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THE CONTENT OF PHENOLIC COMPOUNDS IN UNDERGROUND AND AERIAL PARTS OF DIFFERENT *MENTHA* SPECIES

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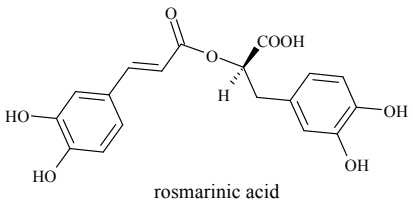
Mentha L., the genus belonging to family Lamiaceae, subfamily Nepetoidae, has high commercial importance in the pharmacy as well as in food industry. Mints are available in all five continents and are important sources of each traditional medicine in the prevention and therapy of plenty of diseases. The most active compounds are essential oil and polyphenols. In the past the secondary metabolites in aerial parts were examined, but there is no evidence about the determination of secondary metabolites in underground parts of mints. Therefore the object of this work was to determine the content of phenolic compounds (total hydroxycinnamic derivatives (THD) and flavonoids) of methanol extracts of rhizomes of different *Mentha* L. and their comparison with leaves extracts. The contents of secondary metabolites were determined using spectrophotometric methods of Slovak Pharmacopoeia. We have detected similar quantities of THD in leaves (0.79 – 2.48 %) and rhizomes (0.96 – 2.18 %), but underground parts were poor in content of flavonoids (< 0.1 %). The free radical scavenging activity using DPPH free radical was investigated as well. A bit stronger antioxidant activity was shown in leaves extracts considering flavonoids content.

Keywords: *Mentha - Rhizomes - flavonoids - THD - scavenging activity (DPPH)*

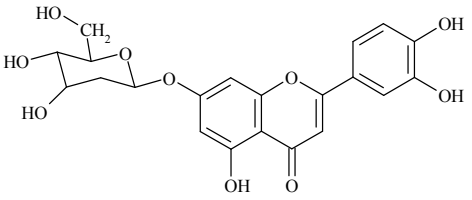
INTRODUCTION

Mints (*Mentha* L., Lamiaceae) are known and classified as aromatic plants with high content of essential oil and are famous medicinal plants in traditional and conventional medicine all over the world. The genus *Mentha* includes 18 species and about 11 named hybrids, placed in four sections (*Pulegium, Tubulosae, Eriodontes, Mentha*). The most used and cultivated mints in the middle Europe are *Mentha* × *piperita* (peppermint) and *Mentha spicata* (spearmint). In the nature of Slovakia, different species of mints can be found, the most common being *M. longifolia, M. verticillata, M. Aquatica,* etc. (Šarić-Kundalić et al., 2009). The main active component is essential oil, presented by monoterpenes and sesquiterpenes, which varies from species to species (Hayes et al., 2007). The main phenolic compounds in mint are phenolic acids (especially rosmarinic

acid) and flavonoids (eriodictyol, luteolin, apigenin and their glycosides) (Guédon & Pasquier, 1994).



rosmarinic acid



luteolin-7-O-glucoside

Several authors have reviewed the literature with respect to the medicinal uses of peppermint. Peppermint has been reported to possess these biological activities: digestive, cholekinetic, choleretic, antispasmodic, antibacterial, antiviral, fungicidal, antioxidant, anti-inflammatory, expectorant, myorelaxant, analgesic as well as insecticidal, aphrodisiac, local anaesthetic, antiemetic, antiulcer, astringent, vasodilatator, etc. (Duke, 2002), (Mckay & Blumberg, 2006). An infusion of dried peppermint leaves was noted to be useful in the treatment of intestinal spasms of the alimentary canal, atony of stomach, flatulence (Flück & Jaspersen-Schib, 1976). Many of these effects depend on phenolic compounds, thanks to its antioxidant activity.
For the medicinal purposes usually used leaves or the whole aerial part is used. Most species of the genus *Mentha* produce long, thin rhizomes, which are not known to use for medicinal or other purpose. This study was based on questions: What secondary metabolites could be found in rhizomes? Could extracts of rhizomes have any fpharmacological activity? Could they be advised for medicinal purposes? The current available literature on the genus *Mentha* L. does not provide any information about contents of secondary metabolites in underground parts. Therefore, in our study we have investigated the presence and the contents of phenol compounds in underground parts of different mints and we have compared the results obtained from rhizomes with the results from leaves.

MATERIAL AND METHODS

Plant material

All mints [*M.* × *piperita* cv. 'Perpeta' (MP), *M. spicata* ssp. *spicata* (MS)*, M. spicata* var. *crispa* (MSC)*, M.* × *villosa* (MV), *M.* × *villosa* cv. 'Snežná' (MVS), *M. longifolia* ssp. *longifolia* (ML), *M. longifolia* var. *lavanduliodora* (MLL)] used in this study were cultivated in the climatic conditions of south-west Slovakia, in the Garden of Medicinal Plants, Faculty of Pharmacy in Bratislava. Cultures were planted on a light sand-loam soil in a sunny location. The leaves were harvested in July 2009, during plant flowering and rhizomes in November 2009. The plants were dried at 32 - 35 °C. Voucher specimens were deposited at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Bratislava.

