Cell. Mol. Biol. 59 (Supp): OL1882-OL1888
Published on November 3, 2013
DOI 10.1170/224

Cellular & Molecular Biology
http://www.cellmolbiol.com

EVALUATION OF ANTIOXIDANT PROPERTIES, ELEMENTAL AND PHENOLIC CONTENTS COMPOSITION OF WILD NETTLE (*Urtica dioica* L.) FROM TUNCELI IN TURKEY

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Abstract

Wild nettle (*Urtica dioica* L.) types were sampled from different geographical regions in Tunceli (Turkey) to determine their mineral, vitamin, phenolic contents and their antioxidant properties. The total phenol varied from 37.419±0.380 to 19.182±1.00 mg of GAEs g^{-1} of dry nettle. The highest radical scavenging effect was observed in Mazgirt parting of the ways 7.5 km with 33.70±0.849 mg mL^{-1}. The highest reducing power was observed in the nettles from Mazgirt parting of the ways 7.5 km. Among the various macronutrients estimated in the plant samples, potassium was present in the highest quantity followed by calcium and phosphate. Kaempferol and resveratrol were not determined in some nettle samples but rutin levels were determined in all samples. Vitamin A concentrations were ranged between 13.64±1.90 and 5.74±1.00 (mg kg^{-1} dry weight). These results show that *Urtica dioica* L. collected from Tunceli in Turkey could be considered as a natural alternative source for food, pharmacology and medicine sectors.

Key words: *Urtica dioica* L., Tunceli, Antioxidant activity, Phenolic Content, Elemental composition, Vitamin.

Article information

Received on January 7, 2013
Accepted on October 11, 2013

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INTRODUCTION

Reactive oxygen species (ROS) are generated spontaneously in cells during metabolism and implicated in the aetiology of different degenerative diseases, such as heart diseases, stroke, rheumatoid arthritis, diabetes and cancer (20). Antioxidant components are microconstituents present in the diet that can delay or inhibit lipid oxidation, by inhibiting the intiation or propagation of oxidizing chain reactions, and are also involved in scavenging free radicals (26).

A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases (38). One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources (27). Antioxidant supplements or antioxidant containing foods may be used to help the human body to reduce oxidative damage or to protect food quality by preventing oxidative deterioration (14,45). Natural plant antioxidants can therefore serve as a type of preventive medicine. Some plants such as *U. dioica* L. can be a good source of antioxidants. *U. dioica* L. extract prevented free radical formation in lipid oxidation (15). *U. dioica* L. water extract had more antioxidant activity than butylated hydroxyanisole (BHA), quercetin, and α-tocopherol (18). As of 2004, 6759 species had been recorded in the data bank of Italian vascular flora, of which 700 are endemic. *U. dioica L.* leaves are also used to treat stomachaches in Turkish traditional medicine (44).

Phenolic compounds are widely distributed in plant foods and therefore important constituents of the human diet. The term of phenolic compounds refers to the main classes of secondary metabolites in plants (25, 16). Several thousand molecules have been identified in various plant species. Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannins), have diverse biological effects, such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects, as a result of their antioxidant activity (11). Phenolic compounds inhibit lipid peroxidation, scavenge free radicals, chelate iron and copper ions, protect lipoprotein cholesterol from being oxidized, and stimulate enzymes involved in detoxification of carcinogenic substances (22).

Trace elements (Mn^{2+}, Se^{3+}, Zn^{2+}, Cu^{2+} and Fe^{2+}) are required in balanced proportions, for most are toxic at high doses (2). Several trace elements are involved in the cellular defense against ROS mainly in their role as cofactors of antioxidant enzymes (30). For instance, calcium is key regulator of many cellular processes including cell signaling and proliferation, metabolism, muscle contraction and bone formation and mineralization (46). Zinc is known to regulate the expression in lymphocytes of metallothionein that have antioxidant activity (35). Magnesium, an ubiquitous element that plays a fundamental role in many cellular reactions, is involved in >300 enzymatic reactions in which food is catabolized and new chemical products are formed (3). The presence of these mineral elements could thus indicate that this plant could be useful in the management of diseases (1).

Vitamins are compounds that cannot be synthesized by humans and thus need to be taken up in the diet. They have a complex biochemistry and play an essential role in human nutrition and health. Vitamin deficiencies cause diseases that can be severe and even lethal in some cases. The antioxidant vitamins that have been the focus of most attention in plants are carotenoids (pro-vitamin A), ascorbate (vitamin C) and tocochromanols (vitamin E, including both tocopherols and tocotrienols) (4).

