



Investigation of Phenolic Compounds and Antioxidant Activity of *Mentha spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* (Briq.) Harley Decoction and Infusion

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Abstract: In the present study, phenolic compounds and antioxidant activity of decoction and infusion of aerial parts of *Mentha spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* (Briq.) Harley were investigated. Phenolic contents of the decoction and infusion were analyzed using LC-MS/MS. Also, the antioxidant activity of the species was determined by three methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, β -carotene linoleic acid assays, and CUPRAC assays. Flavonoids and derivatives were the most abundant components of the *M. spicata* subsp. *spicata* decoction and *M. longifolia* subsp. *typhoides* decoction and infusion, whereas coumaric acids and derivatives were found to be the most abundant components of *M. spicata* subsp. *spicata* infusion. Particularly, the main compounds were determined as follow for *M. spicata* subsp. *spicata* decoction and infusion; caffeic acid and fumaric acid. Rosmarinic acid was detected in high amounts in *M. longifolia* subsp. *typhoides* decoction and infusion. For all the activity assays, infusion and decoction of the samples showed good activity.

Keywords: *Mentha spicata* subsp. *spicata*, *Mentha longifolia* subsp. *typhoides*, antioxidant activity, phenolic compound.

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INTRODUCTION

Lamiaceae (Labiatae) family consisting of about 236 genera and 6,900 to 7,200 species distributed all over the world (1). This family have strong antioxidant properties due to their rich source of polyphenolic compounds (2, 3). The genus *Mentha* (Lamiaceae) is represented in Turkey by 6 species 4 hybrids and 13 taxa (4). *Mentha* species are used as herbal tea and folk medicine for treatment of several disorders (5, 6). *M. spicata* (eşek nanesi) is a medicinally important plant and commonly known as spearmint. Infusion and hydrolate of the aerial parts of *M. spicata* used treatment of colds and flu, respiratory tract problems, gastralgia, hemorrhoids, and stomachache (7). Also, *M. longifolia* is generally known under the name "dere nanesi" and widely used for sore throat, hemorrhoids, shortness of breath, stomachache, sunstroke, headache, cough, and menstrual pain (8).

Many studies have been conducted to investigate the chemical content and biological activities of *Mentha* species (3-6). The studies especially related to essential oil composition and biological activities (9-12). Previous studies have been reported that the extracts of different species possess phenolic content, antioxidant, anti-inflammatory and antimicrobial activities (13-15). Also, there are some reports on total phenolic content and antioxidant activity of infusion of *M. spicata* and *M. longifolia* from different region of the world and the main phenolic compounds were determined as mainly eriocitrin, luteolin-7-*O*-glucoside, rosmarinic acid, luteolin, and caffeic acid (15-23). The results showed that, since locality, climatic and seasonal conditions are effect the chemical constituents of the plants, biological activity results differ. Also previous studies have demonstrated that the phenolic content of herbal infusion is strongly correlated with their antioxidant activity (17-21). The antioxidant activity of phenolic compounds is widely due to their free radical scavenger, singlet and triplet oxygen quencher, metal chelation potential, and hydrogen donor properties (13-16). Therefore, the present study aimed to investigate comparative antioxidant activities as well as phenolic compounds of decoction and infusion of aerial parts of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* Turkey.

EXPERIMENTAL SECTION

Plant materials

The aerial parts of *M. longifolia* subsp. *typhoides* (Briq.) Harley;

Balıkesir: On the road of Çamlık-Turnacı, forest bottoms, 1310 m, 10.9.2015, (herbarium number SS 6809).

The aerial parts of *M. spicata* subsp. *spicata*;

Balıkesir: Sındırgı, Ulus Peak, 1768 m, 21.8.2015, (herbarium number SS 6701).

The species were identified by Dr. Selami Selvi at Balıkesir University. The voucher specimens were deposited at the Herbarium of the Altınoluk Vocational School, Balıkesir University, Balıkesir, Turkey.

Preparation of decoction and infusion samples

4 g of aerial parts of the plant, dried in the shade and chopped into small pieces. For infusion; 2 g of the plant were added to 98 mL of distilled boiling water and allowed to stay for 15 minute. For decoction; 2 g of the plant were added to 98 mL of distilled water and heated together in a steel kettle and allowed to stay for 15 minute after it boiled. The teas were filtered with an ashless filter paper. The filtrates were diluted with 25 mL of distilled water.

