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Determination of phytochemical content by LC-MS/MS, investigation of antioxidant capacity, and enzyme inhibition effects of nettle (*Urtica dioica*)

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Abstract. – OBJECTIVE: *Urtica dioica L. Subsp. dioica* is an annual or perennial herbaceous plant belonging to the Urticaceae family that has an important place in ethnobotany. This study aimed to investigate the phytochemical content and the inhibition effect on acetylcholinesterase (AChE), which interact with beta-amyloid to promote the deposition of amyloid plaques and paraoxonase (PON1). This plays a role in the regulation of HDL and LDL and an antiatherogenic, and antioxidant capacity of *Urtica dioica*.

MATERIALS AND METHODS: Phytochemical content was determined by the liquid chromatography/mass spectrometry (LC-MS/MS), and to assess the enzyme inhibition and antioxidant capacity the spectrophotometer technique was used. The antioxidant capacity of *U. dioica* extracts (methanol, hexane, and water) was determined by applying 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\cdot+}$), 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot+}$), ferric reducing antioxidant power (FRAP), and cupric ion reducing antioxidant capacity (CUPRAC) methods.

RESULTS: The methanol extract of the *U. dioica* exhibited significant inhibition on the AChE (IC50= 0.098 ± 0.011 mg/mL). However, methanol and water extracts of the *U. dioica* did not exhibit the inhibition effect on PON1. The highest activity for $ABTS^{\cdot+}$ was in the hexane extract (55.97%), and for $DPPH^{\cdot+}$ was in the methanol extract (62.42%). Compared to other solvents (hexane and water), the methanol extract of the *U. dioica* showed the highest activity for FRAP and CUPRAC methods. Results (as absorbance) were 0.302 for CUPRAC and 0.147 for FRAP in the methanol extract of the *U. dioica*. The acetohydroxamic acid, gallic acid, caffeic acid, ellagic acid, *p*-hydroxybenzoic acid, and quercetin were qualified and quantified in LC-MS/MS analyses of *Urtica dioica* extract.

CONCLUSIONS: *U. dioica*, which has antioxidant, anti-atherosclerotic and neuroprotective effects, has a natural medicine potential if compared to synthetic drugs used in Alzheimer's patients.

Key Words:
Acetylcholinesterase, Antioxidant, LC-MS/MS, Paraoxonase, *Urtica dioica.*

Introduction

Nettle (*Urtica dioica*) is an annual or perennial herbaceous plant belonging to the Urticaceae family and the genus *Urtica*. It has a broad distribution around the world and in different regions of Turkey. Compared to other green leafy plants, the nettle leaves are very nutritious, easily digested, and contain a high amount of protein, amino acids, minerals (calcium, potassium, magnesium, phosphorus, and iron), vitamin C, provitamin A, and carotenoids. The nettle is one of those plants that is used both for food and medicine and improves health. This plant has been known for a long time as a medicinal plant in many parts of the world. Nettle is effective in the central nervous system and is an alternative plant that detoxifies and boosts the metabolism[1,2]. It was reported[3-6] that *U. dioica* prevented the damage of rat liver tissue structure[3] and treated stomachache[4], rheumatic pain[5], and liver insufficiency[6]. In traditional medicine, it is stated[7,8] that nettle has wide usage in the treatment of diabetes, hypertension, gastrointestinal diseases, and rheumatism pains.

Free radicals have the risk of oxidative damage on the biomolecules. They eventually lead to atherosclerosis, Alzheimer's, cancer, diabetes, aging, and other degenerative diseases[9]. However, there are enzymatic (superoxide dismutase, catalase, paraoxonase) and non-enzymatic (melatonin, hemoglobin, ferritin, bilirubin) mechanisms, neutralizing the free radicals in the body[10]. If these mechanisms do not work regu-

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larly, it is necessary to take dietary antioxidants. Secondary metabolites produced by plants have antioxidant properties. Antioxidants from plants can reduce oxidative damage caused by free radicals and active oxygen[11]. Various phytochemicals from teas, spices, and herbs and their effects on health are studied notably.

