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Antioxidant Activity and Phytochemical Profile of Eight Wild Edible Plants Grown in Afyonkarahisar, Türkiye

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ABSTRACT

In this study, the antioxidant activities, total phenolic content (TPC), total flavonoid content (TFC), and phenolic compounds in the leaves of wild edible plants, grown in Afyonkarahisar, Turkey were investigated. Antioxidant activities were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2´-azine-bis (3-ethylbenz-thiazoline-6-sulfonic acid) diammonium salt (ABTS), and ferric reducing antioxidant power (FRAP) assays, while phenolic acids and flavonoids were identified and quantified by high performance liquid chromatography. The plants included *Lactuca serriola* L. (bitter lettuce), *Thymus vulgaris* L. (thyme), *Sinapis arvensis* L. (mustard), *Malva neglecta* L. (hibiscus), *Amaranthus retroflexus* L. (redroot pigweed), *Tragopogon longirostris bisch* (goat's beard), *Taraxacum officinale* (dandelion), and *Chenopodium album* (baconweed or lamb's quarters). Phenolic acids, including gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid and flavonoids including catechin, apigenin, naringin, rutin and quercetin amounts in plant leaves were determined. All plants showed antioxidant properties but *Tragopogon longirostis bisch*, *Sinapis arvensis* L., and *Thymus vulgaris* L. had higher antioxidant activity than the rest. The highest TPC (2.69 mg/g) belonged to *Tragopogon longirostis bisch*, and the highest TFC (1.84mg/g) belonged to *Amaranthus retroflexus*. *Amaranthus retroflexus* L. had the highest gallic acid and vanillic acid levels. *Malva neglecta* L. had the highest ferulic, chlorogenic, ellagic, and cinnamic acid contents. *Tragopogon longirostris bisch* had the highest *p*-coumaric acid, 4-hydroxybenzoic acid, and 2,5 dihydroxybenzoic acid levels. It was observed that *Malva neglecta* L. had the highest catechin, apigenin, and quercetin contents while *Thymus vulgaris* L. had the highest naringin and rutin levels. These results suggested these leaves could be consumed as the sources of natural antioxidants in human diet.

Keywords: Phenolic, Antioxidant, DPPH, ABTS, FRAP

Afyonkarahisar'da Yetişen Sekiz Yabani Yenilebilir Bitkinin Antioksidan Aktivitesi ve Fitokimyasal Profili

ÖZ

Bu çalışmada, Türkiye'nin Afyonkarahisar ilinde yetişen yabani yenilebilir bitkilerin yapraklarının antioksidan aktiviteleri, toplam fenolik içerikleri (TPC), toplam flavonoid içerikleri ve fenolik bileşenler araştırılmıştır. Antioksidan aktiviteler 2,2-difenil-1-pikrilhidrazil (DPPH), 2,2´-azin-bis (3-etilbenz-tiazolin-6-sülfonik asit) diamonyum tuzu (ABTS) ve demir indirgeyici antioksidan güç (FRAP) analizleri ile ölçülürken, fenolik asitler ve flavonoidler HPLC ile tanımlanıp miktarları belirlenmiştir. Bu bitkiler *Lactuca serriola* L. (acı marul), *Thymus vulgaris* L. (kekik), *Sinapis arvensis* L. (hardal), Malva neglecta L. (ebegümeci), *Amaranthus retroflexus* L. (kızılbacak), *Tragopogon longirostris bisch*

