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Assessment of wild mint from Tunceli as source of bioactive compounds, and its antioxidant Activity

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Abstract

The types of wild mint (*Mentha spicata* L.) were sampled from different geographical regions in Tunceli (Turkey) in order to find out their vitamin, mineral, phenolic contents and their antioxidant properties. The total phenol varied from 77.7±0.242 to 52.34±0.351 mg of GAEs/g of dry mint. The highest radical effect of scavenging was observed in Mazgirt parting of the ways 7.5 km with 6.17±0.245 mg/mL. The highest reducing power and metal chelating were observed in the mint from Cicekli parting of the ways 6.5 km Demirkapı. Among the various macronutrients which were estimated in the plant samples, potassium was presented in the highest quantity followed by calcium and phosphate. Although rutin and resveratrol were not determined in any samples, kaempferol and catechin levels were found out in almost all samples. The concentrations of vitamin A ranged between 42,14±5.70 and 13.61±3.00 (mg/kg dry weight). These results show that plants of mint are quite rich in phenolic compounds, and these have been appeared to have antioxidant activity, which agrees with this work, since the extract showed a higher content of phenolic compounds and higher antioxidant activity and mint may be considered as a natural alternative source for food, pharmacology and medicine sectors.

Key words: *Mentha spicata*, antioxidant activity, phenolic content, elemental composition, vitamin.

Introduction

Many plants are used as spices to enhance food flavor and are consumed in small quantities, contributing in low levels to the nutritional value of the diet. However, as they are secondary metabolism compounds that may have pharmacological activity. There is currently a growing interest in plant extracts as sources of antimicrobial and antioxidant compounds as a means of avoiding potential problems caused by excessive consumption of synthetic additives (1).

Antioxidant supplements or foods that contain antioxidant may be leveraged to help the human body reduce oxidative harm or protect the quality of food by preventing oxidative decay (2). Natural plant antioxidants can therefore serve as a type of alternative medicine. A lot of plants such as *Mentha spicata* L. subsp. *spicata* (mint) is a good source of antioxidants.

The genus *Mentha* (Lamiaceae) is represented by roughly 30 species which grow in temperate regions of Australia, Eurasia and South Africa (3, 4). The genus which has great economic importance in the world because of the cultivation of mint oil from tropical to temperate climates of America, Europe, China and Brazil. The different herbal and food products from Mentha species have been in use since ancient times for the treatment of nausea, irritable bowel syndrome, gall-bladder and bile ducts, herpes, heart burns, indigestion, colic, flatulence, coughs and flu as well as certain skin infections including acne and pigmentation (5, 6, 7) *Mentha spicata* L. (spearmint) is a creeping glabrous, rhizomatous and perennial herb which has a strong aromatic odour. The species have been proved to be useful as digestive and gastro-stimulant (8). *M. spicata* has two subspecies which grow in Turkey and there was not any report on the phenolic profile of *M. spicata* subsp. *spicata*.

Studies on plants from different regions resulted in the innovation of biologically active substances. Therefore the study was conducted in order to investigate the chemical composition and antioxidant activities of extracts from *M. spicata* native from different regions of Tunceli.

Materials and Methods

Collection of Plant Material

The samples of *Mentha spicata* L. were collected at flowering stage from different regions of district of Tunceli, Turkey. Location of the sampling area is presented below (figure 1).

Figure 1. Map of the sampling locations (1 station: Cicekli parting of the ways 18 km-Demirkapi; 2 station: Cicekli parting of the ways 65 km-Demirkapi; 3 station: Mazgirt parting of the ways; 4 station: Mazgirt parting of the ways 4 km; 5 station: Mazgirt parting of the ways 73 km; 6 station: Ovacik parting of the ways 5,1; 7 station: Ovacik parting of the ways 92 km; 8 station: Ovacik parting of the ways 41 Km; 9 station: Pertek parting of the ways 11 km; 10 station: Pulumur parting of the ways 154 km.).

Preparation of the Extracts

The plant samples' aerial parts (2 g) were taken out with 20 ml methanol (MeOH). The organic solvents were vaporised 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 and were used as the phenolic compounds and antioxidant testing (9).

