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Original article

EVALUATION OF ANTIOXIDANT PROPERTIES OF SOME *TRAGOPOGON* SPECIES GROWING IN TURKEY

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Abstract

In the current study, antioxidant activities of the aerial parts and roots of Tragopogon longirostris Bisch. ex Sch. Bip. var. longirostris and T. pratensis subsp. orientalis L. were evaluated. Antioxidant activity of the Tragopogon aqueous methanol extracts were investigated by DPPH scavenging and superoxide anion scavenging assays. Total phenolic and flavonoid contents of the extracts were calculated using Folin-Ciocalteu and aluminum chloride colorimetric methods and determined ranging from 63.45±0.25 to 68.92±0.42 mg/g caffeic acid and from 4±1 to 210±9 mg/g quercetin respectively. Furthermore the phenolic acids; chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, p-coumaric acid and flavonoids; apigenin, luteolin, quercetin, hyperoside, rutin, hesperidin in T. longirostris var. longirostris and T. pratensis subsp. orientalis have been determined qualitatively and quantitatively using reverse phase high performance liquid chromatography. Chlorogenic acid was determined in all samples investigated. The highest content of chlorogenic acid was detected in aerial part extract of T. longirostris var. longirostris as 578.22 ± 4.19 µg/mg.

Key words: *Asteraceae, Tragopogon, Antioxidant Activity, Chlorogenic acid*

Türkiye'de Yetişen Bazı *Tragopogon* Türlerinin Antioksidan Etkileri

Bu çalışmada Tragopogon longirostris Bisch. ex Sch. Bip. var. longirostris ve T. pratensis subsp. orientalis L. bitkilerinin toprak üstü ve köklerinin antioksidan etkileri değerlendirilmiştir. Tragopogon türlerinin sulu metanollü ekstrelerinin antioksidan aktivitesi DPPH ve süperoksit anyon süpürücü aktivite testleri kullanılarak değerlendirilmiştir. Ekstrelerin total fenol ve flavonoit içerikleri Folin-Coicalteu ve aluminyum klorür kolorimetrik yöntemleri kullanılarak sırasıyla 63.45±0.25-68.92±0.42 mg/g kafeik asit ve 4±1 - 210±9 mg/g kersetin olarak belirlenmiştir. Ayrıca T. longirostris var. longirostris ve T. pratensis subsp. orientalis ekstrelerinin fenolik asit; klorojenik asit, kafeik asit, ferulik asit, rosmarinik asit, p-kumarik asit ve flavonoit; apigenol, luteolol, kersetol, hiperozit, rutin, hesperidin içerikleri kalitatif ve kantitatif olarak ters faz yüksek basınçlı sıvı kromatografisi kullanılarak tespit edilmiştir. Klorojenik asit araştırılan tüm türlerde tespit edilmiştir. En yüksek klorojenik asit miktarı T. longirostris var. longirostris toprak üstü kısımlarında 578.22 ± 4.19 µg/mg olarak tespit edilmiştir.

Anahtar kelimeler: *Asteraceae, Tragopogon, Antioksidan Aktivite, Klorojenik Asit*

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INTRODUCTION

The genus *Tragopogon* L. (Asteraceae) is widely distributed throughout Europe, Asia and North Africa with 100 species (1). In Turkey this genus is represented by 21 members (2,3). Among them *Tragopogon porrifolius* L. known as white salsify is used as a vegetable in Europe as well as in Turkey (4,5). The roots and young shoots are used as vegetables (6). Aerial parts of the *T. porrifolius* and also some of other *Tragopogon* species are known as *yemlik* and *teke sakalı* in Anatolia and they are eaten freshly or after cooked (7,8). The genera were also used as herbal medicine. *T. porrifolius* is used, in European folk medicine, for its antibilious, diuretic, laxative effects and in Lebanese folk medicine for treatment of cancer (4,9). In Turkish folk medicine *T. coloratus*, *T. dubius, T. pratensis* ssp. *orientalis*, *T. pterocarpus* and *T. reticulatus* aerial parts are used in treatment of stomach ache, *T. buphtalmoides* var. *buphtalmoides* (latex and herb) and *T. longirostris* (stem and leaves) are used in intestinal disorders as well as antihelminthic activities of some *Tragopogon* species have been recorded (8, 10).

