

PRIRODNE I MATEMATIČKE NAUKE

***in vitro* ANALYSES OF ANTIOXIDANT ACTIVITY OF EXTRACTS OF FIVE SELECTED PLANTS FROM NIGDE, TURKEY**

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Abstract

Agrimonia eupatoria, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale* are commonly consumed and naturally grown in Turkey, especially in Nigde region. The aim of our study was to investigate the antioxidant properties of five plant extracts by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, cupric reducing antioxidant capacity (CUPRAC) and reducing power assay *in vitro*. *Agrimonia eupatoria* showed the highest antioxidant activity compared to other samples using DPPH and CUPRAC assays. *Cichorium inthybus* showed good antioxidant activity in DPPH assay. *Eruca vesicaria* showed good antioxidant activity measured by CUPRAC assay and reducing power assay. *Lepidium sativum* showed good radical scavenging activity only when reducing power assay was used.

Key words: *antioxidant activity, in vitro analyses, DPPH, CUPRAC, reducing power assay*

INTRODUCTION

Generation of free radicals or reactive oxygen species (ROS) give rise to oxidative stress (Sabir, et al., 2013). Over production of ROS in human beings, by endogenous or external sources, such as smoking, pollutants, inflammation, radiation, organic solvents or pesticides, are causes of oxidative stress (Wang, et al., 2012). Oxidative stress plays an important role in chronic diseases, age-related degenerative diseases, heart disease, cancer and in the aging process (Çelik, Özyürek, Güçlü

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& Apak, 2010). The free radicals may be eliminated with endogenous antioxidant systems such as superoxide dismutase, hydrogen peroxide and catalase. Antioxidants could also be found in various fruits and vegetables (Papetti, et al., 2006). Listed plants show antioxidant properties because they contain large amounts of phenolic compounds and flavonoids. Antioxidants have been shown to reduce the risk for chronic diseases, including cancer and cardiovascular diseases (Bursal & Köksal, 2011; Takashima, et al., 2012). *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale* are well known local vegetables and they are consumed daily in Nigde region (Ilahi, Samar, Khan & Ahmad, 2013). This study was designed to determine the antioxidant activities of extracts of *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale* *in vitro*. 1,1- diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method, cupric reducing antioxidant capacity (CUPRAC) and reducing power assay have been used to determine antioxidant activity of these plant extracts. Antioxidant activities of plant extracts at different concentrations have been determined by *in vitro* analysis.

MATERIAL AND METHODS

Plant extracts

Wild plants were collected from Nigde region. Samples containing 10 g of wild plants were kept at -80 °C. Frozen samples were blended and homogenized in 100 ml of 1M phosphate buffer, pH 7.4, for 2 minutes.

These homogenized samples were filtered using filter paper (Whatman, 11 µm). Samples were stored at -20 °C until use (Costa, et al., 2012).

DPPH free radical scavenging activity

The free radical-scavenging activity of fruits was measured using the method described by Brand-Williams, Cuvelier, and Berset (1995), with modifications. 0.06 mM solution of DPPH was prepared with methanol. A methanol solution of the sample extracts at various concentrations (50 µl, 100 µl, 200 µl, 300 µl, 500 µl) was added to 2.5 ml of 0.06 mM methanolic solution of DPPH and allowed to stand for 30 min at 25 °C. The absorbance of the sample was measured at 517 nm against blank samples. A 0.1 mM solution of DPPH in methanol was used as control, whereas ascorbic acid was used as reference standard. All tests were performed in triplicate. Higher DPPH free radical scavenging activity is indicated with lower absorbance of the reaction mixture. A standard column was prepared using different concentrations of DPPH. The percent of DPPH decoloration of the

samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100.$$

Cupric reducing antioxidant capacity (CUPRAC)

The CUPRAC method was used according to Özyürek, 2010, with some modifications (Özyürek, et al., 2010). The CUPRAC method is based on the reduction of a Cu(II) complex by antioxidants, to the Cu(I). 10^{-2} M Cu(II) solution was prepared. To a test tube 1 ml of Cu(II), Nc and NH₄Ac (pH 7) buffer solutions were added. Antioxidant standard solution or samples (x ml) and H₂O (1-x) ml were added to the initial mixture to make the final volume of 4 ml. After 30 min of incubation, absorbance at 450 nm (A_{450}) was recorded against a reagent blank. The molar absorptivity for each antioxidant pertaining to the CUPRAC method was calculated from the absorbance.

