Microsaic Systems

Investigating Polar Phenolic Compounds in Peppermint Rhizomes and Leaves Using 4500 MiD®

Microsaic Systems plc, GMS House, Boundary Road, Woking, Surrey, GU21 5BX, United Kingdom
Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University, Odbojárov 10, 832 32 Bratislava, Slovak Republic
Amedis spol. s.r.o., Kamenárska 7, 821 04 Bratislava 2, Slovak Republic

INTRODUCTION

The genus *Mentha* L. belongs to the large family of Lamiaceae, subfamily Nepetoidae. A lot is known about usage of aerial parts, especially due to the menthol rich essential oil and phenolic compounds as rosmarinic acid and eriocitrin. Peppermint has been reported to possess many biological activities, e.g., digestion-stimulating, choleretic, antiseptic, secretolytic, antibacterial, antiviral, antispasmodic, antioxidant, anti-inflammatory, myorelaxant, and analgesic effects [1,2]. The presence of bioactive secondary metabolites, essential oil components (monoterpenes, sesquiterpenes) and phenolic compounds (flavonoids and phenolic acids) is thought to influence these properties [3]. However, less is known about peppermint rhizomes, which are produced in high quantity every year [4].

The aim of this study was to compare the leaves and rhizomes of peppermint with respect to phenolic compounds using Sykam HPLC-DAD connected with the Microsaic 4500 MiD® mass spectrometer.

EXPERIEMNTAL

M. × piperita L. leaves and rhizomes were acquired from the Medicinal Plant Garden of the Faculty of Pharmacy, Comenius University in Bratislava. Leaves were collected at the flowering time and rhizomes in spring. The plants were dried at 30 - 32 °C. Voucher specimens are deposited at the Department of Pharmacognosy and Botany, Faculty of Pharmacy, Comenius University in Bratislava, Slovakia.

Infusion of dried leaves and rhizomes of *M. × piperita* was prepared according to Pharmacopoeia Bohemoslovaca 4th edition [5]. The infusion was lyophilised. For the HPLC analysis 5 mg of dry extract was dissolved in 1 mL of water.

Qualitative analysis by HPLC-DAD-MS

The HPLC-DAD analyses were performed on HPLC system, equipped with a PDA detector (S3345) and Clarity Software. The HPLC system was connected in series to mass spectrometer 4500 MiD® a single quadrupole with a mass range of 1400 m/z equipped with ESI source (spraychip®). HPLC separation of the peppermint leaves and rhizomes was carried out on a TELOS LU C18 (2), 250x4.6 mm ID, 5 µm at 30°C and a flow rate of 0.8 mL/min. Water (pH 2.59 with HOAc,) and MeCN were used as mobile phase A and B, respectively. The following gradient program was used: 10 % B (0 min), 15% B (10 min), 30% B (20 min), 40% B (40 min), 90% B (45 min) and 10% B (50 min), followed by a column cleaning and re-equilibration step [6].

The MS parameters were as follows: Negative Ion Mode, Tip Voltage -750.0 V, Nebulizer Flow 2500.0 ml/min., Vacuum Interface Voltage 40.0 V, Tube Lens Voltage 10.0 V, Plate Lens Voltage 5.0 V, Ion Guide Voltage 1.0 V, Count Time 0.08 ms, Software Masscape. Nitrogen was used as the nebulising gas.

Quantitative determination of constituents by HPLC-DAD

The quantitative determination of phenolic compounds in *Mentha* leaves and rhizomes water extracts was provided by the method of external standards. Rosmarinic acid was used for the quantification of these both compounds and caffeic acid derivatives and eriocitrin and hesperidin for the quantification of flavonoid glycosides (see Table 2). The examinations of secondary metabolites in mint rhizomes were performed in triplicate. The quantitative results were calculated from calibration curves, expressed as mean values and standard deviation (SD).

RESULTS AND DISCUSSION

Aerial parts of mints are used in food as well as traditional and conventional medicines all over the world. The most famous species is unambiguously the peppermint, aided by the menthol content in essential oil. Secondary metabolites are also important contributors of peppermint usage. Water extracts of the aerial part of peppermint are rich in phenolics such as rosmarinic acid, eriocitrin, luteolin glycosides, apigenin glycoside and caffeic acid derivatives.