In this study, the antioxidant properties of *U. dioica* L. was evaluated by determing 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging activity, reducing power and phenolic compounds. Their mineral, vitamin and phenolic contents compositions were determined.

MATERIALS AND METHODS

Collection of Plant Material

U. dioica L. were collected at flowering stage from different regions of Tunceli, Turkey.

Location of the sampling area is listed below:

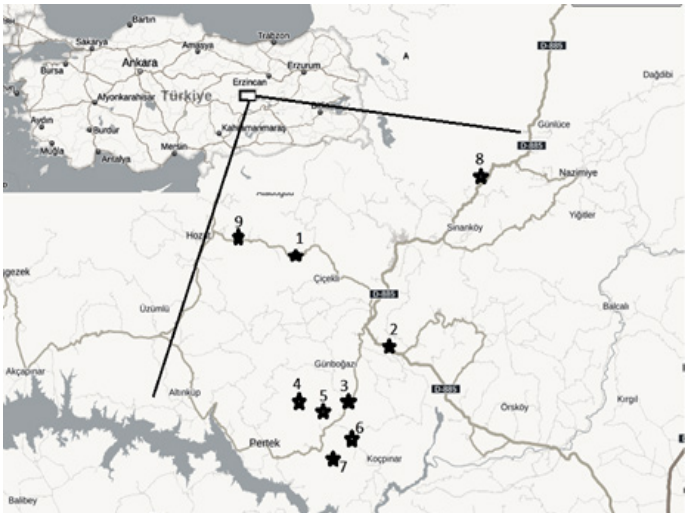

Figure 1. Map of the sampling locations.
1. station: Çiçekli parting of the ways 18 km-Demirkapı; 2. station: Mazgirt parting of the ways 7.5 km; 3. station: Beydamı location 1; 4. station: Beydamı location 2; 5. station: Beydamı location 3; 6. station: Beydamı location 4; 7. station: Beydamı location 5; 8. station: Pulumur parting of the ways 15.4 km; 9. station: Çiçekli parting of the ways 31.1 km (Dervişcemal village)

Preparation of the Extracts

The aerial parts of the plant samples (2 g) were extracted with 20 mL methanol (MeOH). The organic solvents were evaporated to dryness under vacuum at low temperature using a rotary 1649 evaporator. The dried extracts were dissolved in methanol to a final concentration of 25 mg mL^{-1} andused as such for the phenolic compounds and antioxidant testing (43).

Determination of Total Phenolic Content

Singleton et al. (39) method, using Folin-Ciocalteu reagent, was used to determine the total phenolic content. Each plant extract was prepared at a concentration of 1 mg mL^{-1}. The absorbances of all samples were measured at 760 nm against a methanol blank using a spectrophotometer (Shimadzu UV 1800). The standard calibration curve was plotted using gallic acid. The mean of three readings was used and the results expressed as g of Gallic Acid Equivalents (GAE) per 100 g of lyophilised extract (39).

Scavenging Effect on 2,2-diphenyl-1- picrylhydrazyl

The free radical scavenging activity of the mushroom extracts was measured and compared with the activity of butylated hydroxy anisole (BHA) for radical-scavenging ability using the stable radical DPPH (6). The free-radical scavenging activities of extracts and BHA (used as a standard) were measured by decrease in the absorbance of methanol solution of DPPH. The 0.1 mM solution of DPPH in methanol was prepared and 0.5 mL of this solution was added to 1.5 mL of extract solution in methanol at concentrations 100 µg mL^{-1}. Thirty minutes later, the absorbance was measured at 517 nm. Percent inhibition after 30 min was plotted against concentration, and the equation for the line was used to obtain the IC50 value. A lower IC50 value indicates greater antioxidant activity. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ Radical scavenging activity} = ([A_0 - A_1]\, A_0) \times 100$$

where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of the sample and standards.

Reducing power activity assay

Reducing power of nettles were determined by method of Oyaizu (33). Extract of nettle (100 µg mL^{-1}) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1%). The mixture was incubated at 50 °C for 20 min. Aliquots (2.5 mL) of trichloroacetic acid (10%) were added to the mixture and then the mixture was centrifuged at 13.400 g for 5 min. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm using a spectrophotometer. Increased absorbance of the reaction mixture indicates an increase of reduction capability.