(tekesakalı), *Taraxacum officinale* (acıgünek) ve *Chenopodium album*'dur (sirken). Bitki yapraklarında fenolik asitler (gallik asit, ferulik asit, klorojenik asit, *p*-kumarik asit, ellagik asit, vanilik asit, kafeik asit, sinnamik asit, 4-hidroksibenzoik asit, 2,5-dihidroksibenzoik asit) ile flavonoidlerin (kateşin, apigenin, naringin, rutin ve kuersetin) miktarları belirlenmiştir. Tüm bitkiler antioksidan özellik göstermiş fakat *Tragopogon longirostis bisch*, *Sinapis arvensis* L. ve *Thymus vulgaris* L. diğerlerinden daha yüksek antioksidan aktivite değerine sahip olmuştur. En yüksek toplam fenolik madde içeriği (2.69mg/g) *Tragopogon longirostis bisch*'e, en yüksek toplam flavonoid içeriği (1.84mg/g) *Amaranthus retroflexus'*a aittir. *Amaranthus retroflexus* L. en yüksek gallik asit ve vanilik asid içeriğine sahip iken *Malva neglecta* L. en yüksek ferulik, klorojenik, ellagik ve sinamik asit içeriğine sahip olmuştur. *Tragopogon longirostris bisch* en yüksek *p*-kumarik asit, 4-hidroksibenzoik asit ve 2,5 dihidroksibenzoik asidi düzeyini sergilemiştir. *Malva neglecta* L.'nin en yüksek kateşin, apigenin ve kuersetin düzeyi, *Thymus vulgaris* L.'nin ise en yüksek naringin ve rutin içeriğine sahip olduğu görülmüştür. Bulgular, bu bitki yapraklarının insan diyetinde doğal antioksidan kaynakları olarak tüketilebileceğini göstermiştir.

Anahtar Kelimeler: Fenolik, Antioksidan, DPPH, ABTS, FRAP

INTRODUCTION

Medicinal plants contain healthcare components [1]. Phenolic compounds are found in most plants and have antioxidant activity. Their redox properties sustain the antioxidant properties of phenolics. Therefore, phenolic compounds can take a role as reducing agents, oxygen quenchers, and metal chelators [2]. Plants need phenolic compounds for growth, pigmentation, and resistance to pathogens. Plants are exposed to UV-B (280-320nm) radiation, adversely affecting DNA. Plants protect themselves from this radiation by producing phenolic compounds [3]. Natural antioxidants like phenolic compounds can replace synthetic antioxidants against oxidative degradation caused by free radicals [4]. Flavonoids are given as examples of phenolic compounds. Flavonoids, which have high absorption at 250-270nm and 335-360nm, act as good UV screens [5]. The important flavonoid component is quercetin, one of the medicinal plants' most active antioxidants. Phenolics in these plants prevent cancer, cardiovascular diseases, and asthma [6]. Al-Laith et al. [7] studied the antioxidant properties of three wild medicinal plants from Bahrain (*Aizoon canariense* L., *Asphodelus tenuifolius* Cav., and *Emex spinosus* L. Campdera). *E. spinosus* was ranked the highest antioxidant and antiradical activities with an average FRAP value of 1.84 mmol/g and IC_{50} of 10.7 and 7.75 mg/mL for DPPH and ABTS assays, respectively. Günbatan et al. [8] reported that DPPH, ABTS, and TFC of *Malva neglecta* L. were 87.59%, 17.57 mg gallic acid equivalent/g and 42.93 mg rutin equivalent/g, respectively. Kolar et al. [9] demonstrated that *Chenopodium album* had 91.5% DPPH, 18.5 mg ascorbic acid equivalent/g FRAP, 14.9 mg tannic acid equivalent/g TPC, and 0.37 mg quercetin equivalent/g TFC. Ivanov [10] showed that DPPH, FRAP, and TPC of *Taraxacum officinale* grown in Bulgaria were 130.3 mM Trolox® equivalent/g, 131.5 mM Trolox® equivalent/g, and 33.90 mg gallic acid equivalent/g, respectively. Ao and Deb [11] investigated the antioxidant potential of 10 wild edible mushrooms of Nagalan in India. They reported that the highest phenolic content was 18.7 g/100 g, and the highest flavonoid content was 9.3 g/100 g. Shen et al. [12] reported that okra fruit shows antioxidant capacity. Somkuwar et al. [13] reported that gallic acid, vanillic acid, ellagic acid, chlorogenic acid, sorbic acid, coumaric acid, catechin, rutin, quercetin, and kaempferol were major phenolic compounds of red wine grape. White wine grapes contain lower phenolics than red wine grapes. Jagtap et al. [14] reported that *Carica papaya* L. leaves showed antioxidant activity. Yalcin and Schreiner [15] reported that the main phenolics of olive oil were tyrosol and hydroxytyrosol. Free radical scavenging activity of curry leaf (*Murraya koenigii* L.) was approximately 90% [16]. Perea-Dominguaz et al. [17] reported that the TPC of two tomatoes was 91.47 and 57.41 mg gallic acid equivalent/g dry samples. Fellah et al. [18] reported that the highest TPC (152.6 and 125.8 mg gallic acid equivalent/100 g) was recorded in Nabil flowers and Gabsi peels. Infrared treatment caused an increase in the phenolic content of soy [19]. Hassanzadeh and Hassanpour [20] investigated TPC, TFC, DPPH, and FRAP values of thirty-eight genotypes of *Elaeagnus angustifolia* L. grown in Iran. They reported that the TPC of peel and pulp was 268.38-1179 mg gallic acid/100 g and 250.57-820.85 mg gallic acid/100 g, respectively. The TFC of peel and pulp was 23.50-327.50 mg catechin/100 g and 16.50-318.75 mg catechin/100 g, respectively. DPPH of peel and pulp was 49.22-93.11% and 28.59-93.15%, respectively, while FRAP of peel and pulp was 57.00-128.67 mg Fe_2SO_4/100 g and 86.33-160.67 mg Fe_2SO_4/100 g, respectively. Orak [21] investigated phenolics of sixteen red grape cultivars grown in Tekirdağ, Turkey, and reported that TPC ranged from 817 to 3062 µg gallic acid equivalent/mL. Hassanpour and Alizadeh [22] investigated berberry genotypes' antioxidant capacity, TPC, and TFC (*Berberis vulgaris* and *Berberis integerrima)*. They reported that DPPH and FRAP values of genotypes ranged between 20.69-68.33% and 20.2-70.39 TE mmol/L, respectively. Genotypes' TPC and TFC ranged between 263.35-623.07 mg gallic acid equivalent/100 g and 158.33-280.00 mg catechin/100 g, respectively.