Phenolic compounds were determined by LC-MS/MS.

Chemicals

Standard compounds used for LC-MS/MS analysis were as follows: fumaric acid (99%, Sigma-Aldrich), pyrogallol (98%, Sigma-Aldrich), rutin (94%, Sigma-Aldrich), chlorogenic acid (95%, Sigma-Aldrich), gallic acid (99%, Merck), syringic acid (95%, Sigma-Aldrich), t-ferulic acid (99%, Sigma-Aldrich), caffeic acid (98%, Sigma-Aldrich), pelargonin chloride (98%, Sigma-Aldrich), quercitrin (97%, Sigma-Aldrich), salicylic acid (99%, Sigma-Aldrich), *p*-coumaric acid (98%, Sigma-Aldrich), luteolin-7-*O*-glu (99%, AppliChem), rosmarinic acid (96%, Sigma-Aldrich), apigenin (95%, Sigma-Aldrich), kaempferol (96%, Sigma-Aldrich) and isorhamnetin (98%, ExtraSynthese, Genay-France). Stock solutions were prepared as 10 mg/L in methanol. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Calibration solutions were prepared in methanol in a linear range. Dilutions were performed using automatic pipettes and glass volumetric flasks (A class). 0 mg/L curcumin solution was freshly prepared, from which 50 μ L was used as an Internal Standard (IS) in all experiments.

Liquid chromatography-mass spectrometry

LC-MS/MS experiments were performed by a Zivak® HPLC and Zivak® Tandem Gold Triple quadrupole (Istanbul, Turkey) mass spectrometry equipped with a Synergy Max C18 column (250 x 2 mm i.d., 5mm particle size). The mobile phase was composed of water (A, 0.1 % formic acid) in methanol (B, 0.1 % formic acid), the gradient programme of which was 0-1.00 minute 55 % A and 45 % B, 1.01-20.00 minutes 100 % B and finally 20.01-23.00 55 % A and 45 % B. The flow rate of the mobile phase was 0.25 mL/min, and the column temperature was set to 30 °C. The injection volume was 10 µL.

The detailed information on preparation of test solution and evaluation of uncertainty has been reported in the literature (24, 25).

Biological activity

The antioxidant activities were measured based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (24-30), β -carotene linoleic acid assays (24, 26, 30) and cupric (Cu²⁺) ion reducing power assay (CUPRAC) (24, 26, 30-32).

RESULTS AND DISCUSSION

Phenolic contents

The results of the studied phenolic compounds of decoction and infusion of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* by LC-MS/MS are shown in Table 1. All the phenolic compounds of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* decoction and infusion were classified into three groups as flavonoids and derivatives, coumaric acids and derivatives, and simple phenolics and others. Total 20 compounds, composed of 12 flavonoids and 8 phenolic acids were determined in the decoction and infusion of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides*. Caffeic acid (4126.6 mg), quercetagenin-3,6-dimethylether (2141.5 mg) and penduletin (1472.7 mg) were found to be the main phenolic compounds in *M. spicata* subsp. *spicata* decoction, whereas fumaric acid (4220.1 mg), *t*-ferulic acid (1148.7 mg), and caffeic acid (1064.1 mg) were found to be the main phenolic compounds in infusion. In *M. longifolia* subsp. *typhoides*, the main phenolic compounds for decoction were rosmarinic acid (1570.7 mg), luteolin (460.9 mg), and quercetagenin-3,6-dimethylether (420.2 mg). Rosmarinic acid, luteolin and fumaric acid (620.9; 518.2; 489.8 mg, respectively) were found to be the main compounds of *M. longifolia* subsp. *typhoides* infusion.

Flavonoids and derivatives were the dominant group (6303.8 mg) in the decoction of *M. spicata* subsp. *spicata* with quercetagenin-3,6-dimethylether, penduletin, and kaempferol. Coumaric acids and derivatives were represented with 4434.8 mg and caffeic acid (4126.6 mg)

was found to be the dominant compound in decoction *M. spicata* subsp. *spicata*. While simple phenolics and others were detected in scarce amounts (187.4 mg).