The present study aimed to investigate the antioxidant activity of *U. dioica* extracts by using different antioxidant tests, including free radical scavenging ($ABTS^{\bullet+}$ and $DPPH^{\bullet+}$) and reducing power (FRAP and CUPRAC), and evaluate the inhibition of *U. dioica* extracts on acetylcholinesterase (AChE) and paraoxonase (PON1). Additionally, it was determined the phytochemical content of the plant by LC-MS/MS.

Materials and Methods

Chemicals

The following compounds were used as standards in LC-MS/MS analysis: acetohydroxamic acid (98%), catechin hydrate (≥ 99%), vanillic acid (≥97%), thymoquinone (≥97%), resveratrol (99%), gallic acid (98%), caffeic acid (98%), *p*-hydroxybenzoic acid (99%), salicylic acid (99%), oleuropein (≥ 80%), phloridzin dihydrate (≥ 99%), 2-hydroxy-1,4-naphthoquinone (97%), myricetin (≥96%), quercetin (98%), kaempferol (≥97%), and alizarin (97%) from Sigma-Aldrich (Darmstadt, Germany); protocatechuic acid (97%), butein (≥98%), naringenin (≥95%), silymarin (≥95%), and luteolin (≥98%) from Merck (Darmstadt, Germany), syringic acid (97%) and ellagic acid (95%) from Fluka (Buchs, Switzerland); curcumin (≥99.5%) from Supelco (USA). Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), trolox, and trichloroacetic acid (TCA) were obtained from Sigma-Aldrich (Germany), and $CuCl_2$, neocuprin, ammonium acetate (NH_4Ac), potassium ferricyanide [$K_3Fe(CN)_6$], potassium persulfate ($K_2S_2O_8$), and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) were purchased from Merck (Darmstadt, Germany).

Plant Samples

The nettle plant (*Urtica dioica L. Subsp. dioica*) was collected in May at Çelikhan town, Adiyaman, Turkey and identified by a botanist from Inonu University, Vocational School of Health Services, Malatya, Turkey). The herb was washed with distilled water and then dried in the shade at room temperature.

Sample Extraction

The powdered sample (5 g) was extracted separately with 50 mL extraction solvents (water, methanol, and hexane) using the maceration method at room temperature. 1 mg/mL solution was prepared from the dry extract obtained after the filtration and evaporation processes and was used in antioxidant capacity and enzyme inhibition studies. For LC-MS/MS analyses, only the methanol extract was used.

LC-MS/MS Instrument and Chromatographic Conditions

Qualitative and quantitative determination of 24 phytochemicals was carried out by a Nexera model Shimadzu HPLC coupled to a dual MS instrument (Kyoto, Japan). The liquid chromatograph unit was equipped with LC-30AD binary pumps, DGU-20A3R degasser, SIL-30AC autosampler, and CTO-10AS column oven. The separation was performed using an Inertsil ODS-4 C18 3 μm reversed-phase analytical column (150 mm × 4.6 mm). Gradient elution was performed with 0.5 mL/min flow rate at 40°C, and the injection volume was 4.0 μL. The mobile phase consisted of solvent A (water, 5.0 mM ammonium formate and 0.1% formic acid) and solvent B (methanol, 5.0 mM ammonium formate and 0.1% formic acid). The following elution program was applied: 40-90% B at 0-20 min, 90-99% B at 20-23 min, 99-40% B at 23-24 min, and 4% B at 24-29 min. mass spectrometry (MS) detection was performed using a Shimadzu LCMS 8040 model triple-quadrupole mass spectrometer equipped with an ESI source operating in both positive and negative ionization modes. Liquid chromatography-electrospray ionization/mass spectrometry/mass spectrometry (LC-ESI-MS/MS) data were collected and processed by Shimadzu LabSolutions software (Kyoto, Japan). The multiple reaction monitoring (MRM) mode was used to quantify the analytes. The assay of phenolic compounds was performed following two or three transitions per compound, the first one for quantitative purposes and the second and third one for confirmation.

