

Antioxidant Activity, Total Phenol and Total Flavonoid Contents of *Trachystemon orientalis* (L.) G. Don

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ABSTRACT

Objective: For many years, plants have been considered a source of alternative medicine and are used for the treatment of several diseases. These medicinal plants are excellent sources of phytochemicals and antioxidant activity. *Trachystemon orientalis* (L.) G. Don (Boraginaceae) originates from East Bulgaria but can be found mostly in West Caucasia and the Black Sea region of Turkey. Its flowering branches, rhizomes, and leaves are used as food.

Materials and Methods: The antioxidant activity of a *T. orientalis* aqueous infusion was investigated using various antioxidant tests, such as reducing power and radical scavenging activity. The phenolic and flavonoid contents were also determined. Results were compared with natural and synthetic antioxidants.

Results: The results demonstrated that *T. orientalis* (L.) is a good source of antioxidants.

Conclusion: This study suggested that *T. orientalis* extract can be considered a useful natural antioxidant source because of its flavonoid, phenolic, and anthocyanin contents. The food and cosmetic industries might employ *T. orientalis* extract as an alternative additive to other highly toxic synthetic antioxidants.

Keywords: Antioxidant activity, oxidative stress, phenolic compounds, reducing power, radical scavenging activity, flavonoid, *Trachystemon orientalis* (L.) G. Don.

INTRODUCTION

Oxidative stress is defined as the disturbance or lack of balance in the production of free radicals and the antioxidant system (1,2). Disturbances in this normal redox status can cause toxic impacts by producing peroxides and free radicals that damage cell and tissue components such as lipids, proteins and DNA (3,4). In humans, oxidative stress causes many diseases such as tissue injury, cell death, cancer, cardiovascular diseases, arteriosclerosis, neural disorders, Parkinson's disease, Alzheimer's disease, ageing, skin irritations and inflammations, diabetes mellitus and chronic fatigue syndrome (5-8). Antioxidants are known to delay or inhibit the oxidation of substrates even when present

in low concentrations (9). Synthetic antioxidants such as propyl gallate (PG), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) protect lipids found in foods from the harmful effect of oxidation. However, both BHT and BHA are restricted from use because of their toxic and carcinogenic effects (10). Because of the negative effect of synthetic compounds, natural antioxidants rather than synthetic antioxidants are much more preferred by consumers. Thus, the demand for natural and safer antioxidants is growing.

Studies have shown that higher plants are excellent sources of functional compounds such as anthocyanins, vitamins, flavonoids, dietary glutathione and carotenoids

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- which are responsible for antioxidants' function. Therefore, they are considered as important nutraceuticals with many health benefits (11,12). *T. orientalis* is a plant of East Bulgarian origin. In Turkey, it is popularly known as Galdirek, Kaldirik, Hodan, Kalduruk (Bolu), Tamara (Trabzon), Burgi (Artvin) and Zilbit, Ispit (Zonguldak). The plant is chiefly found in the West Caucasia and Black Sea regions and is consumed as a vegetable in Istanbul and other regions of the Black Sea (13,14). A concoction of their fresh roots is used like a tonic on the skin against rheumatism and/or for the healing of inflamed wounds (15). Additionally, it has diuretic effects and can be used as a blood purifier (16). *T. orientalis* is reported to contain nitrate salts, tannins, mucilage, essential oils, resin and saponins (17).

A quantitative assessment of total phenols, flavonoids and anthocyanin contents of *T. orientalis* was carried out in this study. In addition, the reducing power of the aqueous infusion extract, ferric thiocyanate antioxidant activity and The 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the plant was conducted.

MATERIALS AND METHODS

Plant Materials

The leaves of *T. orientalis* were acquired from Istanbul, Turkey in the month of March 2017. The fresh sections were cleaned with water and dried at 20°C, then stored at -20°C until use.

Preparation of Infusion Extract

Plant extract was prepared as a 10% (w/v) aqueous infusion. A dried sample (20g) was extracted with boiling water (100 mL) for 15 min while stirring.

