

ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF SOME ASTERACEAE PLANTS

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Abstract

Crepis foetida subsp. *rhoeadifolia*, *Erigeron caucasicus* subsp. *venustus*, *Hieracium bornmuelleri*, *Leontodon crispus* var. *asper*, *Pilosella hoppeana* subsp. *testimonialis* and *Reichardia glauca* belonging to the Asteraceae family, were evaluated for their antioxidant activities and phytochemical analysis of the tested extracts was carried out in current study. Antioxidant activities were investigated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and measuring malondialdehyde (MDA) levels. Chemical composition of the extracts was investigated by using some standards such as; phenolic acids; chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, *p*-coumaric acid and flavonoids; apigenin, luteolin, quercetin, hyperoside, rutin, hesperidin by validated HPLC method. All the tested extracts exhibited antioxidant activity in DPPH radical scavenging test. The aerial part extracts of *P. hoppeana* subsp. *testimonialis*, *Crepis foetida* subsp. *rhoeadifolia* and both aerial parts and roots of *Leontodon crispus* var. *asper* were determined as the most active. The highest inhibitory effect of *Leontodon crispus* var. *asper* roots was also revealed by measuring MDA levels. According to the phytochemical analysis chlorogenic acid was detected in all species investigated except for *Reichardia glauca* aerial parts.

Key words: Asteraceae, Chlorogenic acid, DPPH, HPLC, MDA

Asteraceae Familyasına Ait Bazı Türlerin Fitokimyasal Analizleri ve Antioksidan Etkileri

Bu çalışma Asteraceae familyasına ait *Crepis foetida* subsp. *rhoeadifolia*, *Erigeron caucasicus* subsp. *venustus*, *Hieracium bornmuelleri*, *Leontodon crispus* var. *asper*, *Pilosella hoppeana* subsp. *testimonialis* ve *Reichardia glauca* türlerinin antioksidan kapasitelerini değerlendirmeyi ve etkiden sorumlu bileşiklerin belirlenebilmesi için ekstrelerin fitokimyasal içeriklerinin karakterize edilmesini amaçlamaktadır. Antioksidan aktivite 1,1-difenil-2-pikrilhidrazil (DPPH) radikal süpürücü etki ve malondialdehit (MDA) seviyelerinin ölçülmesiyle tespit edilmiştir. Test edilen ekstrelerin fitokimyasal analizleri valide edilmiş YBSK yöntemi ile bazı fenolik asit standartları (klorojenik asit, kafeik asit, ferulik asit, rozmarinik asit, *p*-kumarik asit) ve flavonoit standartları (apigenin, luteolin, kersetol, hiperozit, rutin, hesperidin) kullanılarak yapılmıştır. Test edilen tüm ekstreler DPPH radikal süpürücü aktivite göstermiştir. *P. hoppeana* subsp. *testimonialis*, *Crepis foetida* subsp. *rhoeadifolia* toprak üstü ve *Leontodon crispus* var. *asper* kök ve toprak üstü kısımları en aktif ekstreler olarak belirlenmiştir. *Leontodon crispus* var. *asper* kök ekstresi ile MDA seviyesinde en yüksek düşüş sağlanmıştır. Fitokimyasal analiz sonuçları *Reichardia glauca* toprak üstü hariç test edilen tüm ekstrelerin klorojenik asit içerdiğini ortaya koymuştur.

Anahtar kelimeler: Asteraceae, Klorojenik asit, DPPH, YBSK, MDA

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INTRODUCTION

Reactive oxygen species are produced as a result of physiological processes as byproducts of energy production, for the prevention from the invasive microorganisms or as signaling and regulatory molecules (1-2). The deleterious effects of the reactive species occur in the lack of the equilibrium between oxidation and antioxidant defense in the body. Oxidative stress may cause a serious damage in the body, as reactive oxygen species are known to be highly responsible from oxidation of biological molecules; such as lipids, DNA and proteins (2). This oxidation process causes membrane damage, protein modification and DNA damage unless it is compensated (3). Oxidative stress is closely relevant to many degenerative diseases; such as diabetes, cancer, cardiovascular and neurodegenerative diseases (4). In such cases, antioxidant defense of the body needs to be supported by dietary antioxidants. Besides antioxidant vitamins, fruit and vegetable consumption; some plant species which have antioxidant potential may serve a solution to overcome the inadequacy of endogenous antioxidant defense due to their high content of bioactive compounds (3). These findings have led to increased interest in the antioxidant as well as in the plants as potential sources of naturally occurring antioxidants.