Table 1 Phenolic compounds in water extracts of peppermint rhizomes and leaves

Plant part	Compound	RT (Sykam)	[M-H] *m/z*	Identified/proposed Structure
peppermint rhizomes	1	29.7	609	hesperetin-7-*O*-rutinoside (hesperidin)
	2	30.6	359	rosmarinic acid
	3	32.1	717	caffeic acid tetramer
peppermint leaves	1	25.9	595	eriodictyol-7-*O*-rutinoside (eriocitrin)
	2	26.5	593	luteolin-7-*O*-rutinoside
	3	27.6	461	luteolin-7-*O*-glucuronide
	4	28.6	577	apigenin-7-*O*-rutinoside (isorhoifolin)
	5	29.3	717	caffeic acid tetramer
	6	29.6	609	hesperetin-7-*O*-rutinoside (hesperidin)
	7	30.6	359	rosmarinic acid
	8	32.2	717	caffeic acid tetramer

Microsaic Systems

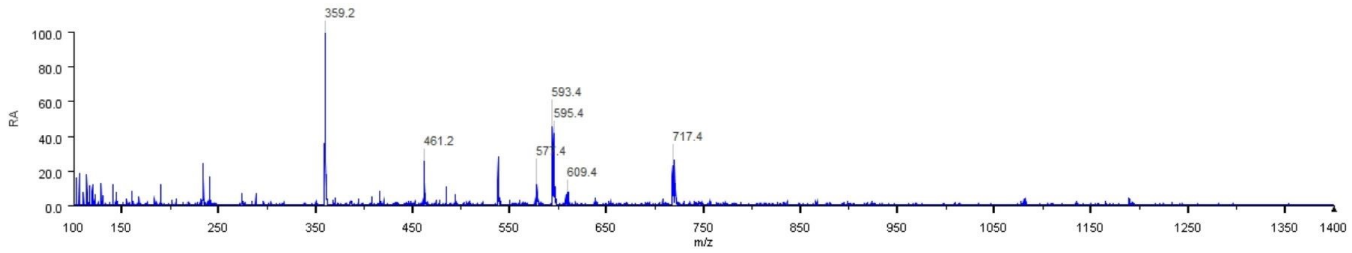

Figure 1. Extracted mass spectrum from injections of peppermint leaves

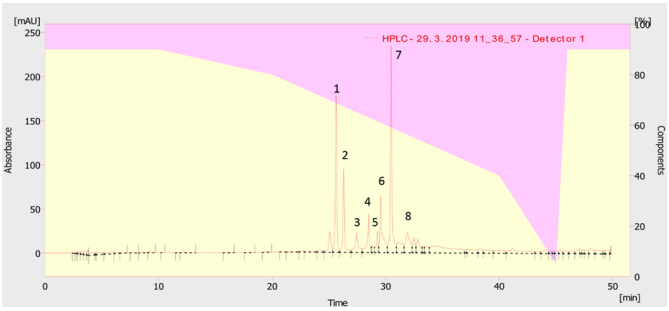

Figure 2 HPLC-DAD chromatogram of peppermint leaves aqueous

Table 2. Quantitative abundance of polar phenolic compounds in water extracts of M. × piperita rhizomes and leaves (µg.mL-1) (performed by HPLC-DAD)

Compound	Mass concentration (µg.mL^{-1})* ± SD	
	rhizomes	leaves
eriodictyol-7-O-rutinoside (eriocitrin)[a]	-	349.5 ± 10.52
luteolin-7-O-rutinoside[a]	-	204.1 ± 9.02
luteolin-7-O-glucuronide[a]	-	58.8 ± 0.77
apigenin-7-O-rutinoside (isorhoifolin)[a]	-	59.1 ± 3.88
caffeic acid tetramer (salvianolic acid B)[c]	-	24.1 ± 3.08
hesperetin-7-O-rutinoside (hesperidin)[b]	19.8 ± 2.48	37.47 ± 3.02
rosmarinic acid[c]	93.8 ± 8.15	286.4 ± 5.69
caffeic acid tetramer (salvianolic acid B)[c]	7.99 ± 1.61	25.0 ± 1.48

