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Polyphenols in northern Hungarian Mentha longifolia (L.) L. treated with ultrasonic extraction for potential oenological uses

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Abstract: The main objective of this study is to investigate three equalized batches of a wild *Mentha longifolia* (ML) population from northern Hungary for extractability by gentle ultrasonic extraction, antioxidant activity, and phenolic constituents. This is the first analytical investigation in a Hungarian population of this species. Antioxidant properties of ML extracts were evaluated with DPPH, photochemiluminescence-$O_2^{\bullet}$ scavenging assay, FRAP, and total polyphenol measurements. HPLC-DAD determination was performed for the main phenolic constituents. The extracts are medium or strong antioxidants in DPPH and FRAP assays and show strong $O_2^{\bullet}$ scavenging capacity. Rutin, quercetin, kaempferol, apigenin, naringenin, rosmarinic, vanillic, gallic, syringic, caffeic, and ferulic acids were identified. All batches were unexpectedly rutin-rich (1700–7500 mg/kg dry herb) and also were rich in rosmarinic acid. Further characteristic traits in the polyphenol profile were a lack of naringenin and (previously not reported) the presence of free kaempferol (up to 30 mg/kg dry herb).

Key words: Antioxidant, Hungary, *Mentha longifolia*, polyphenols, photochemiluminescence, ultrasound

1. Introduction

Wild mint or horsemint (*Mentha longifolia* (L.) L., syn. *Mentha sylvestris* L.) is a perennial herb belonging to the genus *Mentha* of the family Lamiaceae. The genus *Mentha* includes about 20 species with many subspecies and varieties including many medicinal and culinary herbs. These taxa can hybridize easily and thus their differentiation is relatively difficult. In recent years, morphological, cytological, and chemotaxonomic studies made the classification of *Mentha* plants more clear. The genus is indigenous in Eurasia, Africa, and Australia. The species *Mentha longifolia* (ML) is common in Eurasia, the Atlas Mountains, Egypt, the Arabian Peninsula, and South Africa.

In Linnaeus' herbarium in 1753 he determined three varieties of *Mentha spicata*: *M. spicata* var. *viridis*, var. *longifolia*, and var. *rotundifolia*. In Flora Monspeliensis he listed *Mentha longifolia*, but in the second edition of Species Plantarum in 1763 he mentioned var. *longifolia* within *Mentha sylvestris*. Today the name *Mentha longifolia* (L.) L. is accepted. ML has many subspecies and varieties, similarly to other mint plants. The subspecies *longifolia* is best known. Other subspecies, subsp. *noeana*, subsp. *grisella*, and subsp. *typhioides*, are known from Eurasia. Further subspecies are indigenous in South Africa: subsp. *capensis*, subsp. *wissii*, and subsp. *polyadena*. Other subspecies of ML varieties are also described from Central Europe. In 1893, *M. sylvestris* Linnaeus var. *globifera* Waisbecker & Borbás was determined from a sample that originated in Kőszeg (Güns), West Hungary; the specimen was deposited in the Gray Herbarium (GH) under No. GH00415377. A further variety, var. *lavanduliodora*, is grown in the botanical garden of Bratislava (Fialová and Tekeľová, 2008). In the Herbarium of Eszterházy Károly University (EGR) there are more different specimens of *M. longifolia*. The most valuable specimen was collected by M Vrabély in 1863 in the Mátra Mountains (Sass-Gyarmati and Vojtkó, 2010).

Generally, ML contains four main types of bioactive ingredients. These are flavonoids, phenolic acids (predominantly caffeic acid derivatives), essential oil based on a couple of monoterpene ketones (Hajlaoui et al., 2002; Iqbal et al., 2013; Llórens-Molina et al., 2015), and eucalyptol (Murad et al., 2016), as well as various triterpenes including phytosterols.

The most frequent caffeic acid-originated component of the plant is rosmarinic acid (Mimica-Dukić et al.,

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1999; Fialová and Tekeľová, 2008; Pereira and Cardoso, 2012; Stanislavljević et al., 2012; Benedec et al., 2013; Elansary and Mahmoud, 2015). Cinnamic acid (Mimica-Dukić et al., 1999) and caffeic and ferulic acids (Benedec et al., 2013) are abundant, but sinapic, caftaric (Benedec et al., 2013), and chlorogenic acids (Mimica-Dukić et al., 1999) and nepetoidin A and B, salvianolic acid L, and dihydrosalvianolic acid (Pereira and Cardoso, 2012) are also found. No data were found about benzoic acid derivatives, although these are present in many Lamiaceae species.