The limits of detection (LOD) and limits of quantitation (LOQ) of the LC-MS/MS method for phytochemicals were calculated according to following equations:

$$LOD = X + 3SD$$
$$LOQ = X + 10SD$$

where X is the mean concentration of the blank and SD is standard deviation of the blank[12].

Antioxidant Capacity of the Extracts

To determine the antioxidant capacity, the following tests were applied: DPPH free radical, ABTS cation radical scavenging activity, cupric reducing (CUPRAC), and ferric reducing (FRAP) methods[13-15].

Enzyme Inhibition

A spectrophotometric method developed by Ellman et al[16] was used to evaluate the acetyl-cholinesterase inhibitory activities. Paraoxonase inhibitory activity of the extracts of *U. dioica* extracts was determined according to the protocol reported by Necip et al[17].

Results

LC-MS/MS system with high selectivity and sensitivity was chosen for phytochemical analyses in *U. dioica*. LOD, LOQ, linear range, and R^2 were determined for the studied analytes (Table I). Methanol extract of *U. dioica* was used for the determination of phytochemicals by LC-MS/MS. Acetohydroxamic acid, gallic acid, caffeic acid, *p*-hydroxybenzoic acid, ellagic acid, and quercetin from phenolic compounds were quantified in the methanol extract of *U. dioica*. Acetohydroxamic acid (52.54 mg/100 g) was found to be much higher than other phenolic compounds. Catechin hydrate, syringic acid, thymoquinone, resveratrol, protocatechuic acid, salicylic acid, oleuropein, phloridzin dihydrate, 2-hydroxy-1,4-naphthoquinone, myricetin, butein, naringenin, silymarin, luteolin, kaempferol, alizarin, and curcumin were under LOQ, and vanillic acid was not detected (Table II).

The antioxidant capacity of the methanol, water, and hexane extracts of *U. dioica* plant was assayed with the following tests: DPPH free radical scavenging, ABTS cation radical scavenging, cupric reducing (CUPRAC), and ferric reducing (FRAP). Results of antioxidant capacity are given in Table III. DPPH and ABTS results were expressed as percentage radical scavenging activity, and CUPRAC and FRAP results were expressed as absorbance. DPPH radical scavenging activity of 0.2 mg/mL *U. dioica* extract was 62.41 (methanol extract), 12.35 (water extract), and 25.27% (hexane extract). FRAP-reducing activity as absorbance was 0.147 (methanol extract), 0.118 (water extract), and 0.074 (hexane extract) (Table III).

As seen in Table IV, the *U. dioica* methanol extract inhibited 50% of the AChE at 0.098 mg/mL (Figure 1). Water extract showed no inhibition against AChE. Similarly, both methanol extract and water extract exhibited no inhibition against PON 1.

Discussion

The consumption of natural products is increasing to help in the treatment of diseases. So, studies about the effectiveness of plant-derived compounds are of great interest since they explore novel agents with treatment purposes. Phenolic compounds are secondary metabolites from plants and have a role in defense mechanisms against herbivores, pests, pathogens, and various abiotic stress factors in plants. Phenols have some biological and pharmacological activity functions, including antioxidant, anti-inflammatory, anti-tumor, anti-viral, and anti-allergic substances with potential health benefits. It is stated[18,19] that they are effective, especially in the prevention and treatment of chronic diseases in humans, including neurodegenerative, diabetes, cancer, and cardiovascular diseases.