Determination of Total Phenolic Content

The Singleton & Rossi (10) method, using Folin-Ciocalteu reagent, was used in order to find out the total phenolic content.

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

The free radical scavenging activity of the *M. spicata* extracts were measured. It was also compared with the butylated hydroxy anisol (BHA) activity for radical-scavenging ability by using the stable radical DPPH (10).

Reducing power activity assay

Reducing power of mint was determined by using Oyaizu's method (12). Increased absorbance of the reaction mixture indicates an increase of reduction capability.

HPLC Analysis of Phenolic Component

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

Mineral Content Analysis

3 g of dried *M. spicata* was taken and put into ash furnace. Samples were hold on at 480 ºC 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 *M. spicata* was taken, 20 mL of hexan/isopropanol (3:2) added and centrifuged. 1 mL of solution wastaken 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 ºC 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 13.0 statistical software was used for statistical analysis (SPSS Inc.). Data was statistically analysed for means ± standard error. Duncan's multiple range test was used in order 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

Table 1 shows the scavenging activity of the DPPH radical because of its reduction by different *M. spicata* which are isolated from Tunceli. The strongest radical scavenging activity of DPPH was found in the *M. spicata* collected from Pertek parting of the ways 11. km. Total phenolics concentration, which is expressed as mg of GAEs/g of dry mint, ranged from 77.7±0.242 to 52.34±0.351; the highest value was obtained for Maz-

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

Stations	Scavenging activity of DPPH radical IC50 value (mg/mL) *	Total phenolics mg of GAEs/g of dry mint
1	791±0541^{a}	7367±0599
2	81±0724^{c}	7496±0322^{d}
3	937±0849^{a}	7567±1260^{e}
4	821±0343^{d}	7738±0380^{a}
5	1067±0466^{c}	777±0242^{cb}
6	726±0609^{a}	7532±0189^{a}
7	714±0347	7224±0164
8	624±0700^{c}	616±2067^{bc}
9	617±0245^{c}	576±0558^{e}
10	767±0145^{a}	5234±0351^{e}

*The results are expressed as mean ± SE (n = 3) In each column different letters mean significant differences between results ($p < 005$).

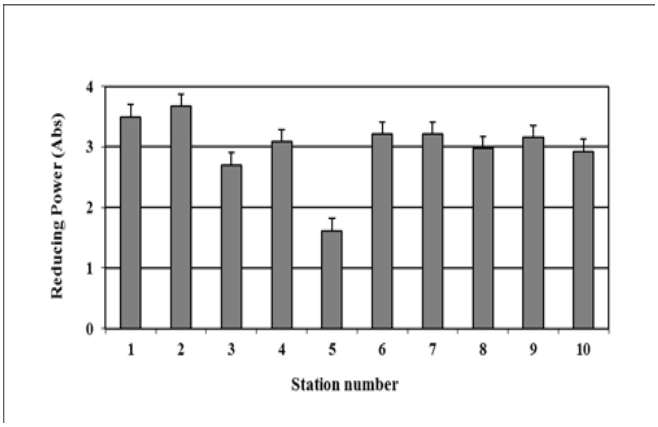

Figure 2. Reducing power of mint collected from different region of Tunceli.

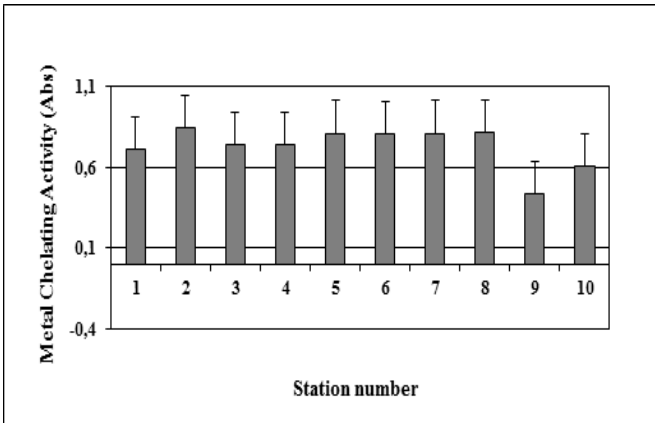

Figure 3. Metal chelating Activity of mint collected from different region of Tunceli.

girt parting of the ways 4 km. The lowest concentration was obtained for *M. spicata* L. from 9. stations (52.34±0.351).