This study compares the antioxidant activities in terms of DPPH, ABTS, and FRAP, TPC, TFC, and phenolic compounds of wild edible plants collected in Afyonkarahisar, Turkey, on 25 May 2017. These plants were *Lactuca serriola* L., T*hymus vulgaris* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch*, *Taraxacum officinale* and *Chenopodium album*. A comparison of the antioxidant activities, TPC, TFC, and phenolic compounds of the leaves of these plants has not been

reported. This study will contribute to literature. The data obtained will be a resource for researchers who will work in this field.

MATERIALS and METHODS

Chemicals

HPLC grade methanol, formic acid, sodium carbonate (purity≥99%), 2,4,6-tri(2-pyridyl)-s-triazine ferric chloride hexahydrate, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (98% purity), ABTS (98% purity), DPPH (95% purity), 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) (98% purity), potassium persulfate, $AlCl_3$, $NaNO_2$, H_2O_2, $CuCl_2$ and $FeSO_4$ were purchased from Merck (Darmstadt, Germany). Folin-Chiocalteu reagent and phenolic standards (*p*-coumaric acid, caffeic acid, chlorogenic acid, ferulic acid, gallic acid, ellagic acid, cinnamic acid, vanillic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin) were purchased from Sigma-Aldrich (St, Louis, MO, USA).

Materials

Lactuca serriola L., T*hymus vulgaris* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch*, *Taraxacum officinale* and *Chenopodium album* L. were collected from their natural habitats (Latitude: 38,5917, Longitude: 31,0286 38° 35′ 30″ North, 31° 1′ 43″ East, Altitude: 1050m) in Afyonkarahisar on 25 May 2017. The leaves of these plants were separated from the plants and used for analysis.

For sample extraction, 1 g of leaves was extracted with 10 mL of methanol in a homogenizer (Daihan WiseTis HG-15D Digital Homogenizer, Seoul, South Korea) for 1 min at 20000 rpm. The homogenate was centrifuged at 3500 rpm for 10 min (Daihan Scientific Co., Ltd., WiseSpin® CF-10 Microcentrifuge, Seoul, South Korea). The extract was separated and dried by a vacuum rotary evaporator (Scilogex RE 100-Pro, USA) at 40°C. The dry residues were dissolved in 90% methanol (10 mL) before analysis.

DPPH Radical Scavenging Activity Assay

DPPH radical scavenging activity assay was performed according to the method reported by Brand-Williams et al. [23]. The determination of antioxidant activity with the DPPH assay is based on the ability of the reaction of the DPPH free radical with hydrogen donors. DPPH radical solution is decolorized after reduction with an antioxidant. So, color difference was calculated to determine antioxidant activity [24].