*

Values (µg.mL-1 in liquid extract) are presented as means ± standard deviation (n=3), calculated as external standards: eriocitrin (a), hesperidin (b), rosmarinic acid (c)

All of these compounds influence the medicinal properties of peppermint extracts (dry or liquid). As the major compounds may be marked eriocitrin and rosmarinic acid [7,8]. It is of interest to determine to determine if the underground parts are also rich in these compounds and in what quantities. Recently, it was found that the antioxidant activities of leaves and rhizomes are comparable [4]. Using liquid chromatography connected to 4500 MiD® mass spectrometer the separation and identification of phenolic compounds of Mentha leaves and rhizomes was performed (see Table 1).

Three phenolic compounds in rhizomes and eight in leaves were identified by comparison with authentic standards and/or literature. The resolution of caffeic acid tetramer (rhizomes: peak 3; leaves: peaks 6 and 8) was not clear, due to the low quality of salvianolic acid B standard). All compounds have been described previously in the genus Mentha L. [7-10].

CONCLUSIONS

By using HPLC-DAD connected to MS (4500 MiD®, a single quadrupole MS) we analysed the infusions of peppermint leaves and rhizomes. We identified and quantified one flavonoid and two caffeic acid derivatives in rhizomes and five flavonoid glycosides and three caffeic acid derivatives in leaves. The main component in rhizomes is the phenolic compound rosmarinic acid. Despite of its three times lower content than in leaves, rhizomes may be considered as a potential source for pharmaceutical research and for use in the food industry. It could be beneficial to prepare and study other kinds of peppermint rhizomes extracts (also non -polar).

REFERENCES

1. Mckay D.L., Blumberg J.B. (2006) A review of the bioactivity and potential health benefits of peppermint tea (Mentha × pipretia L). *Phytother Res.*; **20**, 619-633.
2. Lawrence BM. Mint. The genus Mentha. Medical and Aromatic Plants –Industrial Profiles. Volume 44. CRC Press, Taylor & Francis Group, Boca Raton, London, New York 2007.
3. Mimica-Dukic N., Bozin B. (2008) Mentha L. species (Lamiaceae) as promising sources of bioactive secondary metabolites. *Curr Pharm Des*, **14**, 3141-3150.
4. Fialova S., Tekeľová D., Grančai D. (2012) The content of phenolic compounds in underground and aerial parts of different Mentha species. *Acta Fac Pharm Univ Comen*, **59**, 30-38.
5. Pharmacopoea Bohemoslovaca PhBs IV. (1987) Avicenum, Praha
6. Fialová, S.; Slobodníková, L.; Veizerová, L.; Grančai D. (2015) Lycopus europaeus: phenolic fingerprint, antioxidant activity and antimicrobial effect on clinical Staphylococcus aureus strains. *Nat. Prod Res.*, **29**, 2271-2274.
7. Areias FM, Valentao P, Andrade PB, Ferreres F, Seabra RM. (2001) Phenolic fingerprint of peppermint leaves. *Food Chem*, **73**, 307-311.
8. Dorman DJH, Kosar M, Kahlos K, Holm Y, Hiltunen K. (2009) Phenolic Profile and antioxidant evaluation of Mentha × pipretia L. (peppermint) extracts. *Nat Prod Commun*, **4**, 535-542.
9. Dorman HJD, Kosar M, Kahlos K, Holm Y, Hiltunen R. (2003) Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. *J Agr Food Chem*, **51**, 4563-4569.
10. Fialová S, Prinz S, Zehl M, Tekeľová D, Reznicek G, Grančai D, Kopp B. (2009) Polyphenolic fingerprint of methanolic extracts of Mentha sp. cultivated in Slovakia. *Planta Med*, **75**, SL24.

ACKNOWLEDGEMENTS

Microsaic would like to thank Silvia Bittner Fialova from the Faculty of Pharmacy at Cormenius University in Bratislava for sharing experimental data and also Frantisek Jonas from Amedis spol. Microsaic's distributor in Slovakia for facilitating this collaboration.

microsaic.com +44 (0)1483 751 577 info@microsaic.com