Today more than 50 flavonoids are known from ML. Three subtypes of them are present, namely flavones, flavonols, and flavanones, also in free form and glycosides. Their quantity is variable, from traces up to the 1000 mg/kg dry herb scale. Flavonoids previously described from ML are summarized in Tables 1–4 with their structural formulas and literature references.

Among the flavones, apigenin is the most frequent, in free form and also as numerous glycosides (Mimica-Dukic et al., 1999; Sharaf et al., 1999; Fialová and Tekeľová, 2008; Baris et al., 2011; Stanislavljevic et al., 2012; Benedec et al., 2013; Hawrył et al., 2015). Luteolin is also abundant. Hypolaetin was reported from a Tunisian wild population (Sharaf et al., 1999). Tricetin, its 7-O-methylether, and glycosides of them were detected in the same sample, although tricetin is a rarity in Lamiaceae plants. Regarding flavonols, less variability is observable (see Table 3). Quercetin, kaempferol, and their derivatives, e.g., rutin, are present in variable, not large quantities, particularly in Central European batches. In ML four flavanones are detected (Table 4): naringenin, eriodyctiol, hesperetin, and 4'-methoxy-naringenin. This latter was found as longitin (4'-OMe-naringenin-7-*O*-fucoparanosyl-1→6 glucoside) in a Pakistani sample (Ali et al., 2002) together with novel terpene compounds.

Therefore, the main objective of this work was to investigate a Hungarian population of wild mint for extractability, in vitro antioxidant properties, total polyphenol contents, and main phenolic antioxidants. A further aim was to establish the use of this plant as a potential component of vermouth specialties of the Eger region. The term "vermouth" here means aromatized wine made with at least one species of *Artemisia* and other unspecified plants or extracts, and the beverage has a total alcohol content between 17.5% and 22% v/v, established with the addition of ethanol from oenological origins (European Council, 1991, 2008). The benefits of wine polyphenols were observed to be various, such as a role in the prevention of plaque generation in blood vessels and cardiovascular diseases, and antiradical effects (Perez-Vizcaino et al., 2006). It is possible to enhance these effects with the addition of plant extracts and this can be regarded as the long-term goal of this study.

Table 1. Flavone aglycones previously described from ML, their substitution patterns, and references. C3 bears H in every flavone, so it is not shown in this table. The numbering of the references used in Tables 1–4 for clarity is as follows: 1) Zaidi et al., 1998; 2) Mimica-Dukic et al., 1999; 3) Sharaf et al., 1999; 4) Ghoulami et al., 2001; 5) Ali et al., 2002; 6) Ulubelen et al., 2005; 7) Fialová and Tekeľová, 2008; 8) Baris et al., 2011; 9) Orhan et al., 2012; 10) Pereira and Cardoso, 2012; 11) Stanislavljevic et al., 2012; 12) Benedec et al., 2013; 13) Elansary and Mahmoud 2015, 14) Ertaş et al., 2015; 15) Hawrył et al., 2015.

Flavone aglycones from ML									
C5	C6	C7	C8	C2'	C3'	C4'	C5'	Name(s)	Ref.
OH	H	OH	H	H	H	OH	H	5,7,4'-Trihydroxy-flavone, Apigenin	2; 7; 11; 12; 15
OH	H	OH	H	H	OH	OH	H	5,7,3',4'-tetrahydroxy-flavone, Luteolin	2; 12; 15
OH	H	OH	OH	H	H	OH	H	5,7,8,4'-tetrahydroxy-flavone, Hypolaetin	3
OH	OH	OMe	OMe	H	OMe	OH	H	5,6,4'-trihydroxy-7,8,3'-trimethoxy-flavone, Thymonin	6; 10
OH	OH	OMe	OMe	H	OMe	OMe	H	5,6,-dihydroxy-7,8,3'4'-tetramethoxy-flavone, Pebrellin	4
OH	H	OH	H	H	OH	OH	OH	5,7,3',4',5',-pentahydroxy-flavone, Tricetin	3
OH	H	OMe	H	H	OH	OH	OH	7-methoxy-tricetin, Tricetin-7-methylether	3
OH	OMe	OH	H	OMe	OMe	OH	H	5,7,4'-trihydroxy-6,2',3'-trimethoxy-flavone	4
OH	OMe	OMe	H	H	OMe	OMe	H	5-hydroxy-6,7,3',4'-tetramethoxy-flavone, Belamcanidin	14
OH	H	OH	H	H	H	OMe	H	5,7-dihydroxy-4'-methoxy-flavone, Acacetin	1
OH	OH	OMe	H	H	OMe	H	H	5,6,4'-trihydroxy-7,3'-dimethoxy-flavone	1

Table 2. Flavone glycosides previously described from ML, their substitution patterns, and references.

