

Antioxidant Activities of Traditional Medicinal Plant Species From the Kars (Turkey)

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Fourteen different plant parts consisting of leaves, roots, flowers, and seeds were examined separately of *Rumex patientia*, *Hyoscyamus niger*, *Melilotus officinalis*, *Lamium album*, and *Epilobium angustifolium* plants. All of these are naturally growing and used for medicinal purposes in Kars province. Five different methods were used to determine the antioxidant capacity of these herbal products. The methods were as follows: determination of total polyphenol, total flavonoid, DPPH• (free radical), CUPRAC (cupric reducing antioxidant capacity), and FRAP (ferric ion reducing antioxidant power). The data obtained regarding the antioxidant activity demonstrated that the flower part of the *Epilobium angustifolium* plant obtained from Kars had the highest antioxidant capacity. It was determined that the seeds of the *Hyoscyamus niger* plant had the lowest antioxidant capacity.

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INTRODUCTION

It is estimated that there are approximately 750,000 to 1,000,000 species of plants in the world today, and approximately 20,000 of these plant species are used for medicinal purposes. Of the approximately 9,000 plant species growing in Turkey, only about 500 of them are known to be used in treatment (Baytop 2021). Medicinal aromatic plant species have been used both medicinally and as food from past to present, and their value has increased over time. Extracts obtained from parts of medicinal aromatic plants such as roots, fruits, flowers, and leaves constitute the main ingredient of many medicinal drugs. 80% of the people in the world prefer to use medicinal plants first in the treatment of their diseases. The main ingredient of 1/4 of the drugs used by developed countries is also of herbal origin (Farnsworth *et al.* 1985; Ceylan *et al.* 2019b). Medicinal aromatic plant species are also traded plants. While the majority of these plants are collected from nature, very few of them are produced in cultivated fields. As a result of research on plants, the discovery of new active substances to be used for the treatment of diseases has emerged. As a result of the studies carried out in 1985, it was determined that 2,618 of 3,500 new active substances were of herbal origin. People should make it a principle to protect medicinal plants that are collected from nature. This situation is important for plants to continue their existence (Güler 2004).

Antioxidants are versatile compounds due to their diverse compositions and mechanisms. They can chelate metals, inhibit various enzymes, and eliminate free radicals. These substances are commonly found in plants, with key components being phenolic compounds, carotenoids, vitamin C, and vitamin E, all of which possess antioxidant properties (Rodríguez-García *et al.* 2019; Gonzales *et al.* 2021).

Antioxidants, which act as free radical scavengers, play a crucial role in preventing chronic diseases such as cancer, autoimmune disorders, aging, cataracts, rheumatoid arthritis, cardiovascular issues, and neurodegenerative conditions. They also bolster the immune system. Antioxidant defenses shield the body from the harmful impact of free radicals generated during normal metabolism (Nakilcioğlu and Hışıl 2013; Ceylan *et al.* 2018).

Among phenolic compounds, phenolic acids and flavonoids are well known for their potent antioxidant and various other biological activities, including anticancer, antiviral, antibacterial, and anti-inflammatory effects (Tapiero *et al.* 2002; Tlili *et al.* 2013; Tohma *et al.* 2017; Granato *et al.* 2018; Rahman *et al.* 2018; Ceylan *et al.* 2020).

Although it is difficult to collect medicinal plants from natural environments, the need for these plants is quite high. This study examined the antioxidant activities of the plants collected from Kars province, which are used medicinally and aromatically.

EXPERIMENTAL

Plant Material

The plant material utilized in the study was collected from the Kars province through fieldwork. These plants are both edible and have a history of being used as medicinal remedies by local residents over many years. After collection, the plant materials were dried in an oven at 40 °C before analysis. Approximately 10 grams were weighed separately for each plant species, and approximately 50 mL of methanol was added to each plant. The material was then mixed on a shaker at room temperature for 24 h. The methanolic extracts were obtained after filtration using filter papers and these extracts were used to evaluate their antioxidant properties (Ceylan *et al.* 2019a). These analyses were repeated three times. The determination of total polyphenols, total flavonoids, and antioxidant activities was conducted using spectrophotometric methods, which are commonly employed for assessing natural raw materials. Table 1 provides the Latin names, local Turkish names, and the locations where these plants were found (Table 1).