Methods

Extract: methanol extracts were prepared using 2.00 g of powdered dry leaves or rhizomes and 20 g (2 x 10 g) of solvent. The extraction was provided by sonication 2 x 30 min. The solvent was evaporated after extraction; extract was weighted and again dissolved in methanol for the analyses.

1) The determination of secondary metabolites

Phenol compounds were determined using spectrophotometric methods of the Slovak Pharmacopoeia first edition (Ph.S. I, 2001).

Total hydroxycinnamic derivatives (THD, Arnow´s assay)

The content of THD was determined using a colorimetric method with the Arnow´s reagent at 505 nm (Spectrophotometer Thermo, Electron Corporation Genesys 6, United Kingdom). The percentage contents of THD were calculated and expressed as rosmarinic acid.

Flavonoids (Spectrophotometric assay)

The content of total flavonoids was determined by a spectrophotometric method using aluminium chloride at 392 nm (Spectrophotometer Thermo, Electron Corporation Genesys 6, United Kingdom). The percentage contents were calculated and expressed as luteolin-7-O-glucoside.

2) The determination of free radical scavenging activity

DPPH test

The free radical scavenging activity of methanol extracts was estimated using the DPPH, colour-free radical solution (55 μmol/l). The activity was expressed as SC_{50} ($\mu g.ml^{-1}$), which is the concentration of the test solution required to give a 50% decrease in absorbance from that of a blank solution (Lamaison et al., 1990, 1991), (Nagy et al., 2006). Trolox, ascorbic acid and rosmarinic acid were used as a positive control.

All analyses were made in triplicate. The percentage content of investigated phenolic compounds was calculated with reference to the dry drug weight.

RESULTS

As described above the extraction was performed by sonication using methanol as solvent. After extraction, the solvent was evaporated to obtain dry extract. The amounts of dry extracts are presented in **Table 1**. The extraction yields from rhizomes were about half of extraction yields from leaves. The best yield of dry extract from leaves was detected in *M. piperita* (22.6%), while the highest yield from rhizomes was found in *M. longifolia* var. *lavanduliodora* (12.1%).

Table 1. The yield of extraction using methanol in %

Mentha L.	Yield of extraction [%]	
	leaves	rhizomes
MP	**22.6**	11.8
MS	21.6	11.4
MSC	14.4	7.6
MV	19.1	11.9
MVS	14.9	10.1
ML	17.2	7.6
MLL	16.3	**12.1**

MP - *M.* × *piperita* cv. 'Perpeta', MS - *M. spicata* ssp. *spicata,* MSC - *M. spicata* var. *crispa,* MV- *M.* × *villosa,* MVS - *M.* × *villosa* cv. 'Snežná', ML - *M. longifolia* ssp. *longifolia,* MLL - *M. longifolia* var. *lavanduliodora*

For the determinations of total hydroxycinnamic derivatives expressed as rosmarinic acid and flavonoids expressed as luteolin-7-O-glucoside were performed using spectrophotometrical methods from Slovak Pharmacopoeia 1st edition. The results are found in **Table 2**. The highest content of THD as well as flavonoids was detected in leaves extracts of *M. piperita* (2.48 % and 1.08 % resp.).

Table 2. The percentage contents of THD and flavonoids in leaves and rhizomes of different *Mentha* sp.

Mentha L.	The content *of THD expressed as Rosmarinic acid (λ = 505 nm) [%]± SD		The content* of flavonoids expressed as luteolin-7-*O*-glucoside (λ = 392 nm) [%]± SD	
	leaves	rhizomes	leaves	Rhizomes
MP	**2.48** ± 0,30	1.14 ± 0.05	**1.08** ± 0.11	< 0.01
MS	1.95 ± 0.07	**2.18** ± 0.01	0.14 ± 0.01	< 0.01
MSC	1.15 ± 0.11	0.96 ± 0.08	0.14 ± 0.01	< 0.01
MV	1.32 ± 0.17	1.01 ± 0.29	0.60 ± 0.05	< 0.01
MVS	1.55 ± 0.15	1.01 ± 0.35	0.98 ± 0.06	< 0.01
ML	1.67 ± 0.12	1.98 ± 0.57	0.63 ± 0.06	< 0.01
MLL	0.79 ± 0.02	1.31 ± 0.41	0.27 ± 0.02	< 0.01

*** in dry drug; MP - *M.* × *piperita* cv. 'Perpeta', MS - *M. spicata* ssp. *spicata,* MSC - *M. spicata* var. *crispa,* MV- *M.* × *villosa,* MVS - *M.* × *villosa* cv. 'Snežná', ML - *M. longifolia* ssp. *longifolia,* MLL - *M. longifolia* var. *lavanduliodora***

The antioxidant activity was expressed as concentration of extract scavenging 50% of DPPH free radical (**Table. 3, Figure. 1**). The highest activity was detected in leaves

of *M. piperita*. The scavenging activity was stronger in leaves extract of each investigated mint except *M. longifolia* var. *lavanduliodora*.