Flavone glycosides from ML									
C5	C6	C7	C8	C2'	C3'	C4'	C5'	Name(s)	Ref.
OH	H	O-glu	H	H	H	H	H	Apigenin-7-O-glucoside, Cosmosiin	8
OH	H	O-glc	H	H	H	H	H	Apigenin-7-O-glucuronide	8
OH	H	O-rut	H	H	H	H	H	Apigenin-7-O-rutinoside, Isorhoifolin	8
OH	H	OH	H	H	H	O-glu	H	Apigenin-4'-O-glucoside	11
O-glu	H	OH	H	H	H	OH	H	Apigenin-5-O-glucoside	11
OH	C-glu	OH	C-glu	H	H	OH	H	Apigenin-6,8-C-diglucoside, Vicenin-2	3
OH	H	O-glu	H	H	OH	OH	H	Luteolin-7-O-glucoside, Cynaroside	9; 4; 13
OH	H	O-glc	H	H	H	H	H	Luteolin-7-O-glucuronide	9
OH	H	O-rut	H	H	H	H	H	Luteolin-7-O-rutinoside, Lonicerin	9
OH	H	O-neohes	H	H	H	O-sop	H	Luteolin-7-O-neohesperoside-4'-O-sophoroside	3
O-glu	H	OH	H	H	OH	OH	H	Luteolin-5-O-glucoside, Galuteolin	11
OH	H	H	H	H	O-glu	OH	H	Luteolin-3'-O-glucoside	3
OH	C-glu	OH	H	H	H	OH	H	Luteolin-6-C-glucoside, Isoorientin	3
OH	C-glu	OH	C-glu	H	H	OH	H	Hypolaetin-6,8-C-diglucoside, Lucenin-1	3
OH	H	OH	H	H	O-glu	OH	O-rha	Tricetin-3'-O-glucoside-5'-O-rhamnoside	3
OH	H	OH	H	H	O-rha-rha	OH	OH	Tricetin-3'-O-di-rhamnoside	3
OH	H	OMe	H	H	O-glu	OH	O-rha	7-methoxy-tricetin-3'-O-glucoside-5'-O-rhamnoside	3
O-glu	H	OMe	H	H	H	OH	H	Genkwanin*-5-O-glucoside	11
O-6"-mal-glu	H	OMe	H	H	H	OH	H	Genkwanin*-5-O-[6"-O-malonyl-]-glucoside	11
OH	H	OMe	H	H	H	OH	H	Genkwanin*-4'-O-glucoside, Phegopoline	11

C3 bears H in every case. Mal = malonyl, neohes = neohesperoside. Aglycone labeled with an asterisk (genkwanin) was found to be reported only in glycosides

Table 3. Flavonol constituents previously described from ML, their substitution patterns, and references. Aglycones labeled with an asterisk were found to be reported only in glycosides.

Flavonols from ML										
C3	C5	C6	C7	C8	C2'	C3'	C4'	C5'	Name(s)	Ref.
OH	OH	H	OH	H	H	H	OH	H	Quercetin	11; 12; 13; 15
O-glu	OH	H	OH	H	H	H	OH	H	Kaempferol*-3-O-glucoside, Astragalin	11
O-soph	OH	H	OH	H	H	H	OH	H	Kaempferol*-3-O-sophoroside, Sophoraflavonoside	11
O-rha	OH	H	OH	H	H	H	OH	H	Kaempferol*-3-O-rhamnoside, Afzelin	11
OH	OH	H	O-rha	H	H	H	OH	H	Kaempferol*-7-O-rhamnoside	11
O-6"-mal-glu	OH	H	O-rha	H	H	H	OH	H	Kaempferol*-3-O-[6"-O-malonyl-]-glucoside-7-O-rhamnoside	11
O-glu	OH	H	OH	H	H	H	OH	H	Quercetin-3-O-glucoside, Isoquercitrin	12
O-rut	OH	H	OH	H	H	H	OH	H	Quercetin-3-O-rutinoside, Rutin	12; 15
O-glu	OH	H	O-glu	H	H	H	OH	H	Quercetin-3,7-O-diglucoside	11

Table 4. Flavanone constituents previously described from ML, their substitution patterns, and references. Aglycones labeled with an asterisk were found to be reported only in glycosides.