On the contrary, phenolic compounds of *M. spicata* subsp. *spicata* infusion were characterized by the presence of simple phenolics and others (4280.0 mg) and fumaric acid was found to be the major compound (4220.1 mg). Flavonoids and derivatives of infusion of *M. spicata* subsp. *spicata* were detected in scarce amounts (548.2 mg).

Flavonoids and coumaric acids derivatives were presented almost in equal amount (1771.2 and 1838.8 mg, respectively) in decoction of *M. longifolia* subsp. *typhoides* with rosmarinic acid (1570.7 mg), luteolin (460.9 mg) and quercetageitin-3,6-dimethylether (420.2 mg) were found to be the main components. In the infusion of *M. longifolia* subsp. *typhoides*, flavonoids and coumaric acids derivatives were detected in equal amount (884.3 and 937.6 mg, respectively). Rosmarinic acid (620.9 mg) was detected as main coumaric acid derivative and luteolin (518.2 mg) was detected as main flavonoid.

As a result the amount of phenolic compounds extracted in decoction of *M. spicata* subsp. *spicata* is very high comparison with other extracts. Especially amount of flavonoids were found to be higher than the other extracts. Also, the decoction extracts of both plants were found to contain more phenolic compounds than the infusions.

In the previous studies, aqueous extract of *M. spicata* var. *crispa* characterized by a high content of phenolic compounds such as eriocitrin, naringenin-gluc and rosmarinic acid and antioxidant properties were analyzed by iron reduction and chelation, 1,1-diphenyl-2-picrylhydrazyl radical and iron-ascorbate generated hydroxyl radical scavenging (15). Also, antioxidant activity and phenolic content of aqueous extracts were investigated of *M. spicata* L. from Bulgaria (21). In another study, chemical characterization such as chlorogenic acid, rosmarinic acid, salvianolic acid B and salicylic acid of aqueous extracts were investigated of *M. spicata* from Italy (9). Additionally, total phenolic and antioxidant activity of methanolic extract of different *M. spicata* and *M. longifolia* five Iranian mint accessions were investigated (19). *Dinis et.al.* (23) reported the chemical compounds (eriocitrin, eriodictyol) and acetylcholinesterase inhibitory activity of infusion of *M. spicata* from Portugal. Also, *Sytar et. al.* (20) reported the phenolic compounds and antioxidant activity of methanolic extracts of *M. spicata* L. from Slovakia. Previous studies showed that aqueous extracts of the species had a variety of phenolic contents. The variation might be concerned local, climatic and seasonal differences.

Table 1: Phenolic contents of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* decoction and infusion.

	Parent ion	Daughter ion	Collision energy (V)	<i>M. spicata</i>		<i>M. longifolia</i>	
				Decoction	Infusion	Decoction	Infusion
Flavonoids and derivatives							
pelargonin	271.2	121	34	441.7±449.0	147.4±7.5	73.9±7.5	152.9±7.8
penduletin	345.2	311	25	1472.7±149.3	90.1±9.1	55.5±5.6	-
luteolin	285	132	30	-	-	460.9±118.4	518.2±66.5
apigenin	269	151	22	218.9±17.6	57.9±4.7	51.8±4.2	85.4±6.9
isorhamnetin	315	300	15	36.4±3.2	-	6.2±0.6	-
quercetagetin-3,6-dimethylether	345.1	329.5	16	2141.5±400.9	-	420.2±78.7	43.3±8.1
luteolin-7- <i>O</i> -glucoside	447	284.5	14	206.6±21.0	41.9±2.1	77.9±7.9	32.1±1.6
luteolin-5- <i>O</i> -glucoside	447	289.5	20	283.9±18.3	13.8±0.9	244.3±15.7	13.9±0.9
kaempferol	287	152.3	30	1364.8±96.3	-	363.3±25.6	-
rutin	609	301	16	137.3±8.9	159.5±10.4	-	35.3±2.3
salvigenin	329	295.8	15	-	37.6±2.6	17.2±1.2	-
isoquercetin	463.3	300	25	-	-	-	3.2±0.9
Total (mg/kg dried herb)				6303.8	548.2	1771.2	884.3
Coumaric acids and derivatives							
caffeic acid	179	135	10	4126.6±816.6	1064.1±210.6	215.4±42.6	113.1±22.4
t-ferulic acid	193	133	15	-	1148.7±80.3	10.9±0.8	162.7±11.4
chlorogenic acid	353	191	14	308.2±42.7	102.4±14.2	27.4±3.8	26.3±3.6
rosmarinic acid	359.2	160.5	15	-	-	1570.7±120.4	620.9±47.6
<i>p</i> -coumaric acid	163.2	118.7	14	-	63.1±9.7	14.4±2.2	14.6±2.2
Total (mg/kg dried herb)				4434.8	2378.3	1838.8	937.6
Simple phenolics and others							
gallic acid	168.6	124	13	11.3±0.8	5.6±0.4	4.4±0.3	4.6±0.3
syringic acid	196.7	181.4	12	176.1±11.9	54.3±3.7	55.5±3.7	18.7±1.3
fumaric acid	115	71	8	-	4220.1±292.7	-	489.8±33.9
Total (mg/kg dried herb)				187.4	4280.0	59.9	513.1
curcumin*	369.3	176.9	20				
				10926.0	7206.5	3669.9	2335.0