DPPH was dissolved in 100% methanol to obtain a solution with a concentration at 4.1075 mol/L. The sample extract (400 µL) was added to the DPPH solution (1.6 mL). After incubation in a dark place at room temperature for 30 min, the decrease in absorbance was measured at 517 nm. The DPPH solution (4.1075 mol/L) was used as a control for all samples. The DPPH radical scavenging activity was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left(1 - \frac{\text{absorbance}_{\text{sample}}}{\text{absorbance}_{\text{control}}}\right) \text{x } 100$$

ABTS Radical Cation Decolorization Assay

ABTS radical cation decolorization of samples was determined according to the study reported by Re et al. [25] with some modifications. The determination of antioxidant activity by the ABTS radical cation decolorization assay is based on the neutralization of a radical cation after the one-electron oxidation of the synthetic chromophore ABTS. Antioxidant activity is determined by the change in the absorption spectrum after the reaction [24].

ABTS (1.8 mM) and potassium persulfate (0.63 mM) were mixed and stored in the dark for 24h at room temperature for reaction. This solution was mixed with methanol until an absorbance of 0.70 at 732 nm was obtained. Then, the mixture (1.98mL) was added to the sample extract (20 µL). After 30 min, the absorbance was measured using a spectrophotometer (Optizen pop Uv-Vis Spectrophotometer, South Korea) at 732 nm. A standard curve was prepared by plotting the percentage of radical cation decolorization of Trolox® (standard antioxidant) versus its concentration (0.1-2.5mM). The ABTS radical cation decolorization was expressed as mg Trolox® equivalent (TE) per g sample.

Ferric Reducing/Antioxidant Power (FRAP) Assay

The reducing capacity of samples was performed according to the method reported by Benzie and Strain [26]. Determination of antioxidant activity by the FRAP assay is based on the forming of blue color after reaction of 2,4,6-tri-(2-pyridyl)-s-triazine (TPTZ) with ferric chloride hexahydrate [24]. FRAP reagent was prepared for blank reading at 593 nm. 10 mL of sample was then added to FRAP reagent (300 mL), along with 30 mL H_2O; the final dilution of the sample in the reaction mixture was 1/34. Absorbance readings were taken after 0.5 s and every 15 s. The change in absorbance was calculated for each sample. 100-1000 mmol/L $FeSO_4 7H_2O$ were used for calibration. The results were expressed as mg $FeSO_4$ per g of sample.

Total Phenolic Content

The TPC of samples was determined by using the Folin-Chiocalteu method [27]. Sample extract (300 µL) and Folin-Chiocalteu reagent (750 µL) were mixed and incubated for 5 min. Then 750 µL of Na_2CO_3 (60g/L) was added, and the mixture was incubated in the dark for 90 min at room temperature. The absorbance was measured at 725 nm. Analysis was performed with

working solutions in the 0.0-0.1 mg/mL concentration range prepared from the catechin standard, and a calibration curve was prepared. TPC was expressed as mg catechin equivalents per g of the sample through the calibration curve of catechin.

Total Flavonoid Content

The TFC of samples was determined according to the method of Dewanto et al. [28]. 0.25 mL of the extract was mixed with 1.25 mL of distilled water in a tube, followed by the addition of 75 µL of a 5% $NaNO_2$ solution. After 6 min, 150 µL of a 10% $AlCl_3 6H_2O$ solution was added and allowed to stand for 5 min. Then, 0.5 mL of 1 M NaOH was added. The mixture was brought to 2.5 mL with distilled water and mixed. The absorbance was measured against the blank at 510 nm using a spectrophotometer. TFC was expressed as mg catechin equivalents per g of sample.

Analysis of Phenolic Compounds

The dried sample was dissolved in 1 mL of 100% methanol and filtered through a 0.45 µm nylon filter. Phenolic compounds of samples were analyzed according to the method of Caponio et al. [29] with some modification by using HPLC (Shimadzu Prominence, Kyoto, Japan) equipped with a diode array detector (SPD-M20A) and Zorbax Eclipse C18 column (250 × 4.6 mm, 5 µ). The mobile phase was 3% formic acid (A) and methanol (B). The elution gradient was 95% A/5% B for 3 min, 80%A/20%B in 15 min and isocratic for 2 min, 60%A/40%B in 10 min, 50%A/50%B in 10 min, and 100%B in 10 min until the end of the run. The flow rate was 1 mL/min. The eluates were detected at 278 nm. Quantitative phenolic compound evaluation was performed by using calibration curves of standards. Gallic acid, ferulic acid, chlorogenic acid, coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin, and quercetin were determined. The amount of phenolic compounds was expressed as µg per g of sample.