Table 3. The comparison of DPPH scavenging activity of dry methanol extracts of leaves and rhizomes in different *Mentha sp.*

	AA SC_{50} [µg/ml]* ± SD	
***Mentha* L.**	leaves	Rhizomes
MP	**8.24**± 1.03	18.1 ± 1.86
MS	9.28 ± 0.45	**10.5** ± 0.20
MSC	13.62 ± 1.28	14.3 ± 0.20
MV	10.17 ± 0.13	13.2 ± 0.17
MVS	10.50 ± 1.17	20.5 ± 0.32
ML	9.55 ± 0.12	14.1 ± 0.15
MLL	5.83 ± 0.65	12.6 ± 0.08
Trolox [a]	6.20 ± 0.06	
Rosmarinic acid	1.72 ± 0.13	
Ascorbic acid	1.69 ± 0.03	

*** dry methanol extract; MP - *M.* × *piperita* cv. 'Perpeta', MS - *M. spicata* ssp. *spicata,* MSC - *M. spicata* var. *crispa,* MV- *M.* × *villosa,* MVS - *M.* × *villosa* cv. 'Snežná', ML - *M. longifolia* ssp. *longifolia,* MLL - *M. longifolia* var. *lavanduliodora***

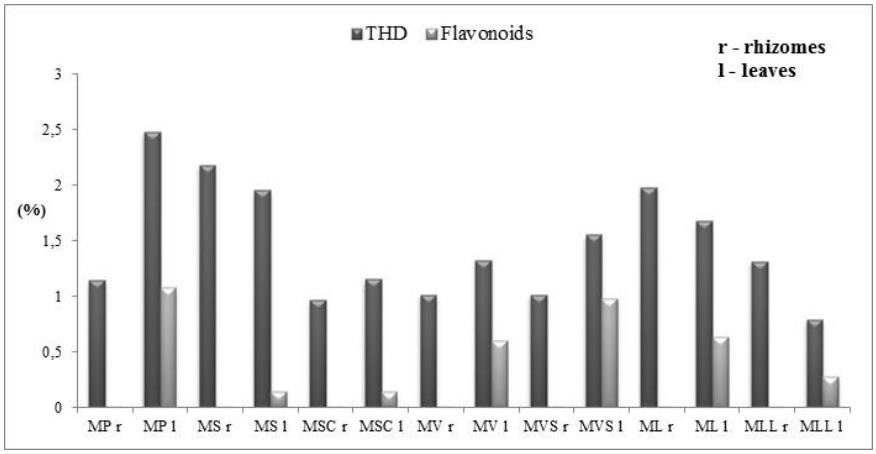


Figure. 1 The comparison of THD and flavonoids contents in rhizomes (r) and leaves (l) in different *Mentha* L. (MP -M. × *piperita* cv. 'Perpeta', MS - *M. spicata* ssp. *spicata,* MSC - *M. spicata* var. *crispa,* MV- *M.* × *villosa,* MVS - *M.* × *villosa* cv. 'Snežná', ML - *M. longifolia* ssp. *longifolia,* MLL - *M. longifolia* var. *lavanduliodora)*

DISCUSSION

The organism disposes own enzymatic mechanisms that eliminate free radicals in the body. Important roles in this process are played by substances with antioxidant effects presented in daily food. Those participate on elimination of pathological free radicals formation. The balance between antioxidant and prooxidant pathways is most appropriate. Long-term precarious balance inclined to the side of the prooxidative processes can be described as redox stress (Rice-Evans et al., 1996). Oxidation stress affects all tissues and all types of macromolecules in the cell (DNA, proteins, carbohydrates, lipids) (Ďuračková, 1998). In recent times, there has been wide research on natural antioxidants, key components in the protection mechanism against free radicals. The group of natural antioxidants is quite broad and includes mainly polyphenols (flavonoids, catechins, phenolic acids and their derivatives). Polyphenol compounds are also present in the species of the genus *Mentha*. The aerial parts of mints present an easily available source of natural antioxidants for medicine and pharmacy (Kähkönen et al., 1999), (Dorman et al., 2009).