Flavanones from ML									
C5	C6	C7	C8	C2'	C3'	C4'	C5'	Name(s)	Ref.
OH	H	OH	H	H	H	OH	H	Naringenin	15
OH	H	O-rut	H	H	H	OH	H	Naringenin-7-O-rutinoside, Narirutin	15
OH	H	O-fuc-glu	H	H	OH	OMe	H	4'-methoxy-naringenin*-7-O-fucopyranosyl-1→6-glucoside, Longitin	5
OH	H	O-rut	H	H	OH	OH	H	Eryodictiol*-7-O-rutinoside, Eryocitrin	15
OH	H	O-rut	H	H	OH	OMe	H	Hesperetin*-7-O-rutinoside, Hesperidin	4

2. Materials and methods

2.1. Plant material

The investigated plant batches are listed in Table 5. They were collected and packed by Pharmaherb Ltd., Bükkszentkereszt, Hungary. Determination was done by microscopy, with a Delta Optical SZ-430 stereomicroscope (MOM, Budapest, Hungary).

2.2. Chemicals

Ethanol and methanol were obtained from VWR (Debrecen, Hungary); deionized water (G = 0.067 μS/cm) was prepared on an Elga PureLab apparatus. HPLC-grade acetonitrile and acetic acid were purchased from Sigma (Munich, Germany). Standards for HPLC measurements were as follows: Phenolic acids: gallic acid a. r. (Fluka, Munich, Germany), caffeic acid a. r. (Fluka), ferulic acid a. r. (Merck, Darmstadt, Germany), rosmarinic acid (Sigma). Flavonoids: quercetin (HPLC) (Sigma), rutin hydrate >95%, (Sigma), apigenin (HPLC) (Sigma), kaempferol (HPLC) (Fluka), naringenin (Sigma).

2.3. Extraction solvents

Four different solvents were used to compare the efficacy in extraction of plant antioxidants. Methanol (MeOH) was used in many studies (e.g., Hajlaoui et al., 2002; Murad et al., 2016), but only as a reference because the application of methanol in the food industry is prohibited. Ethanol (EtOH) is widely used for extraction of plants in industry, and an ethanol : water 70:30 mixture (WA) is used to extract *M. longifolia* phenolics (Mimica-Dukić et al., 1999; Stanislavljević et al., 2012; Benedec et al., 2013) and it is traditionally used in European medicine to make polyphenol-rich tinctures. Ethanol : water 10:90, with pH adjusted to 3.5 (AWA), served as a model of wine as an extractive medium.

2.4. Sample preparation

From each batch of dry herb 1 g was balanced on an analytical scale (Mettler Toledo) and homogenized with 50 mL of solvent by an IKA Turrax T25 Basic homogenizer in the 1st stage (6500 rpm).

2.5. Extraction

Extraction was performed with a one-stage ultrasonic bath treatment of 90 min of the samples and the bath was tempered to room temperature. The apparatus was supplied by Elma (S 60 H Elmasonic). One gram of herb was balanced on an analytical scale and homogenized with 50 mL of solvent (see Section 2.4), then treated by ultrasound for 3 × 30 min with 10 min pauses between the 30-min phases. The extract was then centrifuged (Hettich EBA 21 type, Hettich, Germany) for 5 or 10 min at 6000 rpm and the supernatant was collected with a transfer pipette. Supernatant volumes were between 44 and 47 mL and the losses on extraction were not uniform for the three batches.

2.5.1. HPLC-DAD investigation of phenolic constituents in the extracts

HPLC equipment included the Agilent 1200 Series with an Agilent Eclipse Plus C18 4.6 mm × 250 mm × 5 μm column; injected volume was 10 μL. This type of Agilent apparatus can follow a couple of fixed wavelengths, not the whole spectrum. The detection wavelengths chosen can be seen in Table 6 together with the retention time of the components.

Table 5. Plant materials and their origins.