Phytochemical Tests

The infusion extract was subjected to preliminary qualitative phytochemical analysis for the detection of major chemical groups (Table 1). The details are below:

1. Phenols were analysed after the aqueous solution of *T. orientalis* infusion was filtered using filter paper. A drop of molybdophosphoric acid reagent was added to the sediment and absorbed into NH₃ vapour.
2. Braemer's test for tannins was adopted for tannins determination. A 10 % alcoholic FeCl₃ solution was added to 2-3 mL of infusion extract.
3. Steroids and terpenoids were determined using the Liebermann-Burchardt test. Into one mL of infusion extract solution, one mL of CHCl₃, two / three mL acetic anhydride and one to two drops of 98 % H₂SO₄ were added.
4. Dragendorff's reagent was added to 1mL of infusion extract for alkaloid determination.
5. Bornträger's test for anthraquinones was carried out by heating approximately 50 mg of the infusion extract with 10% FeCl₃ solution and one mL of 37 % HCl. Diethyl ether was used to rinse the cooled extract after filtration, before further extraction with concentrated NH₃.

Determination of Antioxidant Activity of the Extract

Determination of Total Phenolic Compounds

Total phenolic compounds of *T. orientalis* were determined with the Folin-Ciocalteu reagent using Slinkard and Singleton's method (1977), pyrocatechol was used as a standard for phenolic compound (18). Absorbance was measured spectrophotometrically at 760 nm.

Determination of Total Flavonoid Content

Total content of flavonoids in *T. orientalis* was determined spectrophotometrically at 510 nm (19). The results are presented as the mean (±SD) mg of (+)-catechin equivalents per gram of extract.

Determination of the Anthocyanin Content

The anthocyanin composition of *T. orientalis* was estimated by the modified method of Padmavati et al. (20). The anthocyanin concentration was determined spectrophotometrically at 530 and 657 nm. The absorbance of anthocyanin was calculated using the extinction coefficient of 31.6 M⁻¹cm⁻¹.

Ferric Thiocyanate (FTC) Antioxidant Activity

Osawa and Namiki's method was employed for the determination of FTC activity (21). The measurement of absorbance was made spectrophotometrically at 500 nm. α-Tocopherol was used as positive control. All tests were conducted in triplicate and the average was calculated.

FTC antioxidant activity was calculated with the following equation:

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

A represents the control and B is the sample.

Reducing Power

The reducing potential of *T. orientalis* was analysed using Oyaizu's method (22). Absorbance was measured spectrophotometrically at 700 nm, high absorbance values indicate a strong reducing power.

DPPH Radical Scavenging Potential

DPPH radical scavenging activity of antioxidants developed by Brand-Williams et al. (23) was adopted in this study. The equation below was used for the calculation of the DPPH radical activity.

$$\text{DPPH radical scavenging activity (\%)} = (A_0 - A_1/A_0) \times 100$$

A₀ indicates the absorbance of the control and A₁ indicates the absorbance of the sample.

RESULTS

In this study, the presence of tannins, phenols, and anthraquinones was detected in the *T. orientalis* aqueous infusion extract (Table 1).

The level of total phenolic compounds of *T. orientalis* is presented in Table 2. An equivalent of 36 µg of pyrocatechol was obtained in 1 mg/mL of aqueous infusion extract. The concen-

tration of flavonoids in 1 mg of the *T. orientalis* aqueous infusion extract was found to be 29.34 ± 0.62 μg catechin equivalents. This suggests that the antioxidant activities of *T. orientalis* might be due to its high level of flavonoid content. As indicated in Table 2, the anthocyanin level of *T. orientalis* in this study was 0.35 ± 0.06 $\mu\text{mol/g}$ extract.