Statistical Analysis

Statistical differences between plant leaves were evaluated using a one-way analysis of variance (ANOVA) followed by the Duncan test. The difference between groups was significant at $P<0.05$. All data were analyzed using IBM Statistics SPSS 24 (Armonk, New York, USA). The analysis was done in duplicate.

RESULTS and DISCUSSION

DPPH Radical Scavenging Activity of Plant Leaves

The DPPH radical scavenging activities (%) of plant leaves are given in Table 1. Significant differences were found between DPPH radical scavenging activities of plant leaves. % Inhibition ranged from 15.9% (*Chenopodium album)* to 80.7% (*Tragopogon longirostris bisch*). *Tragopogon longirostris bisch* had the highest antioxidant activity, while *Chenopodium album* had the lowest. The antioxidant activity of T*hymus vulgaris* L. was statistically similar to that of *Sinapis arvensis L*. The result of *Lactuca serriola* L. was compatible with that recorded by Liu et al. [30]. Liu et al. [30] reported that the DPPH value of lettuce (*Lactuca serriola* L.*)* grown in Colorado was 69.6-81.6%. DPPH value of *Thymus vulgaris* L. was higher than those (22-55% and 27.5%) reported by Chizzola et al. [31] and Dauqan et al. [32]. DPPH value of *Malva neglecta* L. was lower than that (97.59%) reported by Günbatan et al. [8]. DPPH value *of Chenopodium album* was lower than that (91.5%) reported by Kolar et al. [9].

Table 1. Antioxidant activities of plant leaves (mean ± standard deviation)

Samples	DPPH (%)	ABTS (mg TE/g)	FRAP (mg $FeSO_4$/g)
Lactuca serriola L.	70.2±0.03d	2.13±0.018d	0.94±0.001h
T*hymus vulgaris* L.	77.9±0.15b	6.14±0.018c	8.77±0.001a
Sinapis arvensis L.	76.4±0.15b	10.01±0.018a	2.48±0.008f
Malva neglecta L.	69.5±0.59d	9.52±0.028b	7.74±0.057b
Amaranthus retroflexus L.	29.9±0.48e	8,92±0.030c	7.16±0.011c
Tragopogon longirostris bisch	80.7±1.73a	9.74±0.072ab	6.60±0.004e
Taraxacum officinale	74.3±0.15c	3.30±0.018d	2.43±0.007g
Chenopodium album	15.9±0.01f	8.63±0.035c	7.00±0.006d

*Values followed by the same letter in the same column are not significantly different ($p<0.05$)

ABTS Radical Scavenging Capacity of Plant Leaves

The ABTS radical scavenging capacities of plant leaves are given in Table 1. Significant differences were found between ABTS radical scavenging capacities of plant leaves. ABTS values of plant leaves ranged from 2.13 mg TE/g (*Lactuca serriola* L.) to 10.01 mg TE/g (*Sinapis arvensis* L.). Antioxidant capacity of *Lactuca serriola* L. was statistically similar to that of *Taraxacum officinale* and *Malva neglecta* L. The antioxidant capacity of *Thymus vulgaris* L. was not significantly different from that of *Amaranthus retroflexus* L. and *Chenopodium album.* Antioxidant capacity of *Sinapis arvensis* L. was the highest, while that of *Lactuca serriola* L. was the lowest. Compared with ABTS radical scavenging capacity of *Malva neglecta* L. (17.57 mg gallic acid equivalent/g) reported by Günbatan et al. [8], leaves of *Malva neglecta* L. used in this study had lower ABTS radical scavenging capacity.