Biological activity studies on *Tragopogon* genus as potential sources of bioactive compounds have found to be very limited. Zidorn et al. (11) reported that isolated compounds from *T. porrifolius* exhibited moderate radical scavenging activity against DPPH radical. It has also been recently reported *T. porrifolius* was found to has anticancer, antioxidant and hepatoprotective activities (9). According to the literatue survey a wide range of secondary metabolites including flavonoids; isoorientin, isovitexin, lucenin-1, luteolin, orientin, quercetin-3-*O*-β-D-glucoside, vicenin-1, vicenin-2, vitexin; various types of bibenzyl and dihydroisocoumarin derivatives; chlorogenic acid and 3,5-dicaffeoylquinic acid as well as a number of acylated pentacyclic triterpene saponins from *Tragopogon porrifolius* (6, 11-14), dihydrostilbenes, benzylphtalide, phenylpropanoid and lignan from *T. orientalis* (13), oleanane saponins from *T. pratensis* (15) have been isolated.

Current study aims to evaluate antioxidant properties of two different *Tragopogon* species using DPPH scavenging and superoxide anion scavenging methods. Additionally, to investigate the relationship between the antioxidant activities and chemical content of the tested extracts total phenolic and flavonoid contents were determined with Folin-Ciocalteau and $AlCl_3$ reagents. HPLC analysis were also performed qualitatively and quantitatively by using some standard phenolic compounds such as; chlorogenic acid (CA), caffeic acid (CFA), ferulic acid (FA), rosmarinic acid (RA), *p*-coumaric acid (PCA) and flavonoids; apigenin (A), luteolin (L), quercetin (Q), hyperoside (HY), rutin (R), hesperidin (HE).

EXPERIMENTAL

Plant material

Flowering plants of *T. longirostris* Bisch. ex Sch. Bip. var. *longirostris* and *T. pratensis* subsp. *orientalis* L. were collected from Ankara, Çamlıdere, Turkey in 2005. The taxonomic identification of these plants was confirmed by A. M. Gençler Özkan, Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University, Ankara, Turkey. The voucher specimens are deposited at Herbarium belonging to Faculty of Pharmacy of Ankara University with the corresponding herbarium numbers; AEF 25163, AEF 25162 respectively.

Preparation of the extracts

Air-dried and powdered materials of the aerial parts and roots of the *Tragopogon* species were extracted with 80% aqueous methanol (100 ml) at room temperature for 3 h by continuous stirring seperately. Each extract was filtered and concentrated to dryness under reduced pressure and low temperature (40-50 °C) on a rotary evaporator to give crude extracts.

Chemicals

Ascorbic acid, xanthine, xanthine oxidase, cytochrome c, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene, and α-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO).

Antioxidant and radical scavenging properties

Superoxide radical scavenging assay

Enzymatic formation of superoxide anions was assayed by reduction of cytochrome c as described by McCord and Fridovich (16). The incubation mixture (1.0 mL, total volume) consisted of phosphate buffer (pH= 8.9, 0.1 M), xanthine (50 mµ), cytocrome c (50 mµ), xanthine oxidase (0.32 units/mL) and 100 µL test samples. The reaction was started by addition of xanthine oxidase and was conducted at 30 °C in a heating block. The absorbance was measured spectrophotometrically at 550 nm for cytochrome C reduction. IC_{50} values were determined from a calibration curve.

DPPH radical scavenging assay

DPPH assays were performed according to the method which was previously described by Blois et al (17). Test samples were dissolved in DMSO and mixed with methanol solutions of DPPH (100 mM) in 96-well micro titer plates, following incubation at 37°C for 30 min. DPPH reduction was estimated at 517 nm. For each test sample, different concentrations were tested. Percentage inhibition by the sample treatment was determined by comparison with a DMSO-treated control group. All experiments were carried out in triplicate. The antioxidant activity of each test compound was expressed as an IC_{50} value ± SD, i.e. the concentration in µM that inhibits DPPH absorption by 50 %, and was calculated by linear regression analysis. The radical scavenging activities were expressed as the inhibition percentage and were calculated using the formula:

Radical scavenging activity (%) = $[(A_0 - A_1 / A_0) \times 100]$

Where A_0 is the absorbance of the control (blank, without compound) and A_1 is the absorbance of the compound.

Determination of total phenolic and flavonoid contents of the extracts

The amounts of total phenolics in plant extracts were determined with the Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (18) as modified by Lister and Wilson (19). To 5 mL of each sample (three replicates), 0.25 mL 1/10 dilution of Folin-Ciocalteau's reagent and 0.2 mL of Na_2CO_3 (7.5%, w/v) were added and incubated at 45 ºC for 15 min. The absorbance of all samples was measured at 765 nm using a SPECTRAmax-PLUS384 UV–vis spectrophotometer. Results were expressed as milligrammes of caffeic acid acid equivalent per gramme of dry weight (mg CAE/g dw).