Table 1. Names and Location Information of the Plants Used in the Study

Latin names	Local names (Turkish)	Location
<i>Rumex patientia</i> L.	Evelik	Paşaçayır (Kars)
<i>Melilotus officinalis</i> (L.) Pall.	Kokulu Yonca	Kağızman (Kars)
<i>Lamium album</i> L.	Beyaz Ballıbabası	Sarıkamış (Kars)
<i>Epilobium angustifolium</i> L.	Yakı Otu	Sarıkamış (Kars)
<i>Hyoscyamus niger</i> L.	Kara Banotu	Sarıkamış (Kars)

The Chemicals

The following chemicals were acquired for the study: Methanol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), all

of which were purchased from Sigma Chemical Co. in St. Louis, MO, USA. Additionally, sodium carbonate, acetic acid, neocuproine (2,9-dimethyl-1,10-phenanthroline), aluminum nitrate nonahydrate, and ammonium acetate were obtained from Merck Chemical Co. in Darmstadt, Germany. All of these chemicals used in the study were of analytical grade.

Total Phenolic Assay

The total phenolic content of the plants was determined using the Folin-Ciocalteu assay, as described by Slinkard and Singleton (1977). In this study, various concentrations of gallic acid (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) were used as standards. To perform the assay, 20 μ L of these gallic acid solutions and 20 μ L of methanolic plant samples (at a concentration of 1 mg/mL) were combined with 400 μ L of 0.5 N Folin-Ciocalteu reagent and 680 μ L of distilled water. This mixture was vortexed, and after a 3-min incubation, 400 μ L of a 10% Na_2CO_3 solution was added. The mixture was vortexed again and incubated for 2 h at room temperature. After the incubation period, the absorbance of the mixtures was measured at 760 nm. The concentrations of total phenolic compounds were then calculated as milligrams of gallic acid equivalents per gram of dry weight sample.

Total Flavonoid Assay

The total flavonoid content was determined using the aluminum chloride assay as described by Chang *et al.* (2002). Quercetin was used as the standard for this measurement. In test tubes, 0.5 mL of quercetin concentrations (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) were mixed with 4.3 mL of methanol, 0.1 mL of 10% $\text{Al}(\text{NO}_3)_3$, and 0.1 mL of 1 M $\text{NH}_4\text{CH}_3\text{COO}$. These mixtures were incubated for 40 min, after which their absorbance was measured at 415 nm. The total flavonoid content of the plants was quantified in milligrams of quercetin equivalents per gram of dry weight sample.

Determination Of Antioxidant Activity

The antioxidant activity of the samples was assessed using two methods: the ferric reducing power (FRAP) and cupric reducing power (CUPRAC) methods. In the FRAP method, the total antioxidant capacity was determined by measuring the reduction of a yellow Fe^{3+} -TPTZ complex to a blue Fe^{2+} -TPTZ complex in the presence of electron-donating substances under acidic conditions (Benzie and Szeto 1999). This was achieved by mixing 3 mL of the FRAP reagent with 100 μ L of the test sample or a blank (solvent used for extraction) in a test tube. The maximum absorbance values at 593 nm were recorded over 4 min at 25 $^\circ\text{C}$, and the final absorbance was compared to a standard curve ranging from 100 to 1000 $\mu\text{mol/L}$. The results were expressed as micromoles of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ equivalents per gram of dry matter.

In the CUPRAC method, the antioxidant solution was mixed with a copper(II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7. The developed absorbance was then measured at 450 nm after 60 min. One milliliter of 10 mM CuCl_2 , 1 mL of 7.5 mM neocuproine, and 1 mL of 1 M NH_4Ac were added to test tubes, along with 0.2 mL of the sample and 0.9 mL of H_2O , resulting in a final volume of 4.1 mL. The final absorbance was measured at 450 nm after 1 h, and the results were evaluated in terms of Trolox® equivalent antioxidant capacity (TEAC) (Apak *et al.* 2004).

To assess the scavenging activity of DPPH radical, a method by Molyneux was used (Molyneux 2004). Different concentrations of 0.75 mL of sample extracts were mixed

with 0.75 mL of a 0.1 mM of DPPH solution (dissolved in methanol). These mixtures were incubated at room temperature in the dark for 50 min, and the absorbance was measured at 517 nm using spectrophotometry. Trolox was used as a standard, and the values are expressed as IC₅₀ (mg sample per mL).

Statistical Analysis

All analyses were performed in triplicate and results were shown as mean ± standard deviation (SD). The mean values were statistically analyzed by ANOVA and Duncan's multiplication range test by using SPSS version 23.0. $p < 0.05$ was considered to be significant.