These primary products (i.e. peroxides) can be measured by the ferric thiocyanate method in the linoleic acid system, with

α -tocopherol employed as standard. As a result, high peroxide formation during the emulsion incubation causes a high absorbance value. The effects of varied concentrations (20 and 60 $\mu\text{g/mL}$) of *T. orientalis* of linoleic acid emulsion on lipid peroxidation are given in Table 3. At forty-eight hours of testing, the percentage peroxidation inhibition of 20 and 60 $\mu\text{g/mL}$ of aqueous infusion extracts on the linoleic acid system was 64.57 ± 3.53 % and 75.48 ± 0.62 % respectively. These values were higher than that of 100 $\mu\text{g/mL}$ α -tocopherol (61.19 ± 0.61 %).

More so, a high absorbance value in reducing power test signifies a strong reducing power. The reducing power of *T. orientalis* increased with increasing concentration of the extract (Figure 1). However, its reducing effect was weak when compared

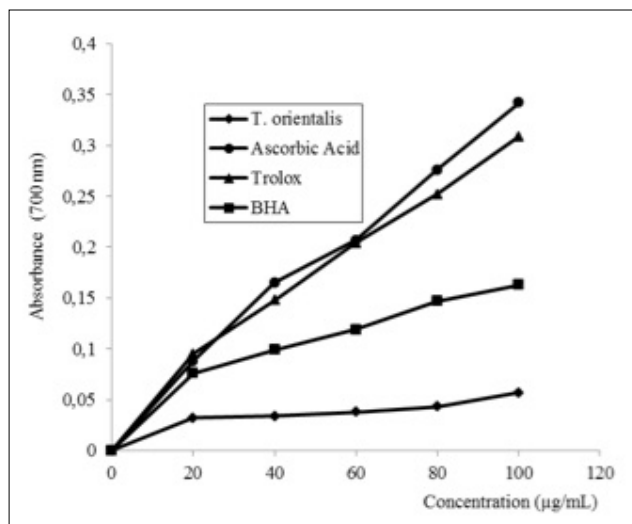


Figure 1. Reducing power of *T. orientalis* (L.) G. Don. aqueous infusion extract.

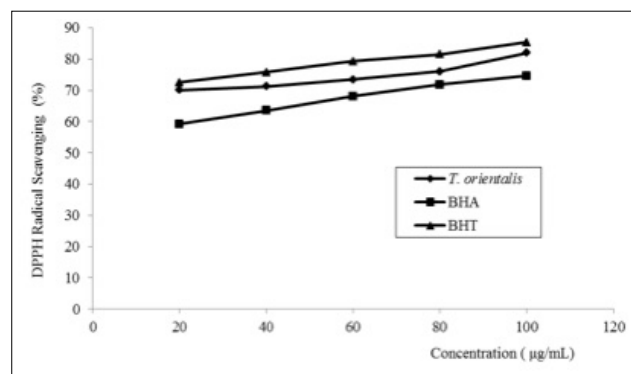


Figure 2. DPPH radical scavenging activity of aqueous extracts of *T. orientalis* (L.) G. Don.

Table 1. Preliminary phytochemical screening of aqueous infusion extract of *T. orientalis* (L.) G. Don

Tested for	Detection*	Test performed
Phenols	++	Phosphomolybdic acid test
Tannins	+++	Braemer's test
Steroids and Terpenoids	---	Liebermann- Burchardt test
Alkaloids	---	Dragendorff's test
Anthraquinones	+++	Bornträger test

* --- = Absent; ++= Moderate; +++ = Abundant

Table 2. Total phenolic compounds (as pyrocatechol equivalent), total flavonoids (as catechin equivalent) and total anthocyanin of *T. orientalis* (L.) G. Don. aqueous infusion extract

Concentration ($\mu\text{g/mL}$)	Phenolic Compounds (μg pyrocatechol/mg extract)	Flavonoids (μg catechin/mg extract)	Anthocyanins ($\mu\text{mol/g}$ extract)
250	29.36 ± 0.87	7.94 ± 0.31	0.35 ± 0.06
500	32.00 ± 0.32	13.92 ± 0.24	-
750	34.17 ± 1.10	21.86 ± 0.74	-
1000	36.00 ± 0.59	29.34 ± 0.62	-

Values: Mean \pm SD (n=3).