Plant material	Herbarium #	Year	Batch #	Supplier	Origin
Mentha longifolia herb	EGR 2011/1	2011	1	Pharmaherb Ltd.	Bükk Highlands, northern Hungary
Mentha longifolia herb	EGR 2011/2	2011	2	Pharmaherb Ltd.	Bükk Highlands, northern Hungary
Mentha longifolia herb	EGR 2012/1	2012	3	Pharmaherb Ltd.	Bükk Highlands, northern Hungary

Table 6. Retention times and detection wavelengths of the identified components.

Retention time, min	Detection wavelength, nm	Component
4.68	270	Gallic acid
14.46	265	Vanillic acid
15.50	265	Syringic acid
18.35	260	Caffeic acid
30.93	320	Ferulic acid
32.90	290	Naringenin
35.67	365	Rutin
38.49	290	Rosmarinic acid
43.61	365	Quercetin
46.59	265	Apigenin
47.18	365	Kaempferol

The method is a gradient elution technique developed for the determination of plant phenolics. Eluent A was HPLC-grade acetonitrile (Sigma) and eluent B was an aqueous solution of acetic acid (0.9% v/v) with flow rate 1 mL/min. The gradient program was as follows: 0–5 min: ratio of B is 5% v/v, 5–26 min: ratio of B increased to 13% v/v, 26–40 min: ratio of B increased to 30% v/v, 40–50 min: ratio of B increased to 50% v/v, 50–53 min: ratio of B decreased to 5% v/v, 53–55 min: ratio of B held at 5% v/v. Quantification of components was based on the area measurement of a 10 mg/L solution of each standard.

2.5.2. Measurement of general radical scavenging activity by DPPH assay

The reagent was DPPH (Sigma) in ethanol, c = 56 mg/L; 0.5 mL of sample and 2.5 mL of reagent were mixed for the assay. Reaction conditions were 4 °C/30 min in the dark. Photometric measurements were performed with a Jasco V-650 apparatus at 517 nm wavelength. Results are given in the frequently used unit of effective concentration, EC_{50} mg/L. This means the concentration referring to the amount of substance reacting with 50% of the initial DPPH concentration within a given reaction time at a given temperature. Positive controls were BHT, thymol, and eugenol. In photochemiluminescence-$O_2^{-\bullet}$ scavenging (PCL) and FRAP assays these substances were used as controls.

2.6. Measurement of radical scavenging capacity against superoxide by PCL

The assay is based on the self-sensitization, radical generation, and radical absorbing reaction of luminol. The competitive reaction of luminol and the antioxidants with $O_2^{-\bullet}$ generated in situ results in a decrease of the luminescence of luminol, which is measured as a voltage signal (Prior et al., 2005) Results are given in mg trolox equivalents/kg dry herb (mg TE/kg d. h.). Measurements were performed with an Analytik Jena PHOTOCHEM device and the reagent kit for lipophilic samples (ACL Kit), which contains methanol (R1), buffer (R2), luminol reagent (R3), and a trolox standard (R4) provided by the supplier.

2.7. Investigation of reducing ability in the Fe^{2+}/Fe^{3+} system (FRAP)

Preparation of 2,4,6-tripyridyl-triazine (TPTZ, Fluka) solution was done as follows: 78.1 mg of TPTZ was added to 20 mL of water in a 25-mL volumetric flask of water, then acidified with 84 µL of cc HCl, and then completed to 25 mL. Preparation of the FRAP reagent was done as follows: to 25 mL of acetate buffer adjusted to pH 3.6 were added 2.5 mL of 20 mM $FeCl_3$ (VWR) and 2.5 mL of TPTZ solution. Sample preparation was done as follows: to 2900 µL of FRAP reagent 100 µL sample was added. Reaction time was 5 min at room temperature. Photometry was performed at 593 nm wavelength. Results are given as mg ascorbic acid equivalents/kg dry herb (mg AAE/kg d. h.), which is frequently used in FRAP assays of plant extracts.

2.8. Measurement of total polyphenol content (TPC)

TPC was determined by Folin–Ciocalteu assay, based on the protocol of Waterhouse (Waterhouse, 2002). Results are given in mg gallic acid equivalents/kg dry herb (mg GAE/kg d. h.)

2.9. Statistical examinations

Pearson-type 2-tailed correlation calculations were performed with SPSS 16.0 for Windows.

3. Results

3.1. Phenolic constituents identified in the extracts

Table 6 shows the retention times and detection wavelengths of the identified constituents.

3.1.1. Flavonoids in the investigated ML batches

Table 7 shows the quantities of the identified flavonoids.