It is known that mints produce a huge net of underground rhizomes and this net enlarges from year to year because of perennial character of these plants. For commercial purposes the yield of aerial part is important, since it is used in pharmacy, cosmetics and culinary, and especially because of essential oil present in leaves. But mints of course contain other active secondary metabolites which are responsible for many pharmacological effects.

This study was aimed to evaluate the contents of main secondary metabolites in leaves and rhizomes of seven mints. There are no indications in available literature about chemical composition or antioxidant activity in rhizomes of mints. In connection to Lamiaceae family, there were investigated rhizomes of some other species i.e. three antioxidant phenylethanoid glycosides were found in rhizomes of *Eremostachys pulvinaris* (forsythoside B, leucosceptoside A and verbascoside) (Delazar et al., 2004). The analysed extracts of leaves and rhizomes were prepared by sonication. For the extraction of phenol secondary metabolites we used methanol. The suitability of methanol as a solvent was recommended for these purposes in the available literature (Dorman et al., 2009), (Areias et al., 2001). The percentage content of THD in leaves' extracts varied from 0.79% to 2.48% and of flavonoids from 0.14 % to 1.08%. We have detected that the presence of THD in rhizomes as well and their contents in rhizomes were approximately equa l (0.96 - 2.18 %) to their contents in leaves, in some cases higher. Also flavonoids were investigated in rhizome extracts using spectrophotometric method and expressed as luteolin-7-*O*-glucoside; because luteolin derivatives have major position of all flavonoids in mints (Fialová et al., 2009). In comparison to leaves we found only trace content of flavonoids in underground parts.

While studying extracts we also examined DPPH radical scavenging activity, the results of which can be found in **table 3**. We suppose that flavonoid's absence reflects in lower free DPPH radical scavenging activity of rhizomes extracts. This is particularly visible in peppermint (MP), horse mint (ML), and apple mint (MV), whose leaves are rich in flavonoids. The antioxidant activity of leaves and rhizomes of mints seems to be associated chiefly with the content of hydroxycinnamic derivatives. High scavenging activity of methanol extract of spearmint (M1) rhizomes compared to other mints could

be caused by a high content of rosmarinic acid, which has been previously detected in leaves of this species (Fialová, 2010). Anyway stronger scavenging activity of leaves extracts is supported by flavonoids content. Scavenging activity in rhizomes extracts do not correlate with the content of THD, therefore we have to consider other antioxidant substances within extracts or their synergic effect.
According to previous scientific research and our knowledge about mints, we are not awaiting any toxicity in rhizomes in spite of not investigating the presence of essential oil components or other compounds in rhizomes. The results of our study should be the reason for rhizomes usage in therapy, or prevention of some disease as in aerial parts mentioned above.

CONCLUSION

This study gives information about phenolic compounds and antioxidant effect of methanol extract of rhizomes in comparison to leaves of different mints. The contents of total hydroxycinnamic derivatives were almost equal in rhizomes and leaves, contrary to total flavonoids expressed as luteolin-7-O-glucoside contents which were higher in leaves. Methanol extracts of rhizomes exhibit quite strong DPPH radical scavenging activity, but lower than that in leaves, probably because of low presence of flavonoids.

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OBSAH FENOLOVÝCH LÁTOK V NADZEMNÝCH A PODZEMNÝCH ČASTIACH RÔZNYCH DRUHOV RODU MENTHA

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Rod *Mentha* L. z čeľade Lamiaceae, podčeľade Nepetoidae, má vysoký komerčný význam vo farmácii, ako aj v potravinárskom priemysle. Mäty sa nachádzajú na všetkých piatich kontinentoch a sú dôležitým súčasťou každej tradičnej medicíny v prevencii a pri liečenie rôznych ochorení. Účinnými zložkami sú silica a fenolové látky. V minulosti boli sekundárne metabolity skúmané najmä v nadzemných častiach, avšak neuvádza sa stanovenie sekundárnych metabolitov v podzemných častiach mäty. Predmetom tejto práce bolo preto stanovenie obsahu fenolových látok (celkové hydroxyškoricové deriváty (THD) a flavonoidy) v metanolových extraktoch podzemkov rôznych druhov rodu *Mentha* L. a ich porovnanie s extraktmi z listov. Obsah sekundárnych metabolitov sa stanovil použitím spektrofotometrických metód Slovenského liekopisu. Zistili sme približne rovnaký obsah THD v listoch (0,79 - 2,48 %) a podzemkoch (0,96 - 2,18 %), avšak podzemné časti neobsahovali takmer žiadne flavonoidy (< 0,1%). Súčasťou práce bolo aj stanovenie antioxidačnej aktivity použitím DPPH voľného radikálu. Silnejšia antioxidačná aktivita sa zaznamenala v extraktoch z listov, pravdepodobne vďaka vyššiemu obsahu flavonoidov.

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