Table 3. Antioxidant activity (FTC) of aqueous infusion extracts of *T. orientalis* (L.) G. Don in comparison to α -tocopherol as reference antioxidant at 48 hours

Compounds	(Inhibition %)
<i>T. orientalis</i> (20 μ g/mL)	64.57 \pm 3.53
<i>T. orientalis</i> (60 μ g/mL)	75.48 \pm 0.62
α -Tocopherol (100 μ g/mL)	61.19 \pm 0.61

Values: Mean \pm SD (n=3). FTC: Ferric Thiocyanate

to the reducing power of positive controls like BHA, Trolox and ascorbic acid.

The DPPH assay is a highly effective and easy to apply spectrophotometric method for understanding the effect of radical compounds, as well as radical scavenging capacities of antioxidants. Figure 2 represents the DPPH radical scavenging activity of *T. orientalis* extract, and that of BHA and BHT as positive control. Both the extract and the standards exhibited scavenging properties according to BHT, *T. orientalis* and BHA had the following activities respectively 85.45 \pm 9.78% > 81.99 \pm 7.45% > 74.61 \pm 9.04%.

DISCUSSION

Reactive oxygen species and reactive nitrogen species are highly reactive oxidizing molecules which are constantly generated during normal cellular activities. For instance, the activity of the mitochondrial respiratory chain and inflammation could generate these compounds, which could lead to damage of other biological molecules such as proteins and DNA. In the last decade, there has been a growing interest in natural antioxidants. Studies shows that diets rich in fruits, vegetables and derived products have been defined to alleviate chronic diseases. These food containing herbs are rich in phytochemical molecules such as vitamins, phenolic antioxidants etc, thus aids normal health and wellbeing (24-27).

Polyphenols are a diverse group of plant secondary metabolites, encompassing subgroups such as tannins and flavonoids among others. They are found throughout the plant kingdom, with their biological function lying mostly within their defensive capabilities against herbivores, pathogens, and UV-B radiation. Tannins are structurally the most complex group of polyphenols, they are large ringed, present in several plant families and are reported to exhibit anticancer or cancer preventive activity and antioxidant capability (28-29). Therefore, *T. orientalis* aqueous infusion extract which is a good source of tannins may possess significant anticancer activity and antioxidant potentials. In recent years, several clinical trials, as well as other evidences have indicate that diseases precipitated by oxidative stress can be prevented or managed with high flavonoids rich foods (30-32). Previous studies by Özen (33) report a phenolic content of 82.1 \pm 1.5 mg pyrocatechol/g dry weight of *T. orientalis*. Moreover, Ayvaz (34) reported a phenol concentration of

68.9 mg pyrogallat/g and 17.5 mg pyrogallat/g respectively in water and ethanolic extracts. Conversely, the phenolic content of *T. orientalis* in this study (at 500 μ g/mL aqueous infusion extract concentration) was below those of the aqueous infusion extracts in earlier reports (32.00 \pm 0.32 mg pyrocatechol/mg). The difference in concentration may be attributed to the concentration of extract used for the qualitative analysis as well as the extraction methods employed.

In many herbs, antioxidant activities correlate positively with phenolic contents (35). Several works of literatures have reported the key role of phenolic compounds in scavenging free radicals, as well as the high scavenging ability of phenol rich plant samples (36-39).

Flavonoids in plants are involved in providing pigmentation for flowers, fruits and seeds to attract pollinators and seed dispersers, improving fertility, aiding germination of pollen, protecting against stress, and acting as signal molecules in plant microbe interactions. Flavonoids are greater antioxidants than natural phenolic compounds (40). Moreover, the antioxidant activity of plant products is also correlated to their total flavonoid content. Thus, it is suggested that the consumption of flavonoid-containing nourishments is beneficial for protection from free radical damage. The flavonoids content quantified from *T. orientalis* in this study at 1000 μ g/mL aqueous infusion extract (29.34 \pm 0.62 μ g catechin/mg extract) was comparable to that of ethanol extract (29.4 mg catechin /g extract), but lower that of aqueous extract (56.88 mg catechin /g extract respectively) reported by Ayvaz (33).