Most prominent in the flavonoid profile of all batches is the dominance of a flavonol, namely rutin, instead of the expected apigenin. Furthermore, the four solvents show highly different efficacies in the extraction of certain flavonoids. The most prominent sign of it is the lack of rutin in AWA extracts of all batches, although rutin is present in the other types of extracts in high quantities, especially in the three extracts made with WA. Rutin can be detected in all batches on the scale of thousand milligrams/kilogram dry herb, with no difference between the two different vintages (see Table 7). Batch No. 2 was found to be the richest in rutin based on the data of its extracts. No. 3 follows it and No. 1 contains relatively the least rutin. Quercetin was also detected in all three batches; it is the flavonoid with the second highest quantity in the investigated plant material, up to 286 mg/kg d. h. Batch No. 1 was observed to be relatively richest in quercetin with regard to the data of its extracts, and No. 2 showed the lowest quercetin contents. This shows an oppositional tendency compared to rutin contents, although significant correlation between the quantities of rutin and quercetin (i.e. a glycoside and its aglycone) in the extracts was not found. The most effective extraction medium of quercetin here is WA since all of the batches had given the highest quercetin contents in their WA extracts. Kaempferol was also detected in all three batches as a minor (<100 mg/kg d. h.) component. It showed the highest quantities (around 20–30 mg / kg d. h.) in batches No. 2 and 3, without strong dependence on the solvent. Apigenin appeared in all 12 extracts without exception, alone among the investigated flavonoids. Thus, this is the only flavonoid that was effectively extracted by AWA and it showed the third highest quantity among the flavonoids identified. It is worth noting that the richest (No. 1) and least apigenin-rich (No. 2) batches were from the same vintage, namely 2011. The differences in the quantity of an important flavonoid of the species in one vintage suggest differences of maturity or technological handling. Naringenin was absent in batches No. 1 and 2 and appeared only as traces in batch No. 3.

3.1.2. Phenolic acids in the investigated ML batches

Table 8 shows the quantities of phenolic acid in ML.

Rosmarinic acid is the dominant representative of the phenolic acids in all of the batches. Its presence is expected, based on data of *Mentha* spp. (Pereira and Cardoso, 2012). The most effective medium to gain extracts rich in the useful antioxidant rosmarinic acid is MeOH, and the second in this sequence is WA (see Table 8). AWA extracts show quantities of rosmarinic acid two orders lower than the extracts made with the three other solvents. Caffeic acid was detected in No. 2 and 3. A survey of the phenolic acid profile of the investigated samples showed that batch No. 1 represents the lowest quality from the

Table 7. Flavonoid components in the samples (mg/kg d. h.). *ND = not detected.

Batch #	Solvent	Components, mg/kg d. h.				
		Rutin	Apigenin	Quercetin	Kaempferol	Naringenin
1	MeOH	3910	144.2	<3	10.34	ND*
2	MeOH	5567	16.41	27.48	ND	ND
3	MeOH	6500	54.39	52.39	20.84	ND
1	EtOH	1858	167.2	155.3	5.567	ND
2	EtOH	5234	19.68	28.93	20.84	ND
3	EtOH	1770	83.81	84.68	15.08	ND
1	WA	5108	125.2	286.4	5.548	ND
2	WA	7571	30.17	134.3	16.66	ND
3	WA	6310	81.22	167.6	28.39	<3
1	AWA	ND	55.76	ND	ND	ND
2	AWA	ND	28.36	28.36	30.38	ND
3	AWA	ND	32.42	<3	4.930	<3

Table 8. Phenolic acids in the samples, mg/kg d. h. *ND = not detected.

Batch #	Solvent	Components, mg/ kg d. h.					
		Rosmarinic acid	Caffeic acid	Ferulic acid	Gallic acid	Syringic acid	Vanillic acid
1	MeOH	6418	ND*	ND	2583	ND	ND
2	MeOH	6481	60.88	ND	1381	ND	ND
3	MeOH	11366	52.39	ND	ND	ND	ND
1	EtOH	2341	60.35	ND	274.1	ND	ND
2	EtOH	6421	ND	ND	1388	ND	ND
3	EtOH	5169	ND	ND	ND	ND	ND
1	WA	5568	ND	ND	ND	ND	ND
2	WA	6777	ND	ND	ND	ND	ND
3	WA	9545	273.3	10.45	ND	ND	ND
1	AWA	52.94	ND	30.59	113.1	56.75	62.17
2	AWA	44.28	ND	ND	393.5	ND	ND
3	AWA	ND	ND	20.97	ND	ND	43.84

point of view of phenolic acid contents and the variety of these components, although it was from the same vintage as batch No 2.

