Chem. 2008; 107(2):861-8.
24. Özen T. Antioxidant activity of wild edible plants in the Black Sea Region of Turkey. Grasas Y Aceites 2010; 61(1):86-94.
25. Ozsoy N, Can A, Yanardag R, Akev N. Antioxidant activity of *Smilax excelsa* L. leaf extracts. Food Chem 2008; 110(3): 571-83.
26. Kumar PS, Sucheta S, Deepa VS, Selvamani P, Latha S. Antioxidant activity in the some selected Indian medical plants. Afr J Biotech 2008; 7(12):1826-8.
27. Kanatt SR, Chawla SP, Sharma A. Antioxidant and radio-protective activities of lemon grass and staranise extracts. Food Bioscience 2014; 6(24):24-30.
28. Sun L, Zhang J, Lu X, Zhang L, Zhang Y. Evaluation to the antioxidant activity of total flavonoids extract from persimmon (*Diospyros kaki* L.) leaves. Food Chem Toxicol 2011; 49(10):2689-96.
29. Kumaran A, Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT - Food Sci Technol 2007; 40(2):344-52.
30. Cakir A, Mavi A, Yildirim A, Duru ME, Harmandar M et al. Isolation and characterization of antioxidant phenolic compounds from the aerial parts of *Hypericum hyssopifolium* L. by activity-guided fractionation. J Ethnopharmacol 2003; 87(1):73-83.
31. Ksouri R, Megdiche W, Falleh H, Trabelsi N, Boulaaba M et al. Influence of biological, environmental and technical factors on phenolic content and antioxidant activities of Tunisian halophytes. C R Biol 2008; 331(11):865-73.
32. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine 1999, 3rd ed., Oxford University Press, Oxford.
33. Fu R, Zhang Y, Guo Y, Liu F, Chen F. Determination of phenolic contents and antioxidant activities of extracts of *Jatropha curcas* L. seed shell, a by-product, a new source of natural antioxidant. Ind Crop Prod 2014; 58:265-70.
34. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature 1958; 181:1199-200.
35. Nono RN, Barboni L, Teponno RB, Quassinti L, Bramucci M et al. Antimicrobial, antioxidant, anti-inflammatory activities and phytoconstituents of extracts from the roots of *Dissotis thollonii* Cogn. (*Melastomataceae*). S Afr J Bot 2014; 93:19-26.
36. Mohamed A, Ali SA, EL-Baz FK, Hegazy AK, Kord MA. Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of *Commiphora myrrha* resin. Ind Crop Prod 2014; 57:10-16.
37. Sun T, Powers JR, Tang J. Evaluation of the antioxidant activity of asparagus, broccoli and their juices. Food Chem 2007; 105(1):101-6.
38. Dastmalchi H, Dorman D, Into L, Raimo H. Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. LWT – Food Sci Technol 2007; 40(9):1655-63.
39. Meng J, Fang Y, Zhang A, Chen S, Xu T et al. Phenolic content and antioxidant capacity of Chinese raisins produced in Xinjiang Province. Food Res Int 2011; 44(9):2830-6.

AKTYWNOŚĆ ANTYOKSYDACYJNA *TRACHYSTEMON ORIENTALIS* (L.) G. DON (OGÓRECZNIKA) UPRAWIANEGO I SPOŻYWANEGO W PROWINCJI ORDU W TURCJI

MELEK COL AYVAZ

Department of Chemistry
Faculty of Arts and Sciences
Ordu University
52200 Ordu, Turkey

tel.: +90452 234 50 10 1669, faks: +90452 233 91 49
e-mail: melekcol@hotmail.com

Streszczenie

Wstęp: Stosowanie produktów naturalnych o właściwościach antyoksydacyjnych jest bardziej korzystne niż antyoksydantów syntetycznych. **Cel:** Celem niniejszej pracy była ocena działania antyoksydacyjnego oraz oznaczanie całkowitej zawartości fenoli i flawonoidów w *Trachystemon orientalis* (ogóreczniku) pochodzącym z Ordu w Turcji. **Metody:** W celu określenia aktywności antyoksydacyjnej w wyciągu wodnym i etanolowym z ogórecznika zastosowano następujące testy *in vitro*: DPPH, ABTS i FRAP. **Wyniki:** Stwierdzono, że wyciąg wodny z *T. orientalis* zawierał więcej fenoli (90 mg GA/g ekstraktu) i flawonoidów (56,88 mg CT/g ekstraktu) w porównaniu do wyciągu etanolowego. Największą aktywność antyoksydacyjną w odniesieniu do kwasu askorbowego określono dla wyciągu wodnego z ogórecznika (244,50 mg/g). Ponadto wykazano, że zawartość fenoli i flawonoidów, a także działanie antyoksydacyjne, było wyższe w wyciągu wodnym niż etanolowym. **Wnioski:** Ogórecznik (*T. orientalis*) może być stosowany jako składnik pożywienia zamiast syntetycznych antyoksydantów. Przedstawione wyniki mogą przyczynić się do zintensyfikowania badań nad użyciem produktów naturalnych w wielu dziedzinach, takich jak produkcja żywności i leków, medycyna alternatywna oraz terapia naturalna.

Słowa kluczowe: *Trachystemon orientalis*, działanie antyoksydacyjne, zawartość fenoli i flawonoidów, wyciąg wodny i etanolowy