Anthocyanins which are a type of antioxidant flavonoids have diverse functions in higher plants. They act as insect and/or animal attractors thanks to the coloration they confer to plants. It is also suggested that they have a protective effect against UV light, infection by pathogens as well as scavenging reactive oxygen species. Furthermore, they confer drought, low temperature and high salinity resistance in plants. In general, anthocyanins find their way into human diet through vegetables, fruit, tea, beans, coffee, cereals, herbs and spice extract. They are famous for their pharmacological effects such as antioxidant, antitumor, anti-inflammatory and antimutagenic activities (41). Comparatively, the anthocyanin content of *T. orientalis* in this study was extremely low (0.35 \pm 0.06 μ mol/g extract), in relation to the 15.2 \pm 0.1 mg cyanidin 3-glucoside/g dry weight reported by Özen (34).

Lipid peroxidation mediated chain reactions are processes initiated by free radicals that result in the production of peroxide implicated in several biological complications (42). The mechanism for spectrophotometric estimation of lipid hydroperoxidation involves the oxidation of ferrous ion to ferric ion, followed by coupling to thiocyanate to produce a complex. By this, the amount of peroxide produced during the initial stages of oxidation (the primary product of lipid oxidation) is quantified. The findings of this study suggest that *T. orientalis* aqueous infusion extract has a high peroxidation inhibition capacity at 20 and 60

µg/mL as compared to α-tocopherol and therefore, may serve as an alternative source of antioxidants.

Reducing power is related to antioxidant activity due to the fact that antioxidant can give off their electrons for the reduction of reactive radicals. Thus, reducing power can be used to give information about antioxidative potentials of prospective antioxidants (43). It can be measured by the direct reduction of $\text{Fe}[(\text{CN})_6]_3$ to $\text{Fe}[(\text{CN})_6]_2$, forming intense Perl's Prussian blue complex measured at 700 nm. An increase in absorbance indicates an increase in reducing capacity as a result of an increase in the formation of the complex (44). The *T. orientalis* aqueous extract in comparison, had a reducing power value (0.05 at 60 µg/mL) less than the 0.2 previously reported by Özen (34). This difference may be attributed to the variation in antioxidant phytochemical level between the sample used, arising from growth conditions and environmental effects on the plants.

A common method for determining antioxidant capability is the DPPH radical scavenging reaction. A reverse in formation of DPPH radicalisation occurs when antioxidants are added to radicals, thus decolourisation occurs. The potential of plant extracts to act as antioxidants depends on the redox properties and electron delocalization of phenolic hydroxyl groups of their constituent polyphenolic compounds. In this study, *T. orientalis* aqueous infusion extract was found to contain a moderate amount of phenolic compounds, and an above average DPPH radical scavenging activity (81.99 %). A previous study (34) reported an approximate 50% inhibition at 100 µg/ml of extract. Also, Ayvaz (33) reported an IC_{50} DPPH radical scavenging potential of aqueous extract of 0.41 mg/mL and 2.6 mg/mL of the ethanolic extract. The DPPH radical scavenging capability of aqueous *T. orientalis* extract can be attributed to its ease in abstracting a hydrogen atom from the hydroxyl group of its phenolic constituents.

CONCLUSION

This study suggests that *T. orientalis* extract can be considered as a useful natural antioxidant source because of its flavonoids, phenolic and anthocyanin contents. Furthermore, it may serve as a cheap and readily accessible source of natural antioxidant. Therefore, the food and cosmetic industries might employ *T. orientalis* extract as an alternative additive to the more toxic synthetic antioxidants.

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Conflict of Interest: The author has no conflict of interest to declare.

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