FRAP of Plant Leaves

The FRAP values of plant leaves are given in Table 1. Significant differences were found between FRAP

values of plant leaves. FRAP values of plants ranged from 0.94 mg $FeSO_4$/g (*Lactuca serriola* L.) to 8.77 mg $FeSO_4$/g (*Thymus vulgaris* L.). *Thymus vulgaris* L. had the highest FRAP value, while *Lactuca serriola* L. had the lowest FRAP value. Lower FRAP value of T*hymus vulgaris* L. was 121.1-339.2 µmol TE/g obtained by Dauqan et al. [32]. FRAP value of *Malva neglecta* L. (7.74mg $FeSO_4$/g) was lower than that (190.3 µmol Fe^2/g) of *Malva neglecta* L. which was grown in Van, Turkey [33]. FRAP value of *Taraxacum officinale* (2.43 mg $FeSO_4$/g) was lower than that of *Taraxacum officinale* (131.5 mM TE/g) reported by Ivanov [10]. FRAP value of *Chenopodium album* (7.00 mg $FeSO_4$/g) was lower than that of *Chenopodium album* (18.5 mg ascorbic acid equivalent/g) reported by Kolar et al. [9].

Total Phenolic Content of Plant Leaves

The TPCs of plant leaves are given in Table 2. A significant difference was found between the TPC of plant leaves. TPC of plants ranged from 0.20 mg catechin/g (*Sinapis arvensis* L.) to 2.69 mg catechin/g (*Tragopogon longirostris bisch*). *Tragopogon longirostis bisch* had significantly higher TPC than other plant leaves. TPC of *Sinapis arvensis* L. was the lowest in all plant leaves. TPC of T*hymus vulgaris* L. was found statistically similar to that of *Malva neglecta* L. and *Amaranthus retroflexus* L. Higher TPC in *Lactuca serriola* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Taraxacum officinale and Chenopodium album* was reported by some researchers. TPC of *Lactuca serriola* L., grown in Netherlands, was 69.67-70.98 mg gallic acid equivalent/g [34], that of *Malva neglecta* L. leaf was 17 mg gallic acid equivalent/g [35], that of *Amaranthus retroflexus* L., grown in India, was 39.636 mg gallic acid equivalent/g [36], that of *Taraxacum officinale* was 33.90 mg gallic acid equivalent/g [10] and 41.47-691.6 mg gallic acid equivalent/g [37], that of *Chenopodium album was* 14.9 mg tannic acid equivalent/g [9] and 18.44 mg gallic acid equivalent/g [38]. Lower TPC (0.219 mg gallic acid equivalent/g) in T*hymus vulgaris* L. was obtained by Dauqan et al. [32].

Table 2. Total phenolic (TPC) and total flavonoid contents (TFC) of plant leaves (mean ± standard deviation)

Samples	TPC (mg catechin/g)	TFC (mg catechin/g)
Lactuca serriola L.	0.46±0.000e	0.06±0.011g
T*hymus vulgaris* L.	2.14±0.003b	1.22±0.006c
Sinapis arvensis L.	0.20±0.000f	0.18±0.000f
Malva neglecta L.	2.17±0.078b	1.39±0.057b
Amaranthus retroflexus L.	2.12±0.039b	1.84±0.001a
Tragopogon longirostris bisch	2.69±0.064a	1.38±0.029b
Taraxacum officinale	0.72±0.002d	0.36±0.011e
Chenopodium album	2.00±0.002c	1.04±0.011d

*Values followed by the same letter in the same column are not significantly different ($p<0.05$)

Total Flavonoid Content of Plant Leaves

The TFCs of plant leaves are given in Table 2. A significant difference was found between the TFC of plant leaves. TFC of plants ranged from 0.06 mg catechin/g (*Lactuca serriola* L.) to 1.84 mg catechin/g (*Amaranthus retroflexus* L.), *Amaranthus retroflexus* L. had significantly higher TFC compared to other plant leaves. TFC of *Malva neglecta* L. was statistically similar to that of *Tragopogon longirostris bisch*. Higher TFC was reported by some researchers. TFC of *Thymus vulgaris* L. was 36.6-44.2 µg quercetin/mg [39], that of *Malva neglecta* L. was 7.21 mg rutin equivalent/g [34] and 42.93 mg rutin equivalent/g [8] and that of *Amaranthus retroflexus* L., grown in India was 25.3 mg quercetin/g [35]. Lower TFC (0.37 mg quercetin equivalent/g) in *Chenopodium album* was reported by Kolar et al. [9].