The aluminum chloride colorimetric method was modified from the procedure reported by Woisky and Salatino (20). Quercetin (QE) was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 5, 10, 25, 50 and 100 µg/mL. The diluted standard solutions (0.5 mL) were separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. using a SPECTRAmax-PLUS384 UV–vis spectrophotometer. The amount of 10 % aluminum chloride was substituted by the same amount of distilled water in blank. Similarly, 0.5 mL of plant extracts or quercetin standard solutions were reacted with aluminum chloride for determination of flavonoid content as described above. For each sample, three readings were taken to get the averaged results. The results were expressed in µg quercetin/g dry weight by comparison with the quercetin standard curve, which was made under the same condition.

HPLC analysis

The HPLC analysis of *Tragopogon* species was carried out according to the Küpeli Akkol et al. (21). This HPLC method was developed and validated to analyse phenolic acids including CA, CFA, FA, RA, COA, A, L, Q, HY, R, HE previously. Agilent LC 1100 model chromatograph (Agilent Technologies, California, USA) was used and diode array detector (DAD) was set at wave length 254 nm. Separation was carried out using a Supelcosil (250 x 4.6 mm; 5µm) column. The mobile phase was made up of acetonitrile (A) and water (B) in gradient elution: initial 0 min, A-B (8:92, v/v), then 0-10 min, linear change from A-B (8:92, v/v) to A-B (18:82), 10-20 min, there is also linear change from A-B (18:82) to A-B (20:80) and the linear gradient elution is from A-B (20:80) to A-B (30:70)

with the range of 20-30 min. This was followed by A-B (30:70) from 30 min to 45 min. The flow rate was 0.5 mL/min and column temperature was maintained at 35 °C. The sample injection volume was 10 μL (22).

RESULTS AND DISCUSSION

The reactive oxygen species (ROS) including superoxide anion radical, hydroxyl radical, singlet oxygen and H_2O_2 have been found to play an important role in the initiation and/or progression of various chronic diseases such as cardovascular diseases, arthritis, cancer, diabetes mellitus, neurodegenerative disorders and aging (22-25). ROS are produced by all aerobic organisms during metabolic processes and there is a balance between the generation of ROS and inactivation of ROS by antioxidant mechanism. All organisms have protection mechanism against free radical damage by enzymes such as superoxide dismutases and catalase or antioxidant compounds including ascorbic acid, tocopherols, and glutathione. Sometimes the mechanism of antioxidant protection becomes unbalanced by exogenous factors as well as endogenous factors. The imbalance between ROS and antioxidant defence mechanisms leads to oxidation of lipids, sugars, proteins and DNA which may result in oxidative damage such as membrane dysfunction, protein modification, enzyme inactivation as well as breakage of DNA strands and modification of its bases (22,23,25,26).

Naturally occuring antioxidants such as vitamin E, vitamin C, carotenoids and phenolics present in diet provide protection from damage caused by uncontrolled production of free radicals (26,27). Epidemiological and experimental studies have revealed that there is a negative correlation between the consumption of phenol-rich foods and beverages and the risk of various chronic diseases (28,29). Phenolics belonging to various classes of compounds such as flavonoids, phenolic acids and other polyphenols are found primarily in fruits, vegetables, spices as well as both edible and non-edible plants (22, 30-34).

Table 1. DPPH radical scavenging and superoxide anion radical scavenging activity of the *Tragopogon* species.

Extracts	DPPH radical scavenging capacity (IC_{50} μg/mL)	Superoxide anion radical scavenging activity (IC_{50} mg/mL)
TPR	280 ±13	9.5 ± 0.5
TPAE	134 ±9	4.5 ± 0.4
TLR	160 ±6	5.0 ± 0.3
TLAE	112 ±4	3.8 ± 0.2
α-tocopherol	13 ± 0.5	0.37 ± 0.05