* Used as internal standard

Antioxidant activity

The antioxidant activities were determined applying DPPH free radical scavenging activity, β -carotene linoleic acid assays and CUPRAC assays. Inhibition of lipid peroxidation and DPPH free radical scavenging effect were determined at 2, 5, 10, and 20 μ L. The results were given in Tables 2 and 3 and Figures 1 and 2. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standard compounds in DPPH and β -carotene linoleic acid assays. In DPPH-free radical scavenging activity assay, decoction and infusion samples of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* at all concentrations showed good activity. In the same way β -carotene linoleic acid assay had good activity results. For the CUPRAC method, decoction and infusion samples of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* had better activity than curcumin, which was used as a standard compound. *M. spicata* subsp. *spicata* samples having 2, 5, 10, 20 μ L concentrations, DPPH and β -carotene methods showed higher activities. For especially the decoction of *M. spicata* subsp. *spicata*, CUPRAC method had better activity. The results are given in the Figure 2.

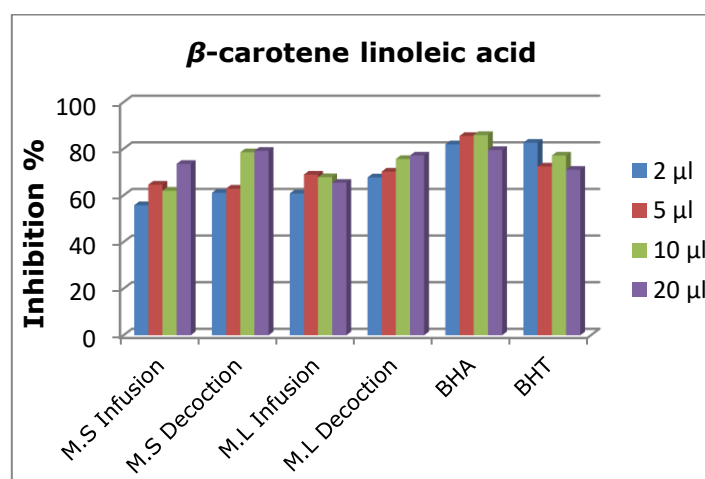
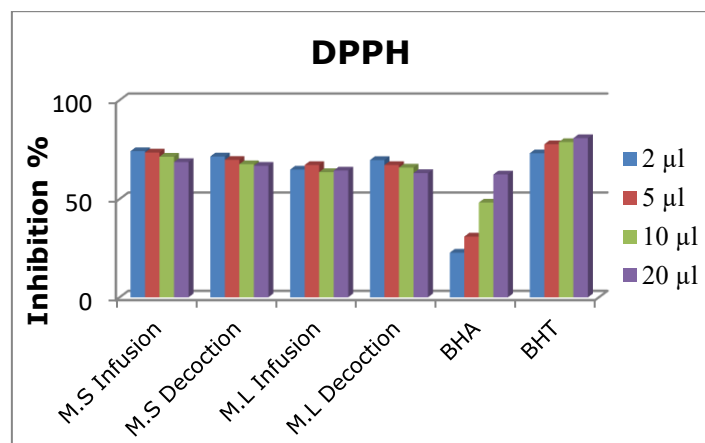
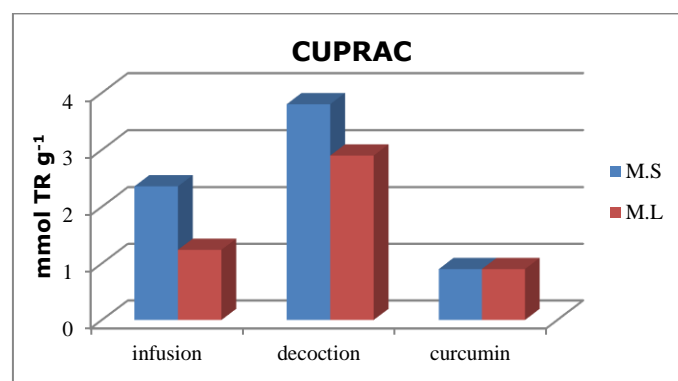
In the literature, the antioxidant activity of 50% aqueous methanol extract of *M. aquatica* L., *M. longifolia* (L.) Huds. subsp. *longifolia*, *M. longifolia* L. subsp. *typhoides* (Briq.) Harley var. *typhoides*, *M. pulegium* L., *M. spicata* L. subsp. *spicata*, *M. spicata* L. subsp. *tomentosa* (Briq.) Harley, was measured by free radical scavenging, hydrogen peroxide (H₂O₂) scavenging and metal (Fe²⁺) chelating assays (17). Also, antioxidant properties were analyzed in mint (*M. spicata*) from Malaysia (18). Our results are compatible with other studies in the literature (15, 18-22).