In the present study, different amounts of acetohydroxamic acid, gallic acid, caffeic acid, *p*-hydroxybenzoic acid, ellagic acid, and quercetin in the methanol extract of *U. dioica* were detected. The results of caffeic acid, ellagic acid, and quercetin were comparable to those previously reported[19,20]. In one study conducted in Poland, Jeszka-Skowron et al[19] quantified syringic acid, protocatechuic acid, p-coumaric acid, ferulic acid, kaempferol, quercetin, and rutin by LC-MS/MS in different parts (root, stem, leaves, and aerial) of nettle. On the contrary, syringic acid, protocatechuic acid, and kaempferol were under LOQ in the present study. Garcia et al[20] found similar amounts of caffeic acid (15-40 mg/g DW) by HPLC in the leaves of *U. dioica* dried with different methods in Italy. On the other hand, Otles and Yalcin[2] found low amounts of quercetin (0.038-0.45 mg/100 g) by HPLC in the roots, leaves, and stalks of *U. dioica*. The differences between the phenolic compounds in

Table I. LAnalytical parameters for LC-MS/MS analysis.

Compounds	Retention time (min)	Precursor ion (m/z)	Product ion (m/z)	LOD (µg/L)	LOQ (µg/L)	Linear regression	Linear range (µg/L)	R^2
Acetohydroxamic acid	0.406	76.15	58	6.90	23.01	y = 216.91x + 6.165.8	20-750	0.9989
Catechin hydrate	2.532	291	139.1	2.05	6.84	y = 1.717.9x - 563.99	10-750	0.9988
Vanillic acid	2.762	168.95	65	84.78	282.61	y = 48.343x + 662.5	250-1.000	0.9993
Syringic acid	3.001	199.1	140.1	2.88	9.61	y = 112.03x + 1.316.1	10-500	0.9994
Thymoquinone	3.337	165	137	7.64	25.47	y = 349.23x - 2.887.4	20-500	0.9971
Resveratrol	3.606	229	135	41.83	139.43	y = 733.34x - 69.955	250-1.000	0.999
Gallic acid	1.278	169.1	124.9	3.92	13.06	y = 305.07x - 1.859.3	10-100	0.9981
Caffeic acid	2.836	179	135	2.87	9.58	y = 1.227.2x - 5.396.5	10-100	0.9948
p-hydroxybenzoic acid	3.555	137.2	93.1	8.92	29.74	y = 3.831.2x - 94.423	40-500	0.9996
Protocatechuic acid	3.556	181	108	2.76	9.20	y = 1.382.2x - 4.393.1	10-500	0.9967
Salicylic acid	3.558	137.2	93	22.88	76.25	y = 3.838.2x - 149.277	75-1.000	0.9977
Oleuropein	3.567	539.1	377	7.17	23.90	y = 324.26x - 5.388.8	40-750	0.9997
Phloridzin dihydrate	3.594	435.1	273.1	81.80	272.67	y = 120.23x - 9.479.5	250-1.000	0.9989
2-Hydroxy-1.4-naphthoquinone	3.664	173.1	145	2.07	6.91	y = 461.45x - 4.553.8	10-500	0.9989
Myricetin	3.644	317	179.1	4.34	14.45	y = 588.4x - 4.990.6	20-500	0.9987
Ellagic acid	3.681	301.1	228.9	23.74	79.14	y = 18.841x + 911.46	100-1.000	0.9967
Quercetin	3.891	301.1	150.9	7.79	25.98	y = 150.09x - 422.87	20-500	0.9997
Butein	3.935	271	134.9	38.50	128.20	y = 62.943x - 2.793	100-1.000	0.996
Naringenin	3.952	271	150.9	68.40	228.10	y = 700.8x - 26.469	250-1.000	0.9997
Silymarin	3.996	481.1	453.1	8.00	26.70	y = 199.91x + 950.97	40-750	0.9997
Luteolin	4.069	285	150.9	6.40	21.40	y = 1.389x - 40.923	40-1.000	0.9988
Kaempferol	4.298	285	117	3.90	13.00	y = 62.513x - 821.08	20-1.000	0.9982
Alizarin	4.594	239	211	15.30	51.10	y = 26.512x - 1721	60-2.000	0.9991
Curcumin	4.672	367.1	216.9	12.80	42.70	y = 1.908.9x - 8.252.1	40-1.000	0.9994

Table II. Results of qualitative and quantitative determination of phytochemicals in *U. dioica* extract by LC-MS/MS.