Also in our study, the highest reducing power and metal chelating activity was found in mint collected from Cicekli parting of the ways 6.5 km Demirkapi (Reducing power: 3,676±0.520; metal chelating activity: 0.848±0.5) (Figure 2, Figure 3).

These results showed us that plants of mint are very rich in phenolic compounds, and these have been shown to have antioxidant activity, which agrees with this work, since the extract showed a higher content of phenolic compounds and higher antioxidant activity.

Table 2 shows concentration of phenolic compounds and vitamin A which was found in ten edible mint types. According to the results, Vitamin A concentrations (mg/kg dry weight) in samples ranged between 42.14±5.70 (Mazgirt parting of the ways) and 13.61±3.00 (Mazgirt parting of the ways 4 km).

The highest kampferol level was found in Mazgirt parting of the ways 7.3 km (24.6±0.40). The highest rutin concentration was found in the samples collected from Ovacik parting of the ways 41 km (2529.9±73.30). Resveratrol concentrations (mg/kg dry weight) in samples were ranged between 44.6±2.45 (Cicekli parting of the ways 6.5 km- Demirkapi) and 1173.0±0.80 (Mazgirt parting of the ways 4 km). Also Catechin concentrations (mg/kg dry weight) in samples were ranged between 2834.6±142.1 (Pulumur parting of the ways 15.4 km-Demirkapi) and 11341.3±123.1 (Ovacik parting of the ways 41 km) (Table 2).

Concentration of mineral found in nine edible mint is shown in Table 3. The highest sodium concentrations was found in Mazgirt parting of the ways 7.3 km (270.4±9.52). The highest potassium (K) concentrations were in Pulumur parting of the ways 15.4 km (19224.7±964.6). The phosphate (P) concentrations (mg kg^{-1}dry weight) in samples were ranged between 4550.01±192.4 (Mazgirt parting of the ways) and 1484.0±124.4 (Pertek parting of the ways 11 km). The highest magnesium concentrations (mg kg^{-1}dry weight) was found in Pertek parting of the ways 11 km (2684.0±196.3). The calcium concentrations (mg kg^{-1}dry weight) in samples were ranged between 11659.0±231.6 (Ovacik parting of the ways 9.2 km) and 7134.8±310.5 (Mazgirt parting of the ways 4 km). Manganese concentrations (mg kg-1dry weight) in samples were ranged between 79.5±8.1 (Ovacik parting of the ways 9.2 km) and 26.92±2.8 (Cicekli parting of the ways 18 km). The highest copper concentrations (mg kg-1dry weight) was 12.6±3.7 (Ovacik parting of the ways 9.2 km). The zinc concentrations (mg kg^{-1}dry weight) varied between 44.2±5.8 (Mazgirt parting of the ways) and 16.7±2.6 (Mazgirt parting of the ways 4 km).

Discussion

Although synthetic antioxidants have long been used, their use has recently been in dispute due to a suspected carcinogenic potential and the general rejection of synthetic food additives by consumers. There is, thus, a growing interest in the search of new, natural antioxidants that would serve as alternative to the synthetic

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

Number of Stations	Vit-A (mg/kg)	Kampferol (mg/kg)	Rutin (mg/kg)	Resveratrol (mg/kg)	Catachin (mg/kg)
1	4107±430	-	-	-	-
2	3263±780	99±131	114320±5320	446±245	44808±16424
3	4214±570	101 ±01	-	4596±2401	38678±15213
4	1361±28	107±014	11604±4340	11730±080	43282±13842
5	2595±640	246±040	9901±1990	5128±3710	34261±1755
6	2741±890	116±0,12	1672±1640	5172±412	43008±14656
7	3230±521	217±024	-	-	33690±13874
8	3107±85	11±007	25299±7330	-	113413±1231
9	251±710	10±008	13090±2980	-	69606±28647
10	1721±490	81±009	16159±2266	-	28346±1421

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

Table 3. Concentration (mg kg^{-1}) of minerals in ten edible mint.