HPLC Analysis of Phenolic Component

2 g of dried nettle was taken and 20 mL of methanol was added. The mixture was centrifuged and the supernatant was filtered through 0.45 µm syringe filter and analyzed by HPLC. The blank solutions were carried out in the same way.

The analyses of kaempferol, rutin, resveratrol and catechin component in nettle samples were done by HPLC. The HPLC system used was Shimadzu Prominence HPLC, equipped with a degasser DGU-20A5, a binary pump LC-20AT, an autosampler SIL-20AHT, a column oven CTO-10ASVP and a diode array detector SPD-M20A. The column used was a Kromasil 100-5C18 (150x4.6 mm, 5µm), operated at 35 °C. An isocratic mode was used and mobile phase was 1% acetic acid in methanol/water/acetonitrile (46:46:8 v/v/v). The flow rate was set to 1 mL/min. The injected volume was 20 µL. Identification and quantitative analysis were done by comparison with standards. HPLC-DAD analysis was carried out in the range between 200 and 500 nm, setting the detector at 265 nm for identification of kaempferol, at 254 nm for rutin, at 306 nm for resveratrol and at 280 nm for catechin.

Mineral Content Analysis

3 g of dried nettle was taken and put into ash furnace. Samples were hold on at 480 °C until obtained white ash. 2 mL concentrated HNO_3 was added to ashes and heated to dryness. This process was repeated once more. Samples were taken final volume with 20 mL 1M HNO_3 after samples cooled. Samples were centrifuged and clear solutions were analyzed by ICP-OES. The blank solutions were carried out in the same way.

Vitamin A

2 g of dried nettle was taken, 20 mL of hexan/isopropanol (3:2) added and centrifuged. 1 mL of solution was taken from the upper phase and 5 mL methanolic KOH

was added. 5 mL distilled water and 10 mL of hexan/isopropanol (3:2) were added, then this solution was incubated at 85 ^{0}C for 30 minutes. Upper phase was taken and left to dryness. Samples were taken final volume with 1 mL acetonitrile/methanol (3:2). The supernatant was filtered through 0.45 µm syringe filter and analyzed by HPLC. The blank solutions were carried out in the same way.

Statical Analysis

SPSS v13.0 statistical software was used for statistical analysis (SPSS Inc., Chicago, IL, USA). Data was statistically analyzed for means ± standard error. Duncan's multiple range test was used to determine the differences between the groups having two parts. One-way analysis of variance was used to determine the differences between the groups having more than two parts.

RESULTS AND DISCUSSION

Table 1- shows the scavenging activity of the DPPH radical due to its reduction by different nettle isolated from Tunceli. The most strong DPPH radical scavenging activity was found in the nettle isolated from Mazgirt parting of the ways 7.5 km.

Total phenolics concentration, expressed as mg of GAEs/g of dry netle, ranged from 37.419 ± 0.380 to 19.182 ± 1.26; the highest value was obtained for Beydamı location 1. The lowest concentration was obtained for *Urtica dioica* L. from 2.stations (19.182±1.26).

In our study, the highest reducing power was found in *Urtica dioica* L. collected from Mazgirt parting of the ways 7.5 km (0.566±0.020) (Figure 2).

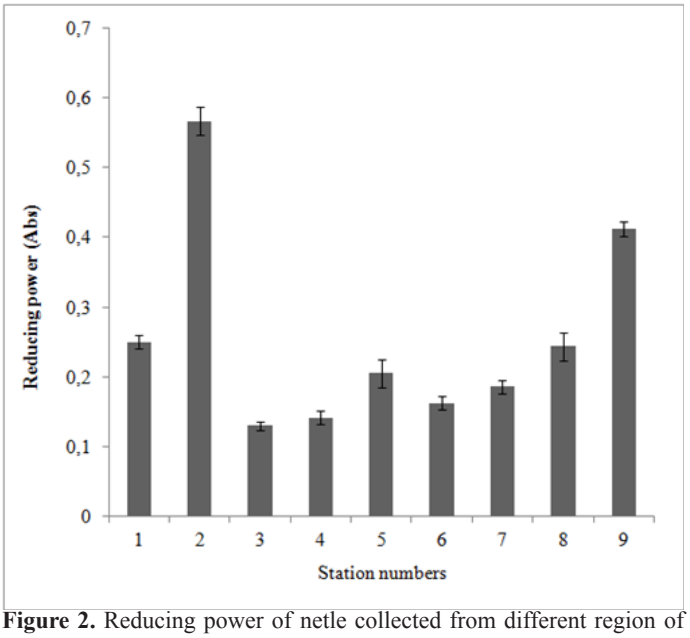

Figure 2. Reducing power of netle collected from different region of Tunceli.