Compounds	Means ± SD (mg/100 g)	Compounds	Means ± SD (mg/100 g)
Acetohydroxamic acid	52.54 ± 1.88	Phloridzin dihydrate	< LOQ
Catechin hydrate	< LOQ	2-Hydroxy-1,4-naphthoquinone	< LOQ
Vanillic acid	Not Detection	Myricetin	< LOQ
Syringic acid	< LOQ	Ellagic acid	6.21 ± 0.60
Thymoquinone	< LOQ	Quercetin	2.31 ± 0.08
Resveratrol	< LOQ	Butein	< LOQ
Gallic acid	2.80 ± 0.03	Naringenin	< LOQ
Caffeic acid	13.48 ± 0.18	Silymarin	< LOQ
p-hydroxybenzoic acid	1.78 ± 0.15	Luteolin	< LOQ
Protocatechuic acid	< LOQ	Kaempferol	< LOQ
Salicylic acid	< LOQ	Alizarin	< LOQ
Oleuropein	< LOQ	Curcumin	< LOQ

Data represent average values ± standard deviation (SD) of three independent samples.

Table III. CAntioxidant capacity of 0.2 mg/mL concentration of *U. dioica* extracts, BHA, BHT, and trolox.

	DPPH	ABTS	FRAP	CUPRAC
	%		Absorbance	
Methanol extract	62.41 ± 5.13	35.34 ± 4.80	0.147 ± 0.017	0.302 ± 0.017
Water extract	12.35 ± 1.87	8.75 ± 0.94	0.118 ± 0.013	0.205 ± 0.012
Hexane extract	25.27 ± 3.06	55.97 ± 3.05	0.074 ± 0.011	0.106 ± 0.013
BHA	79.07 ± 6.39	93.65 ± 4.71	0.748 ± 0.034	0.639 ± 0.010
BHT	46.25 ± 4.21	58.21 ± 2.66	0.520 ± 0.034	0.646 ± 0.021
TROLOX	88.20 ± 4.09	90.03 ± 3.07	0.537 ± 0.028	0.613 ± 0.032

Data represent average values ± standard deviation of three independent samples.

U. dioica plant depend on the soil, the processes after harvesting, the extraction process, and the analysis methods.

According to DPPH, FRAP, and CUPRAC test results, methanol extract showed the highest antioxidant activity. However, the ABTS result of hexane extract was higher than other solvents (methanol and water) extracts. As shown in Table III, the antioxidant capacity of *U. dioica* extracts was compared with those of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and trolox. *U. dioica* methanol extract (62.41%) showed higher activity than BHT (46.25%) but lower than BHA (79.07%) and trolox (88.20%) in DPPH free radical scavenging activity. Hexane extract (55.97%) exhibited an activity close to BHT (58.21%) but lower than BHA (93.65%) and trolox (90.03%) in ABTS cation radical scavenging activity. In the activity tests of FRAP and CUPRAC, all sol-

Table IV. Inhibition effect of *U. dioica* extracts on AChE and PON1

	Inhibition against AChE	Inhibition against PON1
	IC_{50} (mg/mL)	
Methanol extract	0.098 ± 0.011	NI*
Water extract	NI	NI

*No inhibition.

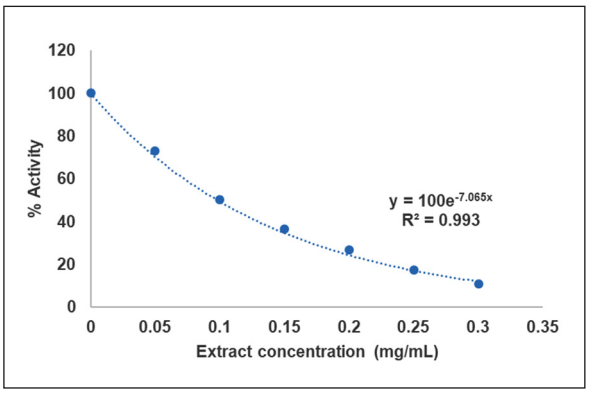

Figure 1. Inhibition curve on AChE activity of *U. dioica* extracts.