Phenolic Compounds of Plant Leaves

The phenolic compounds of plant leaves are given in Tables 3 and 4. Chromatogram of phenolic compounds is shown in Figure 1. Gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid of plant leaves ranged between n.d.-0.625, n.d.-2.398, 0.002-1.446, 0.176-2.897, n.d.-28.093, n.d.-10.675, n.d.-73.951, 0.122-71.000, n.d.-45.432 and 0.249-7.407, respectively, Gallic acid was not determined in *Lactuca serriola* L., *Sinapis arvensis* L., *Malva neglecta* L. and *Chenopodium album.* Ferulic acid was not determined in T*hymus vulgaris* L. Catechin, apigenin, naringin, rutin and quercetin of plant leaves ranged between n.d.-2.398, n.d.-7.677, n.d.-0.247, 0.051-0.856 and 0.074-0.676, respectively, Naringin was not determined in *Lactuca serriola* L., *Sinapis arvensis* L., *Malva neglecta* L., *Amaranthus retroflexus* L., *Tragopogon longirostris bisch* and *Chenopodium album.* 3,4- dihydroxybenzoic acid and epicatechin were not found in plant leaves.

Lactuca serriola L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, rutin and quercetin.
Thymus vulgaris L. had gallic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin.

Sinapis arvensis L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Malva neglecta L. had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Amaranthus retroflexus L. had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, apigenin, rutin and quercetin.

Tragopogon longirostris bisch had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, rutin and quercetin.

Taraxacum officinale had gallic acid, ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, catechin, apigenin, naringin, rutin and quercetin.

Chenopodium album had ferulic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, vanillic acid, caffeic acid, cinnamic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, apigenin, rutin and quercetin.

Table 3. Phenolic acids of plant leaves (µg/g) (mean ± standard deviation)

Samples	Gallic acid	Ferulic acid	Chlorogenic acid	*p*-Coumaric acid	Ellagic acid	Vanillic acid	Caffeic acid	Cinnamic acid	4-hydroxy benzoic acid	2,5-dihydroxy benzoic acid
Lactuca Serriola L.	n.d.	0.055±0.001c	0.014±0.001d	0.176±0.007d	2.586±0.106c	0.108±0.017e	n.d.	0.122±0.015c	2.510±0.010c	0.249±0.013e
Thymus Vulgaris L.	0.016±0.003c	n.d.	0.832±0.023b	0.591±0.015c	0.591±0.009d	0.240±0.023e	0.839±0.070e	0.400±0.007b	1.097±0.017d	1.042±0.017d
Sinapis Arvensis L.	n.d.	0.002±0.000d	0.053±0.005d	0.715±0.017c	4.030±0.174b	7.708±0.371b	73.951±0.998a	0.384±0.093b	3.103±0.031c	1.506±0.039c
Malva Neglecta L.	n.d.	2.398±0.039a	1.446±0.030a	1.446±0.035b	28.093±0.903a	1.208±0.103c	25.584±0.071b	71.000±0.127a	n.d.	2.398±0.091b
Amaranthus retroflexus L.	0.625±0.005a	0.058±0.003c	0.038±0.001d	1.395±0.071b	4.156±0.103b	10.675±0.564a	28.036±0.067b	0.123±0.075c	5.370±0.059b	1.786±0.087c
Tragopogon longirostris bisch	0.134±0.013b	0.004±0.000d	0.187±0.013c	2.897±0.097a	n.d.	1.924±0.317c	9.333±0.097d	0.141±0.091c	45.432±0.307a	7.407±0.107a
Taraxacum officinale	0.024±0.007c	2.113±0.015a	0.074±0.009d	0.257±0.003d	1.936±0.098c	n.d.	0.313±0.017e	0.064±0.009d	0.024±0.009e	2.113±0.019b
Chenopodium album	n.d.	0.160±0.005b	0.002±0.000d	1.136±0.051b	0.018±0.003e	0.679±0.039d	18.217±0.873c	0.129±0.013c	3.007±0.019c	0.664±0.035d

*Values followed by the same letter in the same column are not significantly different (p<0.05)

Table 4. Flavonoids of plant leaves (µg/g) (mean ± standard deviation)