Station	Na	K	P	Mg	Ca	Mn	Fe	Cu	Zn
1	745±621	141883±4752	35018±1876	16276±1864	75668±4526	2692±28	1578±98	518±03	2434±49
2	991±529	134461±5344	19223±2213	15240±1465	81327±3995	341±64	1692±118	79±154	233±32
3	1401±634	144118±4814	455001±1924	20467±2418	73947±2948	434±49	1002±214	68±09	442±58
4	888±498	138985±6529	26525±2334	21566±1952	71348±3105	47375±	1003±209	84±15	167±26
5	2704±952	141060±8658	36130±3168	23822±1876	80326±2415	378±28	946±3856	52±08	183±29
6	522±631	156606±6842	36208±4012	20505±2438	96039±1687	489±61	1765±445	100±18	278±37
7	1156±712	163527±8562	42971±3545	23007±1187	116590±2316	795±81	11581±967	126±37	248±18
8	811±701	172720±5975	35041±2516	25790±2178	102780±6454	299±19	847±314	99±17	394±31
9	999±536	171514±8987	14840±1244	26840±1963	105522±5727	723±63	3262±469	97±26	276±26
10	483±289	192247±9646	26033±2381	21312±1642	99159±1768	360±41	2080±372	115±18	267±11

compounds (13). It is reported by Kizil *et al*. (14) that mineral analysis of mint species show that Ca content ranged from 4396 to 12150 mg kg^{-1}, Cd from 0.210 to 0.220 mg kg^{-1}, Cr from 1585 to 5410 mg kg^{-1}, Cu from 1.76 to 11.52 mg kg^{-1}, Fe from 0 to 531.5 mg kg^{-1}, Mn from 2.37 to 70.82 mg kg^{-1}, Se from 0 to 4.65 mg kg^{-1}, and Zn from 0 to 12.64 mg kg^{-1}. Geographical origin of plants which belong to the same species may result in different concentrations of elements and their bioavailability depending on soil features and environmental pollution (15). The World Health Organization states maximum permissible levels in raw plant materials for cadmium as 0.3 mg kg^{-1}, for chromium as 2 mg kg^{-1}, and for copper as 20 mg kg^{-1} (15, 16). A study showed that Cd, Cr, Cu content of mint species were lower than that of recommended by World Health Organization (16). Mint tea is widely used as herbal tea; and thus, mineral content of its herbs can satisfy daily elemental mineral demand of human body when it is consumed as herbal tea (14).

It has been suggested that antioxidants found in large quantities in fruit and vegetables protect the oxidative stress which are related to diseases. Generally food antioxidants act as reducing agents, reversing oxidation by donating electrons and hydrogen ions (17). The effectiveness of mint leaves, a common herb used in Indian cuisine, as a natural antioxidant for radiation-processed lamb meat was investigated. Mint extract (ME) had good total phenolic and flavonoid contents. It exhibited excellent antioxidant activity, as measured by β-carotene bleaching and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays. It also showed a high superoxide- and hydroxyl-scavenging activity but low iron-chelating ability. A positive correlation was found between the reducing power and the antioxidant activity (18).