vents' extracts showed weak activity compared to BHA, BHT, and trolox (Table III). In previous studies, Çolak et al[21] reported that the ethanol extract (21.53 µg AAE/g) of *U. dioica* showed higher activity than the water extract (10.29 21.53 µg AAE/g) in the FRAP test, whereas ethanol extract (6.20 µg AAE/g) was lower than water extract (13.77 µg AAE/g) in the DPPH test. Besides, in the study of Ertaş et al[22], the methanol extract of *Sedum sediforme* exhibited higher antioxidant activity than petroleum ether, acetone, and water extracts in DPPH, ABTS, and CUPRAC tests. According to these data, it was deduced that antioxidant activity changed depending on the extraction solvent, plant genus, and test. Gülçin et al[23] evaluated the antioxidant capacities of the nettle plant from Erzurum, Turkey, using DPPH method. They found an antioxidant capacity with a value of 37% (ethanol extract), which is lower than the values (62.41% in the methanol extract) obtained in the present study. Florez et al[24] reported that the antioxidant activity values according to DPPH and ABTS tests using different extraction methods in the *U. dioica* plant ranged between 25.8% and 90.2% and between 8.1% and 90.4%, respectively, consistent with the results of the present study. In a study[25] from Giresun, Turkey, the CUPRAC-reducing activity of *U. dioica* extracts (ethanol, chloroform, and hexane) ranged between 0.241 and 0.557 as absorbance, similar to the present study. Antioxidant activity is mostly attributed to the phenolic compounds performing as free radical scavenging, reducing power, and metal chelate activity[21]. The number of phenolic hydroxyl groups plays an important role in the free radical scavenging activity[26].

Overactivity of AChE increases the hydrolysis of the neurotransmitter acetylcholine in the cholinergic system, which causes Alzheimer's disease. Some AChE inhibitors used for this disease have a role in increasing angiogenesis in cardiovascular patients. Some studies[27,28] revealed that the *U. dioica* extract has a neuroprotective effect on neurodegenerative diseases. The anticholinesterase activity of plant extracts was associated with quercetin and ellagic acid presence as well as other phenolic compounds in plants[22,29,30]. Işık[26] reported that *Salvia officinalis* extracts inhibited 50% of AChE at 0.136 mg/mL; the inhibition was lower than the present study (IC_{50}= 0.098 mg/mL).

PON1 enzyme bound to HDL in serum prevents lipoprotein oxidation by hydrolyzing lipid peroxides with oxidized LDL structure with its antioxidant feature[31]. The *U. dioica* extracts exhibited no inhibition on PON1 in the present study. It was reported[32] that the *U. dioica* seed extract had a protective effect on hepatic damage created with ischemia-reperfusion and exhibited a liver protection effect by increasing the activity of paraoxonase, arylesterase, and liver tissue catalase activity.

Conclusions

The phenolic compounds consisting of acetohydroxamic acid, gallic acid, caffeic acid, p-hydroxybenzoic acid, ellagic acid, and quercetin in the *U. dioica* methanol extract were quantified. Methanol extracts exhibited higher antioxidant activity than water and hexane extracts, according to DPPH, CUPRAC, and FRAP tests. In the ABTS test, hexane extract has higher antioxidant activity than other solvent extracts. Results revealed that the *U. dioica* extracts have remarkable antioxidant activity. Additionally, the *U. dioica* extracts show strong inhibition on the AChE but no inhibition on PON1. Oxidative stress induced by free radical formation forms the biological basis of arteriosclerosis, diabetes, neurodegenerative diseases, and cancer. Based on present data, *U. dioica* extract has the effect of free radical inhibitor or scavenger activity as well as an antioxidant which may limit free radical damage, and it may offer great potential opportunities in preventing and controlling neurodegenerative disorders such as Alzheimer's and Parkinson's diseases.