Asteraceae is very large and common family of flowering plants and includes milk thistles, artichokes, chamomile, arnica and marigold. This family is characterized by an impressive phytochemical diversity that includes, most notably, terpenoids (especially sesquiterpene lactones), polyacetylenes, alkaloids, flavonoids and various phenolic compounds (5-6). Therefore it may be assumed that plants from Asteraceae are

worth to investigate owing to their enormous phytochemical content which is established in earlier studies.

In this study, the antioxidant activity of six members of Asteraceae family; *Crepis foetida* subsp. *rhoeadifolia* (M. Bieb.) Celak, *Erigeron caucasicus* Stev subsp. *venustus* (Botsch.) Grierson, *Hieracium bornmuelleri* Feryn., *Leontodon crispus* Vill. var. *asper*, *Pilosella hoppeana* (Schult.) C.H & F. W. Schultz subsp. *testimonialis* and *Reichardia glauca* Matthews were evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and measuring malondialdehyde levels. DPPH is a free radical which has maximum UV absorption at 515 nm. The antioxidant capacity of the samples was determined by measuring the decrease in absorbance of DPPH after reduction (4). Evaluation of antioxidant activity using MDA is a useful method for the determination of lipid peroxidation (7). Phytochemical analyses of the tested extracts were performed by a validated HPLC method. The method, which was developed previously (8), was used and the composition of the extracts was investigated using some standards such as; phenolic acids; 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) and hesperidin (HE).

EXPERIMENTAL

Plant materials

The taxonomic identification of the plants was confirmed by H. Duman. The Voucher specimens are kept in the herbarium of Ankara University, Faculty of Pharmacy (AEF) (Table 1).

Table 1. Collection sites and herbarium numbers of the plants.

Plant species	Collection sites Collection dates	AEF No
<i>Crepis foetida</i> L. subsp. <i>rhoeadifolia</i> (M. Bieb.) Celak	Erzincan-Kemaliye 01.07.2010	25970
<i>Erigeron caucasicus</i> Stev subsp. <i>venustus</i> (Botsch.) Grierson	Kars, Batıkışla 01.07.2006	25977
<i>Hieracium bornmuelleri</i> Feryn.	Kayseri-Erciyes 01.08.2010	25974
<i>Leontodon crispus</i> Vill. var. <i>asper</i>	Kayseri-Erciyes 01.08.2010	25969
<i>Pilosella hoppeana</i> (Schult.) C.H & F.W. Schultz subsp. <i>testimonialis</i>	Kayseri-Erciyes 01.08.2010	25972
<i>Reichardia glauca</i> Matthews	Sivas-Divriği 26.08.2010	25971

Preparation of the extracts

The dried and powdered roots of the plants were extracted with methanol:water (80:20) mixture by continuous stirring at room temperature for 8 hours. After filtration, extracts were concentrated to dryness under the reduced pressure and low temperature (40-50 °C) on a rotary evaporator to give the crude extracts.

Antioxidant and radical scavenging properties

DPPH Radical Scavenging Activity

DPPH scavenging activity tests were carried out according to the method of Brand Williams *et al.* (9) 0.01 g of sample was dissolved in 10 ml DMSO and seven different concentrations (1 mg/ml to 0.015 mg/ml) were prepared with ½ dilutions. 2.9 ml DPPH solution (10^{-4} M in ethanol) was added into 0.1 ml of sample solutions. The mixture was shaken vigorously and incubated 30 minute in 30°C water bath. Absorbance of the resulting solution was measured at 517 nm UV-visible spectrophotometer (Shimadzu). All the assays were carried out in triplicates with propylgallate as a positive control. Percentage of inhibition (DPPH scavenging activity) determined using the formula $100 \times (A_0 - A_t)/A_0$ where A_0 is the absorbance of DPPH

and A_t is the absorbance of the sample.

Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity. For each sample, the concentration of compound required to scavenge 50% (IC_{50}) of free DPPH radical was determined from the linear graph section of the amount of percentage of inhibition against test compound concentration. A trendline equation “ $y = ax+b$ ” was used and IC_{50} was calculated by transforming this equation and the expression x at which y -value is accepted as 50%.

TBARS Assay

The measurement of malondialdehyde (MDA) levels was performed spectrophotometrically by the modified method of Puhl *et al.* (10). The plant extracts were solved in dH_2O and incubated with 8.125 mM $CuSO_4$ solution. Trichloroacetic acid (%0.1) and thiobarbituric acid (%0.67) solutions were added after incubation and the absorbance at 532 nm were recorded. Quantitation of thiobarbituric acid reactive substances (TBARS) was performed by comparison with a standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetraethoxypropane and the results were expressed as nmol/ml.

HPLC analysis

HPLC analyses were carried out according to the K peli Akkol *et al.* (8). As described previously, this HPLC method was developed and validated to analyse phenolic acids; chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, *p*-coumaric acid and flavonoids; apigenin, luteolin, quercetin, hyperoside, rutin and hesperidin.

RESULTS AND DISCUSSION

In the current study, both the root and aerial part extracts of *Crepis foetida* subsp. *rhoeadifolia*, *Erigeron caucasicus* subsp. *venustus*, *Hieracium bornmuelleri*, *Leontodon crispus* var. *asper*, *Pilosella hoppeana* subsp. *testimonialis* and *Reichardia glauca* were evaluated using by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay and by measuring MDA levels.

In Turkish folk medicine usage of *Crepis foetida* subsp. *rhoeadifolia* in heart diseases and for vasodilatation as a decoction was reported (11-12). The investigations about *Crepis* species have revealed that *Crepis foetida*, *C. mollis* and *C. rhoeadifolia* contain phenolic substances (13). *C. rueppellii* has significant hepatoprotective effect (14) and one sesquiterpene lactone glycoside; 11,13-dihydro-taraxinic acid-1'-O-beta-D-glucopyranoside isolated from *C. napifera* is a promising substance against gastric ulcer (15).

Successive investigations of Stanojevic *et al.* (16-17) have reported that *Hieracium pilosella* which is traditionally used for the treatment of urinary problems, wound healing, against bronchitis, bronchial asthma and edema as well among Europe, especially in Serbia, has free radical scavenging activity against DPPH radical and high reactive hydroxyl radical as well as antimicrobial activity. Phenolic content of the plant was suggested to be responsible for the antioxidant activity (17). The plant extracts and a flavonoid isolated from *H. pilosella* exhibited antiproliferative effect as well as antioxidant and antipseudomonal effects (18). Significant anti-inflammatory and gastroprotective activity but low antioxidant activity for *H. gymnocephalum* was reported (19).

Although traditional usage of *Pilosella* species is not widely searched, there have been a few activity studies about these species. Ugur *et al.* have reported that *P. sandrasica*, an endemic species to Turkey, has antimicrobial effect against some multi-resistant microorganisms (20).

Leontodon hispidus of which antioxidant, anti-inflammatory and cytotoxic activities were previously reported, is also a promising remedy as a natural antioxidant (21-22).

Among *Reichardia* species; *R. tingitana*, which is traditionally used against colic, constipation and conjunctivitis in Arabia (23) and *R. picroides* used as depurative for intestine and to relieve the pain of insect bites in Italy (24), are the ones reported to be used as traditional medicine. According to the activity studies, *R. tinctana* exhibited insecticidal (25), antioxidant (26), and slight antibacterial activity (27), however it was found to be inactive against *Herpes simplex* virus type 1 (28) and at *in vitro* immunomodulatory activity assays as well (6).