Table 1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and Total phenolic contents (TPC) of nettle collected from different region of Tunceli (Turkey).

Stations	Scavenging activity of DPPH radical IC50 value (mg mL^{-1})	Total phenolics mg of GAEs g^{-1} of dry nettle
1	12.93±0.724^{c}	25.498±0.322^{d}
2	33.70±0.849^{a}	19.182±1.260^{e}
3	12.64±0.343^{d}	37.419±0.380^{a}
4	22.64±2.264^{b}	35.055±1.630^{ab}
5	12.48±0.466^{c}	31.875±0.242^{cb}
6	32.63±0.609^{a}	35.933±0.089^{a}
7	13.48±0.339^{c}	28.740±1.757^{dc}
8	12.96±0.700^{c}	32.117±2.067^{bc}
9	12.64±0.245^{c}	19.756±0.558^{e}

The results are expressed as mean ± SE (n = 3). In each column different letters mean significant differences between results ($p < 0.05$). * Total phenolics; mg of GAEs g^{-1} of dry netle.

Table 2. Concentration of phenolic compounds and vitamin found in nine edible nettle. Results are expressed as mg of phenolics per kg of dried nettle.

Number of Stations	Vit-A (mg kg^{-1})	Kampferol (mg kg^{-1})	Rutin (mg kg^{-1})	Resveratrol (mg kg^{-1})
1	8.69±2.30	-	1550.60±128.40	-
2	5.93±1.80	5.20±1.30	471.20±53.20	-
3	7.88±1.70	-	251.60±29.60	-
4	9.13±2.00	-	364.60±43.40	10.80±0.80
5	6.60±1.40	3.00±0.40	184.60±19.90	67.20±7.10
6	7.95±1.90	-	186.70±16.40	-
7	5.74±1.00	11.30±1.90	647.10±73.30	13.00±2.00
8	8.01±2.10	-	450.30±29.80	14.40±2.30
9	13.64±1.90	-	2425.60±226.60	6.40±0.50

The results are expressed as mean ± SE (n = 3).

Table II- shows concentration of phenolic compounds and vitamin A found in nine edible nettles. According to the results, vitamin A concentrations (mg/kg dry weight) in samples ranged between 13.64±1.90 (Dervişcemal village) and 5.74±1.00 (Beydamı location 5). (Table II).

The highest kampferol level was found in Beydamı location 5 (11.30±1.90). Rutin was found in all samples. The higest rutin concentration was found in the samples collected from Demirkapı region (2425.60±226.60). Resveratrol concentrations (mg kg^{-1} dry weight) in samples were ranged between 67.20±7.10 (Beydamı location 3) and 6.40±0.50 (Derviscemal village). There were any trace of kaempferol, resveratrol in samples from Demirkapı region, Beydamı location 2 and Beydamı location 4. However rutin was detected in these samples. There was not resveratrol, but there were kaempferol and rutin in the samples from station 2 (Table II).

Concentration of phenolic compounds and vitamin A found in nine edible nettle is shown in Table III. The highest sodium concentration was found in Beydamı location 3 (301.00±26.70). The highest potassium (K) concentration was in Mazgirt parting of the ways 7.5. km (16262.50±1002.20). The phosphate (P) concentrations (mg kg^{-1}dry weight) in samples were ranged between 5123.30±4145.20 (Beydamı location 1) and 2411.30±297.70 (Dervişcemal village). The highest magnesium concentration (mg kg^{-1}dry weight) was found in Pülümür parting of the ways 15.4 km (2328.30±198.70). The calcium concentrations (mg kg^{-1}dry weight) in samples were ranged between 11512.20±1206.80 (Pulumur parting of the ways 15.4 km) and 9433.3±755.7 (Beydamı location 2). Manganese concentrations (mg kg^{-1}dry weight) in samples were ranged between 28.80±3.90 (Mazgirt parting of the ways 7.5 km) and 14.00±1.80 (Beydamı location 3). The highest copper concentration (mg kg^{-1}dry weight) was 6.90±0.70 (Beydamı location 2). The zinc concentrations (mg kg^{-1}dry weight) varied between 28.20±1.60 (Beydamı location 2) and 14.80±1.10 (Mazgirt parting of the ways 7.5 km).