Conflict of Interest
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Authors' Contribution
Y. Uğur: conceptualization, methodology, formal analysis, investigation and writing original draft; A. Güzel: formal analysis, writing, review and editing. All authors approved the final version of the manuscript.

Availability of Data and Materials
Not applicable.

Ethics Approval
his article does not contain any study with human participants or animals performed by any of the authors.

Informed Consent
Not applicable.

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References

1) Dhouibi R, Affes H, Ben Salem M, Hammami S, Sahnoun Z, Zeghal KM, Ksouda K. Screening of pharmacological uses of Urtica dioica and others benefits. Prog Biophys Mol Biol 2020; 150: 67-77.
2) Otles S, Yalcin B. Phenolic compounds analysis of root, stalk, and leaves of nettle. ScientificWorldJournal 2012; 2012: 564367.
3) Lebedev AA, Batakov EA, Kurkin VA, Lebedeva EA, Zapesochnaya GG, Avdeeva EV, Simonova GV, Volotsueva AV. The antioxidative activity of a complex hepatoprotective preparation. Silybokhol Rast Res 2001; 37: 69-75.
4) Sezik E, Yeşilada E, Honda G, Takaishi Y, Takeda Y, Tanaka T. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. J Ethnopharmacol 2001; 75: 95-115.
5) Sezik E, Yeşilada E, Tabata M, Honda G, Takaishi Y, Fujita T, Tanaka T, Takeda Y. Traditional medicine in Turkey VIII. Folk medicine in east anatolia; Erzurum, Erzincan, Ağri, Kars, Iğdir provinces. Econ Bot 1997; 51: 195-211.
6) Yeşilada E, Honda G, Sezik E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in Turkey. IV. Folk medicine in the Mediterranean subdivision. J Ethnopharmacol 1993; 39: 31-38.
7) Guarrera PM. Traditional phytotherapy in Central Italy (Marche, Abruzzo, and Latium). Fitoterapia 2005; 76: 1-25.
8) Ziyyat A, Legssyer A, Mekhfi H, Dassouli A, Serhrouchni M, Benjelloun W. Phytotherapy of hypertension and diabetes in oriental Morocco. J Ethnopharmacol 1997; 58: 45-54.
9) La Fata G, Weber P, Mohajeri MH. Effects of vitamin E on cognitive performance during geing and in Alzheimer's disease. Nutrients 2014; 6: 5453-5472.
10) Sarker U, Oba S. The response of salinity stress-induced A. tricolor to growth, anatomy, physiology, non-enzymatic and enzymatic antioxidants. Front Plant Sci 2020; 11: 559876.
11) Gülçin I, Oktay M, Küfrevioğlu Öİ, Aslan A. Determination of antioxidant activity of lichen Cetraria islandica (L.) Ach. J Ethnopharmacol 2002; 79: 325-329.
12) Uğur Y. Extraction and quantification of melatonin in cornelian cherry (Cornus mas L.) by ultra-fast liquid chromatography coupled to fluorescence detector (UFLC-FD). Acta Chromatogr 2022; published online ahead of print 2022: https://doi.org/10.1556/1326.2022.01052.
13) Miller HE. A simplified method for the evaluation of antioxidants. J Am Oil Chem Soc 1971; 48: 91-92.
14) Elmastaş M, Gülçin İ, Beydemir Ş, Küfrevioğlu Öİ, Aboul-Enein HY. A study on the in vitro antioxidant activity of juniper (Juniperus communis L.) fruit extracts. Analytical Letters 2006; 39: 47-65.
15) Apak R, Guclu K, Ozyurek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC Method. J Agric Food Chem 2004; 52: 7970-7981.
16) Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
17) Necip A, Işık M, Güzel A, Takım K, Kaygısız F. LC-MS/MS analysis, antioxidant properties and inhibition effect on some important metabolic enzymes of Nicotiana rustica L. KSU J Agric Nat 2021; 24: 930-938.
18) Hu W, Sarengaowa, Guan Y, Feng K. Biosynthesis of phenolic compounds and antioxidant activity in fresh-cut fruits and vegetables. Front Microbiol 2022; 13: 906069.
19) Jeszka-Skowron M, Zgoła-Grześkowiak A, Frankowski R, Grześkowiak T, Jeszka AM. Variation in the content of bioactive compounds in infusions prepared from different parts of wild Polish stinging nettle (Urtica dioica L.). Molecules 2022; 27: 4242.