Determination of reducing power

The reducing power of the extracted *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus* and *Taraxacum officinale* plant samples were determined according to the method of Oyaizu (1986), with modifications. To various concentrations of the plant extracts (10-100 µg/ml) we added 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide solution before incubation for 30 min at 50 °C. After incubation 2.5 ml of 10% TCA was added to the reaction mixture. The content was then centrifuged at 6000 rpm for 10 min. Then, the absorbance of the reaction mixture was measured at 700 nm. Ascorbic acid (10-100 µg/ml) was used as positive control. Higher the absorbance of the reaction mixture greater is the reducing power.

RESULT AND DISCUSSION

Radical scavenging power

DPPH is a synthetic radical which is commonly used in in vitro determination of anti-radical activity. The obtained results of DPPH assay show that the radical scavenging activity of tested samples was dose-dependent. The extract of *Agrimonia eupatoria* showed the highest DPPH inhibition in comparison to other samples. Lower DPPH inhibition showed *Taraxacum officinale* and *Cichorium inthybus*. *Eruca vesicaria* and *Lepidium sativum* showed the lowest DPPH inhibition. The differences among *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus* and *Taraxacum officinale* are not significant when they were used

at low concentration (50–300 µl). The concentrations of 300 µl and 500 µl show significant difference. Results of DPPH assay are shown in table 1.

Reducing power

Antioxidant compounds cause the reduction of ferric (Fe^{3+}) form to the ferrous (Fe^{2+}) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding FeCl_3 to the ferrous (Fe^{2+}) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Bursal & Köksal, 2011). A higher absorbance indicates a higher ferric reducing power.

As shown in table 3, ethanol extracts of *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus* and *Taraxacum officinale* showed increased ferric reducing power with the increased concentration of standard antioxidants. Among the extracted plants, *Agrimonia eupatoria* showed better activity in comparison to the other extracts. *Agrimonia eupatoria* is known to have antioxidant activity (Copland, et al., 2003; Venskutonis, Škėmaitė & Sivik, B., 2008). Also other extracts showed antioxidant activity. *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus* and *Taraxacum officinale* showed almost same reducing power (Hu & Kitts, 2005; Heimler, Isolani, Vignolini & Romani, 2009; Shirwaikar, Patel, Kamariya, Parmar & Khan, 2011).

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC assays have been used by many researchers to determine reducing power of antioxidant compounds (Özyürek, et al., 2010). This method is based on Cu^{2+} – Cu^+ reduction by antioxidants in the presence of neocuproine. In this assay, a higher absorbance indicates a higher cupric ions (Cu^{2+}) reducing power. According to results of the present study, reducing power of extracts of *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus* and *Taraxacum officinale* decreased in order of *A. eupatoria* > *T. officinale* > *E. vesicaria* > *C. inthybus* > *L. sativum*. Metal ions can cause lipid peroxidation that can produce free radicals and lipid peroxides (Velebný, Hřčková & Königová, 2010). Therefore, metal chelating activity indicate antioxidant and antiradical properties. Decreased absorbance of the reaction mixture indicates higher metal chelating capability. Every extract showed decreasing absorbance, where *Agrimonia eupatoria* and *Taraxacum officinale* had the highest absorbance.

As a result of CUPRAC assay, *Agrimonia eupatoria* showed the highest absorbance, while the second best absorbance was shown by *Taraxacum officinale*. All samples showed antioxidant activity. The obtained results of CUPRAC assay are shown in table 2.