Synthetic antioxidants have long been used, but their use has recently come into dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by consumers. There is, therefore, a growing interest in the identification of new, natural antioxidants that would serve as alternatives to the synthetic compounds (24). Nettle (*Urtica dioica* L.) is both an annual and perennial herb, distinguished with stinging hairs. Among *Urtica* species, *U. dioica* and *U. urens* have already been known and therefore consumed for a long time as medicinal plants in many parts of the world. The isolated major flavonoid glycosides have been determined to be immune stimulatory, anticarcinogenic, anti inflammatory, antioxidant and antiallergenic activities (9).

It has been suggested that antioxidants found in large quantities in fruits and vegetables protect the oxidative stress related diseases. Generally food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions (23). Estimation of total phenolic content and DPPH stable free radical method are easy, rapid and sensitive method to evaluate the antioxidant activity of a specific compound or plant extracts (28). DPPH is one of the compounds that possess a proton free radical and shows a maximum absorption at 517 nm. When DPPH encounter proton radical scavengers, its purple colour fades rapidly. This assay determines the scavenging of stable radical species of DPPH by antioxidants (10). The Folin–Ciocalteu method is the screening of natural antioxidants, being considered the best method for determination of total phenolic content (41). The ferric ions (Fe^{3+}) reducing antioxidant power (FRAP) method was used to measure the reducing capacity of nettle. The FRAP method is based on a redox reaction in which an easily reduced oxidant (Fe^{3+}) is used in stoichiometric excess and antioxidants act as reductants (5). Increased absorbance of the reaction mixture indicates an increase of reduction capability (19). In a study, total phenolic content of nettle tea bag which includes all parts of nettle (root, stalk, and leaves) was 2.5 mg GAE/g dry matter (32). In the reducing power assay, the presence of antioxidants in the samples would result in the reducing of Fe^{3+}-Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (13). Increasing absorbance at 700 nm indicates an increase in reductive ability. It should be noted that phenolic compounds act more effectively as hydrogen suppliers and therefore they act as an effective antioxidant (17). Gulcin et al. (18), reported that nettle had a powerful antioxidant activity against free radical $DPPH^{\cdot}$, superoxide anion, hydrogen peroxide and showed metal chelating activity. Similarly, Toldy et al. (42) reported that nettle supplementation reduced the free radical concentration and increased the DNA binding of AP-1 in the brain of Wistar rats (36). In our results, the total phenol varied from 37.419±0.380 to 19.182±1.260 mg of GAEs g^{-1} of dry nettle. The highest radical scavenging effect was observed in Mazgirt parting of the ways 7.5 km with 33.70±0.849 mg mL^{-1}. The highest reducing power was observed in Mazgirt parting of the ways 7.5 km with 0.566±0.020 *Urtica dioica* L. was found to be an effective antioxidant in different in vitro assay including reducing power, DPPH radical and total phenolic.

In general, the antioxidant activity of plant extracts is associated with specific compounds or classes of compounds, such as flavones, flavonols and proanthocyanidins in plant materials (40). Most of the antioxidant substances in plants are phenolic compounds. Phenolic substances serve as oxidation terminators by scavenging radicals to form resonance stabilized radicals (36). Many literature reports showed a simple relationship between the content of phenolic compounds and the antioxidant capacity of plant extracts (7, 37). Rutin, also called rutoside or quercetin-3-rutinoside, is a glycoside flavonoid with outstanding antioxidant properties. It is superior to vitamin E in the trolox equivalent antioxidant capacity (TEAC) assay and accumulate in several plant species. Although sometimes erroneously called vitamin P, rutin and other flavonoids are not vitamins because, despite their beneficial effects, they have not been shown to be essential for human health (34). Kaempferol is a natural flavonoid isolated from tea, mushrooms, broccoli, and other plant sources (45). Yildirim et al., (45) investigated the concentration of phenolic compounds in three wild edible mushrooms in Tunceli, Turkey. Kaempferol and catechin were not detected whereas resveratrol was found in small amounts (1.1 mg/kg) in *P. eryngii* collected from Pulumur. Rutin level was 4.4 mg kg^{-1} in *P. eryngii* collected from Pulumur and 9.4 mg kg^{-1} of dry mushroom for Ovacik. Otles et al., (32) analysed phenolic compounds of root, stalk, and leaves of nettle. The authors found any trace of gallic acid, syrin-

Table 3. Concentration of minerals in nine edible nettles. Results are expressed as mg of phenolics per kg of dried nettle.