R: Root; AE: Aerial part

In current study, as shown in Table 1, the methanolic aqueous extracts of aerial parts and the roots of TL and TP were revealed to be effective towards DPPH and superoxide anion radicals. DPPH radical scavenging effects of the plant extracts were examined at four different concentrations (31.25, 62.5, 125, 250 μg/mL). Superoxide radical scavenging effects of the plant extracts on superoxide anion were also tested at four different concentrations as follow 1.25, 2.5, 5, and 10 mg/mL. As can be seen in Table 1 aerial part extracts were found to be more active than root extracts against both DPPH and superoxide anion radicals. TL aerial part extract showed the highest capacity in DPPH as well as superoxide anion radical scavenging activity test models with IC_{50} value of 112±4 μg/mL and 3.8 ± 0.2 mg/mL respectively (Table 1). Relatively high levels of scavenging activity was also detected in aerial part extract of TP on superoxide anion (IC_{50}=4.5±0.4 mg/mL) and DPPH radical (IC_{50}=134±9 μg/mL). Both in DPPH and superoxide anion radical scavenging activity test, the lowest activity was measured by TP root extracts with 280±13 μg/mL and 9.5 ± 0.5 mg/mL of IC_{50} values.

Table 2. Total phenol and flavonoid contents of the *Tragopogon* species.

Extracts	Total phenol content (µg CAE/g dw)	Flavonoid content (QE mg/g dw)
TPR	65.37 ± 0.30	5 ± 2
TPAE	68.92 ± 0.42	210 ± 9
TLR	66.33 ± 0.24	4 ± 1
TLAE	63.45 ± 0.25	63 ± 3

Total phenol contents of the extracts which were calculated as caffeic acid equivalent were found to be very close to each other (Table 2). However the highest content was determined in TP aerial part extracts with value of 68.92 ± 0.42 µg/g followed by TL root (66.33 ± 0.24 µg/g), TP root (65.37 ± 0.30 µg/g) and TL aerial part (63.45 ± 0.25 µg/g) extracts. Total flavonoid content ot the investigated *Tragopogon* species were determined ranging from 4 ± 1 mg/g to 210 ± 9 mg/g quercetin equivalent. Table 2 shows that TP aerial parts contain the highest flavonoid content (210 ± 9 mg/g QE).

Table 3. Content of standards in plant samples (µg/mg).

Species		1	2	3	4	5	6	8	9	10	11	12
TL	R	249.90 ± 0.64	-	-	-	-	-	-	-	-	-	-
	AE	578.22 ± 4.19	-	-	-	-	-	-	-	-	-	-
TP	R	191.74 ± 0.58	-	-	-	-	-	-	-	-	-	-
	AE	502.06 ± 2.26	-	-	-	-	-	-	-	-	48.47 ± 0.11	-

1:Chlorogenic acid; 2:Caffeic acid; 3:*p*-Coumaric acid; 4:Ferulic acid; 5:Rutin; 6:Hyperoside; 7:Hesperidin; 8: Rosmarinic acid; 9:Quercetin; 10:Luteolin; 11:Apigenin.

HPLC results have revealed that chlorogenic acid was detected as the only phenolic acid in all tested extracts among the used standards. Amount of chlorogenic acid was determined in root extracts of TL and TP aerial parts as well as roots as 578.22 µg/g, 502.06 µg/g and 249.90 µg/g, 191.74 µg/g respectively. Results are shown in Table 3. TL aerial part extract was detected to contain chlorogenic acid in higher percentage when we compared with the other tested extracts. Luteolin was also determined as flavonoids in aerial parts of TP (48.47 µg/g) among the investigated standards (Table 3).

CONCLUSION

Current study revealed that *Tragopogon* species have radical scavenger activities against DPPH and superoxide anion radicals. The scavenging effect of plant extracts against DPPH radical are found to be similar to those obtained on superoxide anion radical. The strongest activity was established by TL aerial part extract among the tested extracts followed by TP aerial part extract in both test models. However the highest phenol and flavonoid contents were found for the extract of aerial parts of TP. On the other hand according to the HPLC results TL aerial part extract contain the highest amount of chlorogenic acid followed by TP aerial part, TL and TP root extract respectively similar to radical scavenging activity test results. Chlorogenic acid that one of the most naturally existing phenolic compounds found in numerous plant species is known to have anti-inflammatory, anti-nociceptive as well as strong antioxidant activities (36-39). Chlorogenic acid looks like one of the major component of *Tragopogon species* according to HPLC results. Previous studies have also revealed that *Tragopogon* species rich in phenolic compounds such as dihydroisocoumarines, bibenzyl derivatives, flavonoids, lignans, stilbene derivatives (6,11-15). Most of these polyphenols are known to have antioxidant properties. It could be suggested that based on these results, chlorogenic acid probably one of the responsible compound contributing antioxidant activity of *Tragopogon* species.

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