Samples	Catechin	Apigenin	Naringin	Rutin	Quercetin
Lactuca serriola L.	n.d.	n.d.	n.d.	0.051±0.009d	0.074±0.007c
T*hymus vulgaris* L.	0.015±0.001c	1.431±0.097c	0.247±0.074a	0.557±0.013b	0.102±0.009c
Sinapis arvensis L.	0.078±0.009c	0.648±0.075d	n.d.	0.076±0.007d	0.080±0.003c
Malva neglecta L.	2.398±0.073a	7.677±0.105a	n.d.	0.125±0.035c	0.676±0.103a
Amaranthus retroflexus L.	n.d.	0.601±0.056d	n.d.	0.051±0.007d	0.088±0.007c
Tragopogon longirostris bisch	0.444±0.051b	2.307±0.093b	n.d.	0.548±0.073b	0.548±0.091b
Taraxacum officinale	2.113±0.093a	0.074±0.009e	0.137±0.093b	0.856±0.091a	0.083±0.007c
Chenopodium album	n.d.	0.002±0.000e	n.d.	0.079±0.003d	0.094±0.005c

*Values followed by the same letter in the same column are not significantly different (p<0.05)

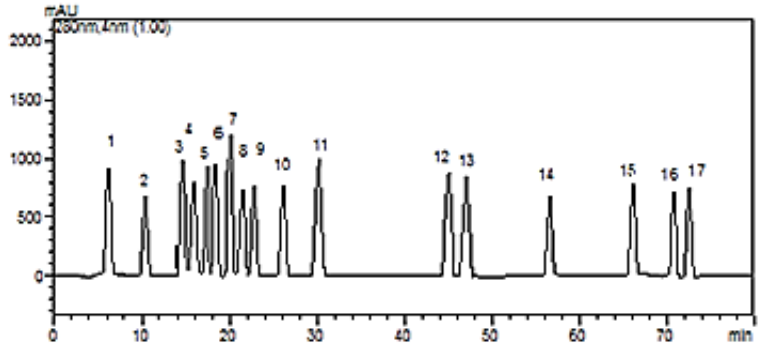

Figure 1. Chromatogram of phenolic compounds (1. gallic acid, 2. 3,4- dihydroxybenzoic acid, 3. 4-hydroxybenzoic acid, 4. 2,5-dihydroxybenzoic acid, 5. chlorogenic acid, 6. vanillic acid, 7. epicatechin, 8. caffeic acid, 9. *p*-coumaric acid, 10. ferulic acid, 11. rutin, 12. ellagic acid, 13. apigenin, 14. cinnamic acid, 15. quercetin. 16. naringin, 17. catechin).

Correlation

Pearson correlation coefficients between DPPH, ABTS, FRAP, TPC, and TFC in plant leaves are given in Table 5. There were high correlation coefficients between ABTS and FRAP, ABTS and TPC, and FRAP and TPC. This result indicated that ABTS could be used as a replacement of FRAP for determining antioxidant activity. Furthermore, ABTS and FRAP could be analyzed for determining TPC.

Table 5. Pearson correlation coefficients between antioxidant activity, total phenolic content, and total flavonoid content

	DPPH	ABTS	FRAP	TPC	TFC
DPPH	1.000	0.567	0.504	0.578	0.182
ABTS	0.567	1.000	0.910 **	0.898 **	0.291
FRAP	0.504	0.910 **	1.000	0.919 **	0.576
TPC	0.578	0.899 **	0.919 **	1.000	0.598
TFC	0.182	0.291	0.576	0.598	1.000

**p<0.01

CONCLUSION

Tragopogon longirostris bisch had the highest radical scavenging activity, TPC, *p*-coumaric acid, 4-hydroxybenzoic acid and 2,5-dihydroxybenzoic acid. *Sinapis arvensis* L. had the highest radical caution decolorization and caffeic acid. *Thymus vulgaris* L. had the highest ferric reducing antioxidant power, as well as naringin and rutin. *Amaranthus retroflexus* L. had the highest TFC, gallic acid and vanillic acid levels. The antioxidant potential of plants was positively correlated with TPC. *Malva neglecta* L. was a rich source of phenolic compounds with high antioxidant properties. The high yield of ferulic acid, chlorogenic acid, ellagic acid, cinnamic acid, catechin, apigenin, and quercetin could make this plant a valuable source of commercial production. Results indicated that *Tragopogon longirostris bisch, Sinapis arvensis* L.*,* T*hymus vulgaris* L.*, Amaranthus retroflexus* L. *and Malva neglecta* L. had a high capacity to prevent diseases caused by the overproduction of radicals.

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