Table 1. Free radical scavenging activity using 1,1- Diphenyl-2-picrylhydrazyl radical (DPPH)

Sample	Concentration in assay μl	% age scavenging of DPPH radical \pm S.E.M*
<i>Taraxacum officinale</i>	50	31,87 \pm 0,5
	100	35,49 \pm 0,3
	200	38,03 \pm 0,4
	300	41,55 \pm 0,6
	500	45,07 \pm 0,5
<i>Agrimonia eupatoria</i>	50	35,70 \pm 0,9
	100	46,51 \pm 1
	200	51,55 \pm 0,7
	300	58,11 \pm 10
	500	62,65 \pm 0,7
<i>Cichorium inthybus</i>	50	35,50 \pm 0,7
	100	38,99 \pm 0,5
	200	42,47 \pm 0,6
	300	44,65 \pm 0,5
	500	52,28 \pm 0,9
<i>Eruca vesicaria</i>	50	33,94 \pm 0,4
	100	35,77 \pm 0,4
	200	38,29 \pm 0,4
	300	41,18 \pm 0,5
	500	42,61 \pm 0,5
<i>Lepidium sativum</i>	50	34,24 \pm 0,6
	100	38,11 \pm 0,5
	200	38,30 \pm 0,6
	300	39,51 \pm 0,4
	500	41,83 \pm 0,4
Ascorbic acid**	60	89,68 \pm 0,16
	30	73,86 \pm 0,09
	15	41,53 \pm 0,05
	8	20,92 \pm 0,03

*: Standard mean error. **: Standard antioxidant

Table 2. The absorbance of CUPRAC assay

	50 μl	100 μl	200 μl	300 μl	500 μl
<i>Taraxacum officinale</i>	0,91 \pm 0,04 ^c	1,58 \pm 0,03 ^d	2,00 \pm 0,07 ^c	3,02 \pm 0,03 ^b	4,40 \pm 0,03 ^a
<i>Agrimonia eupatoria</i>	0,90 \pm 0,02 ^c	1,51 \pm 0,01 ^d	2,14 \pm 0,01 ^c	3,17 \pm 0,01 ^b	4,59 \pm 0,02 ^a
<i>Cichorium inthybus</i>	0,46 \pm 0,01 ^e	0,72 \pm 0,01 ^d	1,04 \pm 0,01 ^c	1,60 \pm 0,02 ^b	2,47 \pm 0,02 ^a
<i>Eruca vesicaria</i>	0,82 \pm 0,02 ^e	1,27 \pm 0,01 ^d	1,71 \pm 0,01 ^c	2,34 \pm 0,01 ^b	2,73 \pm 0,01 ^a
<i>Lepidium sativum</i>	0,45 \pm 0,01 ^e	0,74 \pm 0,01 ^d	0,95 \pm 0,01 ^c	1,42 \pm 0,01 ^b	2,06 \pm 0,01 ^a

Table 3. The absorbance of reducing power assay

	10 μ l	25 μ l	50 μ l	75 μ l	100 μ l
<i>Taraxacum officinale</i>	0,400 \pm 0,03	0,701 \pm 0,03	1,401 \pm 0,07	1,405 \pm 0,03	1,428 \pm 0,04
<i>Agrimonia eupatoria</i>	0,390 \pm 0,02	0,689 \pm 0,02	1,408 \pm 0,01	1,438 \pm 0,01	1,498 \pm 0,01
<i>Cichorium inthybus</i>	0,363 \pm 0,02	0,684 \pm 0,01	1,39 \pm 0,01	1,395 \pm 0,01	1,415 \pm 0,02
<i>Eruca vesicaria</i>	0,389 \pm 0,01	0,696 \pm 0,01	1,424 \pm 0,02	1,425 \pm 0,01	1,445 \pm 0,01
<i>Lepidium sativum</i>	0,399 \pm 0,01	0,724 \pm 0,01	1,431 \pm 0,01	1,447 \pm 0,01	1,441 \pm 0,01
Ascorbic acid	0,497 \pm 0,02	0,957 \pm 0,05	1,453 \pm 0,03	1,670 \pm 0,05	1,896 \pm 0,03

The results of the absorbance of reducing power assay obtained are presented in table 3. All extracts showed antioxidant activity, with no significant difference.