RESULTS AND DISCUSSION

Many different antioxidants exist in plants, and it's a challenging task to measure each of these antioxidants individually. Plant extracts are chemically complex, typically consisting of a mixture of numerous compounds with varying functional groups, polarity, and chemical behavior. This complexity can result in diverse results depending on the specific testing methods used. Therefore, it is more informative and practical to employ various tests to evaluate the antioxidant capabilities of each component, as suggested by previous studies (Huang *et al.* 2005; Zalibera *et al.* 2008).

The CUPRAC, FRAP, total flavonoid, and total polyphenol findings calculated from the results obtained from the methanol extractions are shown in Table 2, while the DPPH activity result graph is shown in Fig. 1. In the study, antioxidant activity levels were determined as a result of analyzes for 14 different samples using total polyphenol, CUPRAC, FRAP, DPPH, and total flavonoid methods for plants collected from Kars province. UV spectrophotometry was used. The *Epilobium angustifolium* plant had the maximum antioxidant capacity among all analyzes. The seeds of the *Hyoscyamus niger* plant had the minimum antioxidant capacity among all analyzes.

As a result of the total polyphenol method, the antioxidant activity of the flower part of the *Epilobium angustifolium* plant was measured as 23.42 ± 0.51 mg GAE/g sample, and its value was higher than the other plants. The fruit of *Rumex patientia* (18.68 ± 0.59 mg GAE/g) plant and the seed of *Epilobium angustifolium* (15.15 ± 0.98 mg GAE/g) also had good activity level. According to the total polyphenol method, the seed part of the *Hyoscyamus niger* plant had the least activity and its activity value was 0.66 ± 0.10 mg GAE/g sample. This sample was followed by the leaf of *Melilotus officinalis* (1.42 ± 0.07 mg GAE/g) and the leaf of *Rumex patientia* (2.45 ± 0.02 mg GAE/g), respectively.

According to the total flavanoid method, the best value was found in the flower part of the *Epilobium angustifolium* plant, and its activity was measured as 8.31 ± 0.71 mg quercetin/g sample. The second and third highest value plant parts are the leaf of *Lamium album* plant and the fruit of *Rumex patientia* plant. The seed of the *Hyoscyamus niger* plant has the lowest activity and its activity was found as 0.05 ± 0.00 mg quercetin /g sample.

According to the FRAP analysis, the highest activity was found in the flower of the *Epilobium angustifolium* plant. Its activity value was measured as 138.55 ± 9.18 μ mol FeSO₄.7H₂O/g sample. The seed of *Epilobium angustifolium* plant and the fruit of *Rumex patientia* plant had high activity. The seed of *Hyoscyamus niger* plant has lower activity than the seed of *Epilobium angustifolium* and the fruit of *Rumex patientia* plant. The seed

of *Hyoscyamus niger* plant was measured as 0.68 ± 0.04 $\mu\text{mol FeSO}_4 \cdot 7\text{H}_2\text{O/g}$ sample. The plants with the lowest activity were *Melilotus officinalis* leaf and flower.

Table 2. Flavonoid Contents, FRAP, CUPRAC, and Phenolic Contents

Samples *	Total Phenolics (mg GAE/g)	Total Flavonoid (mg QE/g)	FRAP ($\mu\text{mol Fe/g}$)	CUPRAC (mmol TEAC/g)
<i>Melilotus</i> fl.	2.55 ± 0.43^c	1.62 ± 0.01^c	$5.71 \pm 0.07^{a,b}$	0.04 ± 0.005^a
<i>Melilotus</i> l.	1.42 ± 0.07^b	2.71 ± 0.11^d	$4.53 \pm 0.13^{a,b}$	0.05 ± 0.009^a
<i>Hyoscyamus</i> s.	0.66 ± 0.10^a	0.05 ± 0.00^a	0.68 ± 0.04^a	0.02 ± 0.007^a
<i>Hyoscyamus</i> fl.	7.16 ± 0.45^e	1.65 ± 0.03^c	36.55 ± 0.71^d	0.03 ± 0.001^a
<i>Hyoscyamus</i> l.	2.98 ± 0.13^c	3.72 ± 0.13^e	12.95 ± 0.28^c	0.10 ± 0.004^b
<i>Lamium</i> fl.	10.00 ± 0.42^g	2.68 ± 0.11^d	56.62 ± 0.92^f	0.19 ± 0.014^c
<i>Lamium</i> l.	5.78 ± 0.36^d	6.22 ± 0.64^h	56.52 ± 1.00^f	$0.22 \pm 0.002^{c,d}$
<i>Epilobium</i> l.	13.36 ± 0.29^h	4.96 ± 0.31^f	96.57 ± 1.84^g	0.56 ± 0.032^e
<i>Epilobium</i> fl.	23.42 ± 0.51^k	8.31 ± 0.71^i	138.55 ± 9.18^j	1.03 ± 0.082^g
<i>Epilobium</i> s.	15.15 ± 0.98^i	3.28 ± 0.34^e	120.31 ± 6.91^i	0.82 ± 0.014^f
<i>Rumex</i> s.	$7.32 \pm 0.21^{e,f}$	1.10 ± 0.09^b	44.37 ± 2.10^e	$0.23 \pm 0.003^{c,d}$
<i>Rumex</i> l.	2.45 ± 0.02^c	4.65 ± 0.28^f	$8.99 \pm 0.30^{b,c}$	$0.06 \pm 0.001^{a,b}$
<i>Rumex</i> fr.	18.68 ± 0.59^j	5.58 ± 0.08^g	112.81 ± 4.59^h	0.53 ± 0.035^e
<i>Rumex</i> r.	8.00 ± 0.45^f	0.51 ± 0.02^a	55.11 ± 1.50^f	0.25 ± 0.009^d