Stations	Na (mg kg^{-1})	K (mg kg^{-1})	P (mg kg^{-1})	Mg (mg kg^{-1})	Ca (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
1	84.70±7.20	15109.20±970.10	3713.40±232.30	1661.10±125.50	10038.90±1245.90	19.70±3.20	4.60±0.80	19.40±1.20
2	80.40±6.90	16262.50±1002.20	4337.90±336.90	2116.20±133.90	10148.50±1102.40	28.80±3.90	4.00±0.50	14.80±1.10
3	242.40±19.90	15526.30±790.60	5123.30±4145.20	2144.50±178.90	10061.80±995.70	26.40±4.40	3.10±0.20	28.00±1.90
4	222.90±21.20	14529.20±669.30	4268.40±361.80	2170.00±150.20	9433.30±755.70	21.80±2.90	6.90±0.70	28.20±1.60
5	301.00±26.70	15611.30±776.30	4653.00±3128.70	2251.40±145.30	10142.50±899.40	14.00±1.80	5.10±1.00	25.50±1.30
6	365.80±33.70	15104.50±869.70	4543.20±396.80	2296.00±200.60	10086.10±1002.80	21.90±2.20	3.80±0.20	23.70±1.30
7	166.30±9.90	14941.30±698.90	4754.10±301.60	2270.80±206.80	10084.00±884.90	18.10±2.10	10.00±0.90	26.10±2.00
8	44.90±3.50	16045.70±1102.50	2971.70±254.80	2328.30±198.70	11512.20±1206.80	22.20±1.80	4.30±0.30	19.10±2.20
9	25.10±2.10	13649.50±774.60	2411.30±297.70	1645.50±123.50	10086.70±956.20	24.10±2.00	5.10±0.30	22.80±2.00

The results are expressed as mean ± SE (n = 3).

gic, fumaric, vanillic, isorhamnetin, catechin, caffeic, and chlorogenic acid in the root samples, but there were rutin, ellagic acid, ferulic, and naringin were detected. In the live samples, there were no trace of gallic acid, fumaric, and catechin, however, myricetin, quercetin, rutin, ellagic, caffeic, and chlorogenic acid were detected. In stalk samples there were any gallic acid, vanillic, and catechin, however, myricetin, isorhamnetin, ferulic and naringin were detected. Our results show any trace of kampferol and resveratrol in samples from Demirkapı region, Beydamı location 2 and Beydamı location 4. However rutin was detected in these samples. From 2 station in samples kampferol and rutin were detected but not resveratrol.

Chahardehi et al., (10) suggests that *Urtica dioica* as a potential source of natural antioxidants. They suggests that phenolic compounds do not make a major contribution to the antioxidant activity of the extracts. There were no correlation between the antioxidant activity and total phenolic contents. The presence of non-phenolic antioxidants such as vitamin C and A and β-Carotene are also effective in antioxidant power (12). The results from this study show that nettle, as other medicinal plants, provides an elevated vitamin A content.

In recent years, there has been a growing interest in trace element concentrations in the environment and they are considered a factor indispensable for its proper functioning. These elements are present in enzymes and activate them, thereby in an essential way influencing the biochemical process in cells (31). Fruits and vegetables are safe and valuable sources of minerals (29). Na^+ and K^+ take part in ionic balance of the human body and maintain tissue excitability, carry normal muscle contraction, help in formation of gastric juicein stomach (8). Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus (21). In our study, among the various macronutrients estimated in the plant samples of different wild edible nettle potassium was present in the highest quantity (16262.50±1002.20 mg kg^{-1}) followed by calcium (11512.20±1206.80 mg kg^{-1}) and phosphate (5123.30±4145.20 mg kg^{-1}).

Wild nettle types were sampled from different geographical regions in Tunceli, Turkey exhibited different levels of the trace element content, vitamin A, antioxidant activities and phenolic compound contents. *Urtica dioica* L. was found to be an effective antioxidant in different in vitro assay including reducing power, DPPH radical and total phenolic. Our study suggested that *Urtica dioica* L. could be considered as a natural alternative source for food, pharmacology and medicine sectors.

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