3.2. Antioxidant properties, total polyphenol contents, and their correlations

For results of antioxidant capacity assays, see Table 9.

3.2.1. Antiradical activity measured with DPPH

As evaluation of antioxidant properties of wild mint, results of DPPH assays were published both for different extracts and for essential oils. Here, this assay shows that all of the extracts have medium scavenging activity against the DPPH radical, since none reached the EC_{50} value of thymol or BHT, but none of them are weak ($EC_{50} \geq 10,000$ mg/L). The strongest antiradical activity was shown by the extracts of batch No. 3 without regard to the solvent (see Table 9), which suggests that better extraction yields of antioxidants can be reached with shorter storage of the herb and also shows some differences in the maturity and freshness of the three batches. Results of the DPPH assay show significant correlation with TPC ($R = 0.888$ and $P \geq 0.01$) and also with superoxide scavenging activity ($R = 0.718$; $p \geq 0.01$), findings that reinforce that the antiradical ability of the plant originates from the phenolic constituents. On the other hand, no significant correlation was found between DPPH results and the measured quantity of any individual constituent of the plant.

3.2.2. Superoxide scavenging activity

All of the extracts showed strong activity as measured by PCL assay; their values are higher than the results of thymol and BHT. The results of PCL assay highly depend on the type of the extraction solvent. WA was observed to have the best efficacy to extract materials with strong PCL activity; extracts made with it have uniform high superoxide scavenging activity. The second highest efficacy was shown by MeOH. The results of the antisuperoxide assay are also batch-dependent since batch No. 3 showed the highest activity in all of its extracts. This finding is in accordance with the results of the DPPH assay, where batch No. 3 also showed the strongest antiradical activity (see Table 9). The two antiradical activity parameters are in significant correlation with total polyphenol content and also with each other.

3.2.3. Ferrous reducing ability

The extracts showed strong ferrous reducing ability with only one exception. This kind of antioxidant activity, in the case of the methanolic extracts, exceeds the FRAP results of thymol, and all WA extracts have higher values than those of BHT. The results of the MeOH extract of batch No. 2 are closer to the results of the eugenol control (902030 AAE) than those of BHT (21020 AAE). The FRAP results have no significant correlation with total polyphenol content, but significant correlation was found with the rutin content measured from the extracts: $R = 0.647$ and $P \geq 0.05$. Rutin-rich batch No. 2 shows the strongest FRAP activity. Literature data of rutin show that this flavonol glycoside shows quite a strong antioxidant activity as measured with FRAP; it is equivalent to Trolox (Lee et al., 2016). From the point of view of the efficacy of solvents to gain extracts with high FRAP activity, WA was found to be most and AWA least effective. This is consistent with the antiradical activity results, where the same efficacies were observed.

Table 9. Antioxidant properties and TPC of extracts.

Batch #	Solvent	DPPH, EC_{50}	Antiradical effect	FRAP, AAE	$Fe^{2+/3+}$ reducing effect	TE mg/kg	$O_2^{\cdot-}$ trapping effect	TPC GAE, mg/kg
1	MeOH	5856	Medium	15,891	Strong	14,366	Strong	20,111
2	MeOH	5887	Medium	60,333	Very strong	6439	Strong	26,853
3	MeOH	2341	Strong	27,134	Strong	14,588	Strong	47,516
1	EtOH	7242	Medium	11,684	Strong	7149	Strong	21,241
2	EtOH	7416	Medium	40,007	Strong	5180	Medium	14,261
3	EtOH	2313	Strong	18,074	Strong	14,147	Strong	40,724
1	WA	6038	Medium	21,701	Strong	14,720	Strong	33,901
2	WA	5551	Medium	31,078	Strong	14,745	Strong	39,159
3	WA	2517	Strong	35,246	Strong	15,435	Strong	38,465
1	AWA	7425	Medium	2840	Medium	7338	Strong	22,501
2	AWA	7185	Medium	21,779	Strong	6581	Strong	19,348
3	AWA	2392	Strong	17,859	Strong	12,773	Strong	46,986
Thymol	EtOH	1039	Strong	15,775	Strong	6093	Strong	
BHT	EtOH	278	Strong	21,020	Strong	6011	Strong	
Eugenol	EtOH	24.5	Very strong	90,230	Very strong	338,227	Very strong	