* *Melilotus* fl., flower of *Melilotus officinalis*; *Melilotus* l., leaf of *Melilotus officinalis*; *Hyoscyamus* s., seed of *Hyoscyamus niger*; *Hyoscyamus* fl., flower of *Hyoscyamus niger*; *Hyoscyamus* l., leaf of *Hyoscyamus niger*; *Lamium* fl., flower of *Lamium album*; *Lamium* l., leaf of *Lamium album*; *Epilobium* l., leaf of *Epilobium angustifolium*; *Epilobium* fl., flower of *Epilobium angustifolium*; *Epilobium* s., seed of *Epilobium angustifolium*; *Rumex* s., seed of *Rumex patientia*; *Rumex* l., leaf of *Rumex patientia*; *Rumex* fr., fruit of *Rumex patientia*; *Rumex* r., root of *Rumex patientia*

^{a-k} different letters within columns indicate statistically significant differences ($P < 0.05$); total phenolic and flavonoid contents and antioxidant activity shown as the mean \pm standard deviation (SD).

According to the CUPRAC method, the highest activity was found in the flower of the *Epilobium angustifolium* plant, and its activity value was measured as 1.03 ± 0.082 mmol Trolox/g sample. The seed and leaf of *Epilobium angustifolium* were also found to have good activity. The lowest activity was observed in the seed of *Hyoscyamus niger* plant, and it was measured as 0.02 ± 0.007 mmol Trolox/g sample. It was found that the flower of *Hyoscyamus niger* and the flower of *Melilotus officinalis* had very little activity.