In *Erigeron* genus; *E. canadensis* is used in folk medicine as diuretic, tonic, antifungal and astringent to control of bleeding (29). In Turkish folk medicine, this plant is also utilized for its diuretic, antidiarrheic activities, and control of bleeding (30). There are reports about antihypertensive, antioxidant and anticoagulant activities of this species (29, 31-32). *E. breviscapus* is another species, which is used as traditional medicine, especially in China. It is used to treat cardiovascular diseases (33), circulatory problems (34), and as an anti-inflammatory agent for rheumatism, hemiparalysis, hyperpiesia, hepatitis, adenolymphitis, and enteronitis (35). It has been reported that this species had moderate antibacterial activity and high antifungal activity (34) as well as antioxidant (33) and γ -aminobutyric acid transaminase and succinic semialdehyde dehydrogenase inhibitory effects (36). *E. floribundus* is traditionally used for several purposes such as pain relief of rheumatism, gout, cystitis, nephritis, dysmenorrhoea, dental pain, headache (37), and the treatment of some skin disorders (38). It is reported that this species showed central and peripheral analgesic, anti-inflammatory activities (37), and immunomodulatory effect

(39). Another member of *Erigeron* genus, which is used against anti-inflammatory ailments traditionally, is *E. multiradius*. A study aimed to justify the traditional use of this species has shown that it possessed anti-inflammatory activity owing to its flavonoid content (35). Although there are a few phytochemical studies about *E. acris*, it is traditionally used for healing dental and arthritic pains as well as an ethnoveterinary remedy (40). Nalewajko-Sieliwoniuk *et al.* (40) have determined that the phenolic compounds were responsible for the antioxidant activity of this species. *E. annuus* is used especially in traditional Chinese medicine for the treatment of indigestion, enteritis, epidemic hepatitis, hematuria (41-42) and as a hypoglycemic agent (43). According to bioactivity assays; *E. annuus* exhibited glycation and aldose reductase inhibitory activities (41, 45-46) as well as antioxidant and neuroprotective activities (42-43, 46). Phenolic content of this species was

established to be responsible for antioxidant potential (42, 46). Besides, two flavonoid derivatives isolated from this species were found to be anti-inflammatory agents as nitric oxide inhibitors (43).

All the tested extracts were found to possess antioxidant activities in DPPH radical scavenging methods in the present study. Tables 2 and 3 show the inhibitory effects of the plant extracts on DPPH radical and MDA levels respectively. The aerial part extracts of *P. hoppeana* subsp. *testimonialis* and *Crepis foetida* subsp. *rhoeadifolia* exhibited anti-superoxide anion formation with 0.231 mg/ml and 0.261 mg/ml of IC₅₀ values respectively. Scavenging activity against DPPH radical was determined relatively high for aerial part and root extract of *Leontodon crispus* var. *asper* as well as root extract of *Hieracium bornmuelleri*, with 0.327 mg/ml, 0.385 mg/ml and 0.461 mg/ml of IC₅₀ values respectively, when compared with propylgallate as a positive control (IC₅₀ = 0.491 mg/ml).

Table 2. IC₅₀ values of plant extracts. The results are given as molar concentrations.

Plant names	Parts used	IC ₅₀ (mg/ml)
<i>Crepis foetida</i> subsp. <i>rhoeadifolia</i>	R	1.545
	AE	0.261
<i>Erigeron caucasicus</i> subsp. <i>venustus</i>	R	2.297
	AE	0.704
<i>Hieracium bornmuelleri</i>	R	0.461
	AE	0.939
<i>Leontodon crispus</i> var. <i>asper</i>	R	0.385
	AE	0.327
<i>Pilosella hoppeana</i> subsp. <i>testimonialis</i>	R	0.231
	AE	0.864
<i>Reichardia glauca</i>	R	1.143
	AE	1.473

R: Root, AE: Aerial parts

In TBARS assay, as shown in Table 3, a remarkable decrease in the levels of MDA was observed by *Leontodon crispus* var. *asper* roots as 2.268 nmol/ml. *P. hoppeana* subsp. *testimonialis* root, *Crepis foetida* subsp.

rhoeadifolia and *Leontodon crispus* var. *asper* aerial parts also lowered MDA levels significantly, which were determined as 3.91 nmol/ml, 4.546 nmol/ml and 7.31 nmol/ml respectively, as shown in Table 3.

Table 3. Comparison of the MDA levels of the plant extracts.