According to Tepe *et al*. (19), plants of the Lamiaceae family are very rich in phenolic compounds, and these have been shown to have antioxidant activity, which agrees with this work, since the methanol extract showed a higher content of phenolic compounds and higher antioxidant activity. Hosseinimehr et al. (20), reported that mint had a powerful antioxidant activity against free radical DPPH. They found that IC_{50} values of mint extracts for free radical scavenging activity to be 0157mg mL^{-1}. Moreover, they found 84±7 mg g^{-1} total phenolic compound of mint. Scherer *et al*. (1) also reported that *Mentha spicata* methanolic extract showed a strong antioxidant activity, according to the proposed classification (AAI = 2.08). In our results, the total phenol varied from 77.7±0.242 to 52.34±0.351 mg of GAEs/g of dry mint (Table 1). The highest radical scavenging effect was observed in Pertek parting of the ways 11. km 6.17±0.245 mg/mL (Table 1). The highest reducing power (3.676±0.520) metal chelating activity (0.848±0.5) (Figure 2, Figure 3) were observed in Cicekli parting of the ways 6.5 km Demirkapi of *M. spicata* was found to be an effective antioxidant in different in vitro assay including reducing power, metal chelating, DPPH radical and total phenolic.

Most of the antioxidant substances in plants are phenolic compounds. Phenolic substances serve as oxidation terminators by scavenging radicals to form resonance stabilized radicals (21).

In a present study was designed to evaluate the antioxidants compound and activities in *Mentha spicata* methanol extracts. Results on the total phenolics (12.±1.22 mg GA/ 100 g DW) and total flavonoids (1.61±0.12 mg CE /100 g DW) contents in the methanol extracts of *M. spicata* showed significant difference ($p<0.05$). Free radical scavenging activity of *Mentha spicata* was found high (22).

In a similar study showed that the total phenol of nettles 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) (23).

There has been a growing interest in trace element concentrations in the environment and they are considered as an indispensable factor for its proper functioning. These elements are presented in enzymes and activating them, thereby is an essential way of influencing the biochemical process in cells (24). Fruit and vegetables are safe and valuable sources of minerals (25). Calcium deficiency causes rickets, back pain, osteoporosis, indigestion, irritability, premenstrual tension and cramping of the uterus (26). 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 juice in stomach (27). In our Study, among the various macronutrients estimated in the plant samples of different wild edible mint potassium was presented in the highest quantity (19224.7±964.6 mg kg^{-1}) followed by calcium (11659.0±231.6 mg kg^{-1}) and phosphate

(4550.01±192.4 mg kg^{-1}).

Phenolic compounds usually are found in the dermal tissues of the plant body. They also have a defensive chemical role against pathogens and predators. Humans cannot produce phenolic compounds and thus these compounds must be mainly taken in through the daily diet. Lamiaceae plants usually have good antioxidant capacity and activities which are closely related to the content of total phenolics (28). Dhifi et al. reported that based on the absorbance values of the extract solutions reacted with the Folin-Ciocalteu reagent and compared with the standard solutions of gallic acid equivalents (GAE), total phenolics of *M. spicata* leaves exhibited an amount of 10.38 mg GAE/g DW, which was about two times higher than the total polyphenols of roots (data not shown) (29). The data reported for total polyphenols were close to those described in similar aromatic and medicinal herbs (30).

According to Dhifi et al. (29), apigenin was detected to be the major flavonoid component in *M. spicata* leaves, showing the levels of 38.4 mg/100 g DW. Epicatechin, rutin and catachin were found with appreciable level in leaves (17.5, 15.3 and 13.6 mg/100 g DW, respectively). Naringenin value was also found 6.8 mg/100 g DW. The amounts of the detected compounds were close to those reported in similar aromatic and medicinal herbs (31). According to the results of several studies carried out, widespread plant in nature are considered to be useful agents for the prevention of diseases (32,33,34).

Wild mint 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. *Mentha spicata* L. was found to be an effective antioxidant in different in vitro assay including reducing power, metal chelating, DPPH radical scavenging and total phenolic. The present study confirmed the antioxidant activity of mint, as well. Our study suggested that *Mentha spicata* L. could be considered as a natural alternative source for food, pharmacology and medicine sectors. A further isolation and purification of *M.spicata* phenolics and volatiles will be associated with an application in food industry as antioxidants, additives for food preservation and for enhancing their nutritional value and organoleptic quality. Furthermore, a pharmacological study could be established through in vivo assays in order to discover new anticarcenogenic, anti-inflammatory, antimutagenic and other activities of *M. spicata*. Such results could be used in pharmaceutical formulations, leading to new human drugs from this medicinal plant.

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