4. Discussion

ML is a widely distributed mint species in Eurasia. Most investigations dealing with the chemistry and bioactivities of ML (often nonspecified subspecies) were performed on batches from Iran (Nickavar et al., 2008; Motamed and Naghibi, 2010), Pakistan (Ali et al., 2002; Ahmad et al., 2012; Iqbal et al., 2013), Arabia, and North Africa (Sharaf et al., 1999; Ghoulami et al., 2001; Hajlaoui et al., 2002; Murad et al., 2016). Subspecies *longifolia* (Baris et al., 2011), *noeana* (Ertaş et al., 2015), and *typhioides* (Serteser et al., 2009) were also collected in Turkey's eastern regions for different investigations. In these countries the plant serves as herbal tea and folk medicine. In Arab countries such as Saudi Arabia, ML is often cultivated (under the names habek, habak, or hasawy).

Regarding Europe, ML is less known, contrary to its abundance; instead of it other *Mentha* taxa are widely used. Nevertheless, increasing interest in ML and its constituents, extractability, processing, bioactivity, and potential food or phytotherapeutic uses is observable in publications from Poland (Bertoli et al., 2011; Hawrył et al., 2015), Serbia (Mimica-Dukić et al., 1999; Stanislavljević et al., 2012), Romania (Spiridon et al., 2011; Benedec et al., 2013), and Slovakia (Fialová and Tekeľová, 2008). Most of these samples are wild-grown, but in Poland experiments are also done with in vitro, callus, and garden cultures (Bertoli et al., 2011). It is noteworthy to mention the demonstration of the hepatoprotective effect of three polyphenolic fractions obtained from wild-growing ML in Serbia (Mimica-Dukić et al., 1999). Other works dealt with the optimization of the drying method to reach the best yield of phenolics (Stanislavljević et al., 2012), or the development in TLC of flavonoids of mints (Hawrył et al., 2015).

Contrary to this, publications about the extractability or constituents of wild mint populations from Hungary were not found and no data were found about antioxidant or other bioactivities of any Hungarian ML batch. There have been floristic data collected from the Pannonian biogeographic region. The importance of ML is that this mint species can be found in natural areas and it is not rare, so it can be collected widely in Hungary, and if its therapeutic or food use broadens it can become a more important species locally. The main phenolic constituents of the investigated northern Hungarian ML batches are slightly different from the available data about this species: the main ones are rosmarinic acid and rutin. No literature data were found about ML with similarly high rutin contents. The rosmarinic acid levels in the investigated batches are similar to the data of widely cultivated mint taxa *M. spicata* and *M.* × *piperita*. In Spanish batches of *M. spicata* leaves, rosmarinic acid was found to be present at 1500–4020 mg/kg dry base (Areias et al., 2001). In *M.* × *piperita* it reached 7100–14,300 mg per kg d. h. (Wang

et al., 2004). Further minor differences compared to the literature were also found. Free kaempferol is present in all of the batches; it was found not to be reported in the literature on ML. In batch No. 3 vanillic and syringic acid were minor constituents. No data of their presence in ML were found. Another characteristic trait of the samples is the lack of naringenin. A survey of Hungarian populations of ML is suggested, based on our results. In northern Hungary the collection of samples by the authors is in process. Results of antiradical activity, total polyphenol, and HPLC assays of the extracts suggest that wine cannot be expected to be an efficient extraction medium for ML polyphenols. AWA (water-ethanol 90:10 mixture, acidified) extraction solvent was prepared as a model of wine. AWA extracts of the three batches showed the weakest antioxidant activities; they did not contain rutin or caffeic acid and they were poor in rosmarinic acid compared to the extracts made with the three other solvent types. Thus, it seems to be more useful to use the WA (ethanol-water 7:3) extract of ML instead of the addition of the plant itself to wine when the goal is to use the ML's antioxidants in vermouth. The WA extract of the plant can be used instead of pure ethanol when the total alcohol content is adjusted to the required interval (17.5% to 22% v/v). Usage of WA extracts is proposed because they show higher antioxidant activities and total polyphenol content and furthermore are richer in rosmarinic acid and rutin than the extracts made with pure ethanol. Elaboration of these suggestions requires further work, e.g., antioxidant and organoleptic evaluation of vermouth batches made of wines treated with ML extracts and their comparison with control batches.

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