Plant names	Parts used	MDA level (nmol/ml)
<i>Crepis foetida</i> subsp. <i>rhoeadifolia</i>	R	19.155
	AE	4.546
<i>Erigeron caucasicus</i> subsp. <i>venustus</i>	R	17.09
	AE	30.52
<i>Leontodon crispus</i> var. <i>asper</i>	R	2.268
	AE	7.31
<i>Hieracium bornmuelleri</i>	R	37.74
	AE	24.56
<i>Pilosella hoppeana</i> subsp. <i>testimonialis</i>	R	3.91
	AE	53.16
<i>Reichardia glauca</i>	R	35.51
	AE	37.74

R: Root, AE: Aerial parts

HPLC analysis results have revealed that chlorogenic acid was present in all tested aerial part and root extracts except *R. glauca* aerial parts (Table 4). The highest chlorogenic acid content was found to be in *H. bornmuelleri* root extract with value of $2366.41 \pm 6.398 \mu\text{g/g}$, followed by *P. hoppeana* subsp. *testimonialis* aerial part extract with $1826.894 \pm 8.850 \mu\text{g/g}$ value. In the case of total flavonoid content, aerial part extracts of *P. hoppeana* subsp.

testimonialis, *Crepis foetida* subsp. *rhoeadifolia* and *Erigeron caucasicus* subsp. *venustus* were found to contain luteolin. Apigenin was detected in aerial parts of *P. hoppeana* subsp. *testimonialis*, *Erigeron caucasicus* subsp. *venustus*, *H. bornmuelleri* and *Leontodon crispus* var. *asper* in low amount as shown in Table 4. *Erigeron caucasicus* subsp. *venustus* was also determined as the only species that contain quercetin and rutin.

Table 4. Content of phenolic compounds in the plant samples ($\mu\text{g/g}$)

Material	Parts used	Chlorogenic acid	Luteolin	Quercetin	Apigenin	Rutin
<i>Crepis foetida</i> subsp. <i>rhoeadifolia</i>	AE	206.21 ± 0.24	37.59 ± 0.02	-	-	-
	R	155.41 ± 0.11	-	-	-	-
<i>Erigeron caucasicus</i> subsp. <i>venustus</i>	AE	451.70 ± 0.80	38.61 ± 0.08	43.31 ± 0.10	4.42 ± 0.41	119.06 ± 0.23
	R	138.07 ± 0.32	-	-	-	-
<i>Hieracium bornmuelleri</i>	AE	1269.267 ± 3.80	-	-	4.21 ± 0.23	-
	R	2366.41 ± 6.398	-	-	-	-

<i>Leontodon crispus</i> var. <i>asper</i>	AE	199.29± 1.57	-	-	low amount	-
	R	502.62± 2.52	-	-	-	-
<i>Pilosella hoppeana</i> subsp. <i>testimonialis</i>	AE	1826.90± 8.85	197.29± 0.47	-	7.15 ± 0.16	-
	R	954.03± 3.52	-	-	-	-
<i>Reichardia glauca</i>	AE	-	-	-	-	-
	R	672.33± 5.10	-	-	-	-

R: Root, AE: Aerial parts, low amount: quantity of the compound is determined under LOQ levels

CONCLUSION

Phenolic compounds or polyphenols, constitute one of the most numerous and widely distributed groups of substances in the plant kingdom (47) and display a broad spectrum of pharmacological activities (48). It has been recently reported that, several drugs such as anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective have antioxidant and/or radical scavenging mechanism as a part of their activities (49). Thus, there has been an increasing interest in natural antioxidants from plant sources which are pharmacologically potent and have low or no side effects in recent years (50). In the current study antioxidant potentials of the plant extracts were tested by using two different antioxidant models. Radical scavenging activities were measured against DPPH

radical and all investigated species were found to have radical scavenger activity. Reduced activity on MDA levels was also determined significantly by some species as mentioned in results section. Phytochemical analyse have revealed that the tested extracts contain high amount of phenolic compounds and chlorogenic acid is the only phenolic acid which was measured in all species except *R. glauca* aerial part extracts. According to the results, it can be suggested that antioxidant activity of plant samples is probably due to their phenolic contents and chlorogenic acid as well as flavonoids may be, in part, responsible compounds for this activity. Furthermore, chlorogenic acid seems to be a common phenolic acid in many Asteraceae plants.

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