Table 2: Inhibition (%) of DPPH and lipid peroxidation of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides*, BHA and BHT

			2 μ L	5 μ L	10 μ L	20 μ L
DPPH	<i>M. spicata</i>	Infusion	74.2±0.2	73.5±0.5	71.4±1.2	68.7±1.6
		Decoction	71.5±1.1	69.8±2.2	67.6±2.0	66.8±2.4
	<i>M. longifolia</i>	Infusion	64.9±12.7	67.2±4.0	63.6±7.4	64.4±0.8
		Decoction	69.7±0.8	67.2±1.4	65.9±0.6	63.1±0.8
β -carotene linoleic	<i>M. spicata</i>	BHA	22.7±2.1	30.9±4.1	48.2±3.9	62.4±2.9
		BHT	73.1±2.6	77.7±0.7	78.8±0.8	80.8±1.6
		Infusion	55.8±9.9	64.6±2.4	62.0±8.6	73.5±1.4
		Decoction	61.1±5.8	62.9±4.4	78.5±4.2	79.1±2.4
	<i>M. longifolia</i>	Infusion	60.8±6.5	68.9±4.2	67.8±4.5	65.4±6.3
		Decoction	67.7±8.5	70.2±1.7	75.6±4.1	77.1±4.3
		BHA	81.9±1.9	85.5±1.7	85.9±2.4	79.5±4.1
		BHT	82.6±5.0	72.4±11.8	77.1±2.9	71.0±1.0

Table 3: Antioxidant activity of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* extracts (CUPRAC)

CUPRAC (mmol TR g ⁻¹)	<i>M. spicata</i>	<i>M. longifolia</i>
Infusion	2.36±0.09	1.24±0.13
Decoction	3.8±0.00	2.9±0.04
Curcumin	0.9	0.9

**Figure 1:** Antioxidant activities of decoction and infusion *M. spicata* subsp. *spicata* (M.S) and *M. longifolia* subsp. *typhoides* (M.L), BHA and BHT (DPPH and β-carotene linoleic acid assays).**Figure 2:** Cu²⁺ reducing power (CUPRAC) assay of the extracts and curcumin.

CONCLUSION

In conclusion, we examined and reported the main phenolic components and antioxidant activity of decoction and infusion of *M. spicata* subsp. *spicata* and *M. longifolia* subsp. *typhoides* in Turkey. *M. spicata* subsp. *spicata* was found to be the richest species in terms of phenolic compounds. Especially caffeic acid was found to be major compound in decoction and infusion of this species. The results indicated that of phenolic contents of decoction and infusion of the samples are an important factor for the antioxidant capacities. In addition, when the results compared with the literature, the content of phenolics can vary greatly, depending on the locality, climatic, and seasonal conditions. Thus, *Mentha* species is very important species which are commonly used in folk medicine, food industry, and herbal tea throughout the world.

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