20) Garcìa L, Ceccanti C, Negro C, De Bellis L, Incrocci L, Pardossi A, Guidi L. Effect of drying methods on phenolic compounds and antioxidant activity of Urtica dioica L. leaves. Horticulturae 2021; 7: 10.

21) Çolak S, Çömlekcioğlu N, Aygan A. Investigation of antioxidant and antimicrobial activities of Urtica dioica L. plant extracts. Eurasian J Bio Chem Sci 2020; 3: 206-212.

22) Ertaş A, Boğa M, Yılmaz MA, Yeşil Y, Haşimi N, Kaya MŞ, Temel H, Kolak U. Chemical compositions by using LC-MS/MS and GC-MS and biological activities of Sedum sediforme (Jacq.) Pau. J Agric Food Chem 2014; 62: 4601-4609.

23) Gülçin I, Küfrevioglu OI, Oktay M, Büyükokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J Ethnopharmacol 2004; 90: 205-215.

24) Flórez M, Cazón P, Vázquez M. Antioxidant extracts of nettle (Urtica dioica) leaves: evaluation of extraction techniques and solvents. Molecules 2022; 27: 6015.

25) Külcü DB, Gökışık CD, Aydın S. An investigation of antibacterial and antioxidant activity of nettle (Urtica dioica L.), mint (Mentha piperita), thyme (Thyme serpyllum) and Chenopodium album L. plants from Yaylacık Plateau, Giresun, Turkey. Food Sci Tech 2019; 7: 73-80.

26) Işık M. Anticholinergic, antioxidant activity and LC-MS/MS analysis of ethanol extract from Salvia officinalis L. Int J Life Sci Biotech 2020; 3: 51-61.

27) Patel SS, Mahindroo N, Udayabanu M. Urtica dioica leaves modulates hippocampal smoothened-glioma associated oncogene-1 pathway and cognitive dysfunction in chronically stressed mice. Biomed Pharmacother 2016; 83: 676-686.

28) Bisht R, Joshi BC, Kalia AN, Prakash A. Antioxidant-rich fraction of Urtica dioica mediated rescue of striatal mito-oxidative damage in MPTP-induced behavioral, cellular, and neurochemical alterations in rats. Mol Neurobiol 2017; 54: 5632-5645.

29) Fan P, Hay AE, Marston A, Hostettmann K. Acetylcholinesterase-inhibitory activity of linarin from Buddleja davidii, structure-activity relationships of related flavonoids, and chemical investigation of Buddleja nitida. Pharm Biol 2008; 46: 596-601.

30) Işık M, Beydemir Ş, Yılmaz A, Naldan ME, Aslan HE, Gülçin İ. Oxidative stress and mRNA expression of acetylcholinesterase in the leukocytes of ischemic patients. Biomed Pharmacother 2017; 87: 561-567.

31) Aşkın U, Karataş F, Türköz Y, Aydın S. Paraoksonaz-1 enziminin bazı kinetik özelliklerinin incelenmesi ve ghrelin hormonu ile ilişkisi. Ann Health Sci Res 2012; 1: 21-28.

32) Kandis H, Karapolat S, Yildirim U, Saritas A, Gezer S, Memisogullari R. Effects of Urtica dioica on hepatic ischemia reperfusion injury in rats. Clin (Sao Paulo) 2010; 65: 1357 1361.