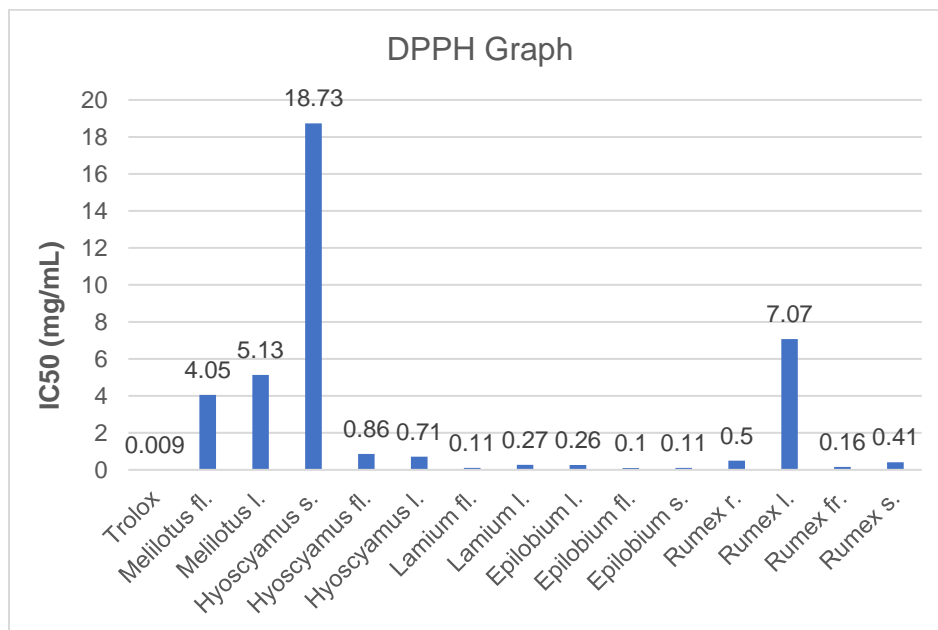


Fig. 1. DPPH for plant extracts

Finally, in the DPPH method applied, the highest activity was found in the flower of the *Epilobium angustifolium* plant, and the activity value was measured as 0.1 mg/mL. The seed of *Epilobium angustifolium* plant and the flower of *Lamium album* plant also had high activity. The lowest activity was found in the seed of the *Hyoscyamus niger* plant and the activity value was measured as 18.73 mg/mL. Also, The leaf of *Rumex patientia* and the leaf of *Melilotus officinalis* showed little activity.

In a study similar to this one, it has been determined that antioxidant structures can prevent many diseases with their mechanism of action. Researchers have recently shown more interest in flavonoids and polyphenols, which are natural antioxidants and are present in plants (Moon and Shibamoto 2009; Frankel and Finley 2008).

In the leaf part of *Rumex patientia*, the total polyphenol was 18.97 ± 0.33 mg GAE/g, total flavonoid was 0.78 ± 0.01 mg quercetin/g, the value in the Frap test was 0.78 ± 0.03 μ mol FeSO₄·7H₂O/g, the value in the Cuprac test was 3.16 ± 0.24 mmol TEAC/g, and the DPPH activity is 3.255 mg/mL (Ceylan *et al.* 2019a). The root, leaf, fruit and seed parts of *Rumex patientia* plant were examined separately. The total polyphenol value, the value in the Cuprac test and the DPPH value were found to be lower in the leaf. The values in the total flavonoid and Frap test were higher.

The total polyphenol was 95 ± 2.42 mg GAE /g and total flavonoid was 119 ± 4.61 mg quercetin/g (Mhalla *et al.* 2017). In this study, the total polyphenol and total flavonoid values in the leaf part of *Rumex patientia*, another species of the *Rumex* genus, were lower.

Miliauskas *et al.* (2004) found that the phenolic content of *Melilotus officinalis* methanol extract (4.3 mgGAE/g) was higher compared to this study. Radical scavenging activity determined by the DPPH capacity determination method was reported as 75.9% (methanol).

In the study conducted by Bubueanu *et al.* (2013), the total phenol content of the *Lamium album* plant was measured as 290 ± 7 mg/100 mL. DPPH activity of the plant was measured as 19.29 mg/mL (EC₅₀). In this study, total polyphenol activities in flower and leaf of *Lamium album* plant were measured as 10.00 ± 0.42 mg GAE/g and 5.78 ± 0.3642 mg

GAE/g sample, respectively. The DPPH activity value was found to be 0.11mg/mL (IC₅₀) in the flower and 0.27 mg/mL (IC₅₀) in the leaf.

In another study, the activities of *Epilobium angustifolium* plant were determined by total polyphenol and DPPH. The total polyphenol activity of *Epilobium angustifolium* plant was 1.94±0.06 Mmol GA/Dm³ and DPPH activity was 3.68±0.02 Mmol Trolox/Dm³ (Nowak *et al.* 2021).

Antioxidant activity and phenolic compounds in the above-ground part of the *Hyoscyamus niger* plant were evaluated by Hajipoor *et al.* (2015). The FRAP activity value of the *Hyoscyamus niger* plant was measured as 287.5±3.64 (mmolFe²⁺/g dry extract), while the EC₅₀ activity of the plant was measured as 377±1.21 µg/mL.

CONCLUSIONS

In the study, 14 plant samples, including leaves, flowers, fruits and roots, obtained from plants collected from Kars province, were extracted with methanol. Five different methods were used to determine the antioxidant capacity of these treated plant samples. The methods used to determine the antioxidant capacity are: Total polyphenol determination, CUPRAC (Cu⁺² reducing antioxidant capacity), FRAP (Fe³⁺ reducing antioxidant power), total flavonoid and DPPH• removal method.

1. The findings of the study show that these plant extracts contain compounds with antioxidant activity. It may be advantageous to replace synthetics with natural antioxidants. According to these results, methanol extracts of these plants may be a strong natural antioxidant source. Due to their antioxidant capacity, these plant species can be used as natural resources in the cosmetic and pharmaceutical industries.
2. There is a large amount of collecting activities of medicinal plants in natural habitats. In order for these plants to survive, it is necessary to raise awareness of the public about medicinal aromatic plants.

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