In this study, all of the plant extracts demonstrated scavenging of stable 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical, reducing ability of cupric ions (Cu^{2+}) and reducing power of Fe^{3+} . *Agrimonia eupatoria* showed the highest antioxidant activity among all samples. Other samples showed antioxidant activity respectively *Taraxacum officinale*, *Eruca vesicaria*, *Cichorium inthybus* and *Lepidium sativum*. There are previous reports available for the antioxidant activity of *Agrimonia eupatoria* (Copland, et al., 2003; Gião, Pereira, Fonseca, Pintado & Malcata, 2009; Gião, et al., 2012). It is supported by the study of Venskutonis, Škėmaitė & Ragažinskienė (2007), who studied radical scavenging capacity of *Agrimonia eupatoria* and *Agrimonia procera*. Both plants are good antioxidant agents against oxidative stress.

The antioxidant activities of plants depend on the amount of phenolic compounds they contain. In our study, extract of *Agrimonia eupatoria* has high antioxidant activity because of the presence of high amount of phenolic compounds. Hydroxyl groups, which are found in the chemical structure of phenolic compounds can provide components needed for a radical scavenger (Nahak & Sahu, 2010). Also in another study, *Agrimonia eupatoria* showed antioxidant activity of *covolvulus hystrix vahl* and its chemical properties were attributed to the high contents of phenolic compounds (El Raheim Mohammed Donia, et al., 2011). The relationship between the antioxidant activity and phenol content was supported by antioxidant and antibacterial activities of selected varieties of thai mango seed extract (Khammuang & Sarnthima, 2011). Our findings are supported by earlier reports that plants, which contain phenols, tannins and flavonoids have antioxidant activity (Ilahi, et al., 2013). In the present study, extract of *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale* showed antioxidant activities. The antioxidant potential can be predicted by high contents of phenolic compounds based on the previous studies. The reducing properties are generally dependent on

the presence of reductones, which have been shown to exert antioxidant activity by breaking the free radical chain by donating a hydrogen atom. The reducing capacity of *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, and *Taraxacum officinale* compared to standard ascorbic acid (10-100 µg/ml) at 700 nm are shown in table 3. Studied extracts showed antioxidant activity with no significant difference noticed. These results suggest that all samples have reducing power but not as efficient as standard ascorbic acid. It is inferred result of the present research study that among the five plants: *Agrimonia eupatoria*, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale*, extract of *Agrimonia eupatoria* have showed highest potential antioxidant activity for scavenging 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical and CUPRAC.

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***in vitro* ISPITIVANJE ANTIOKSIDATIVNOG DJELOVANJA EKSTRAKATA PET ODABRANIH BILJAKA SA PODRUČJA NIGDE, TURSKA**

Sažetak

Agrimonia eupatoria, *Lepidium sativum*, *Eruca vesicaria*, *Cichorium inthybus*, *Taraxacum officinale* se najčešće konzumiraju i prirodno rastu u Turskoj, pogotovo na području Nigde. Naš cilj je bio istražiti antioksidativno djelovanje ekstrakata pet biljaka na osnovu praćenja sposobnosti hvatanja 1,1-difenil-2-pikrilhidrazil (DPPH) slobodnog radikala, određivanja sposobnosti redukcije bakar (II) iona (CUPRAC) i testa redukcijske snage *in vitro*. Između pet uzoraka, *Agrimonia eupatoria* pokazala je najveće antioksidativno djelovanje u poređenju sa rezultatima ostalih uzoraka dobivenih po metodama DPPH i CUPRAC. Rezultati DPPH testa pokazali su dobro antioksidativno djelovanje *Cichorium inthybus*. *Eruca vesicaria* pokazala je dobro antioksidativno djelovanje pomoću DPPH testa. *Lepidium sativum* pokazala je dobro antiradikalno djelovanje pomoću testa redukcijske snage.

Ključne riječi: *antioksidativno djelovanje, in vitro ispitivanje, DPPH, CUPRAC, test redukcijske snage*