LC-MS ANALYSIS AND ANTIOXIDANT ACTIVITY OF PHENOLIC COMPOUNDS FROM TWO INDIGENOUS SPECIES OF *MENTHA*. Note I

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Abstract

Two indigenous species of *Mentha*: *M. viridis* L. *var. crispa* (Schard.) Briq. and *M. longifolia* (L.) Huds. were studied in order to evaluate the phenolic profile and the natural antioxidant capacity. The polyphenolic content was determined by using the Folin-Ciocalteu reagent. The antioxidant activity was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The determination of polyphenolic compounds was performed by HPLC-MS. In *M. viridis var. crispa.* aerial parts extract the phenolic acids were predominant, while in *M. longifolia* extract, the flavonoids were majoritary. These two extracts contain a large amount of phenolic compounds, and show moderate antioxidant activity.

Rezumat

Au fost studiate două specii indigene de mentă: *Mentha viridis* L. *var. crispa* (Schard.) Briq. şi *M. longifolia* (L.) Huds, în vederea determinării profilului fenolic cu ajutorul reactivului Folin-Ciocâlteu şi prin analiză HPLC-MS şi a capacităţii antioxidante prin utilizarea metodei radicalului difenilpicrilhidrazil (DPPH). În extractul obţinut din părţile aeriene de la *M. viridis var. crispa* au predominat acizii fenolici, în timp ce în extractul de *M. longifolia,* flavonoidele au fost majoritare. Aceste două extracte conţin o cantitate mare de compuşi fenolici şi prezintă activitate antioxidantă moderată.

Keywords: *Mentha sp*., polyphenols, DPPH radical

Introduction

Mentha viridis L. *var. crispa* (Schard.) Briq. and *M. longifolia* (L.) Huds. from the spontaneous flora of Romania, belong to the *Mentha* genus in the *Lamiaceae* family and they are widely used in traditional medical practice [3,5,11]. The aerial parts of *Mentha sp*. have digestive stimulant, carminative, antispasmodic, stomachic and diuretic properties. The *Lamiaceae* family is a rich source of polyphenolic compounds and therefore, they could have antioxidant

properties [4,5,6]. The biologically-relevant activities have often been quoted in conjunction with antioxidant properties, which can in turn be related with the polyphenolic content of plant material [2,5,10]. In this regard and in order to complete the basis for a scientific rationale of the therapeutic use of these two Romanian medicinal plants, we evaluated the phenolic profile of aerial parts of *M. viridis var. crispa* and *M. longifolia* by HPLC-MS analysis and the antioxidant activity.

Materials and Methods

Samples preparation

The aerial parts of *Mentha viridis* L. *var. crispa* (Schard.) Briq. (Voucher No. 1270) and of *Mentha longifolia* (L.) Huds. (Voucher No.1271), in the blooming phase, were collected in July 2011 (Cluj, Romania). Voucher specimens were deposited in the Herbarium of the Department of Pharmaceutical Botany of the Faculty of Pharmacy, Cluj-Napoca, Romania. The powder obtained from the aerial parts was extracted with 70% ethanol, at 60°C. The samples were cooled down and centrifuged at 4500 rpm, and the supernatant was recovered [2,7,9,14].

HPLC–MS analysis was performed on an Agilent 1100 HPLC Series system (Agilent, USA) using the chromatographic conditions previously described [2,7,8,13]. Quantitative determinations were performed using an external standard method. Calibration curves in the 0.5-50 mg mL^{-1} range with good linearity (R^2>0.999) for a five points plot were used to determine the concentration of polyphenols in plant samples [2,7, 8,13].

Determination of polyphenolic compounds content (total polyphenols, flavonoids and caffeic acid derivatives). The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocâlteu assay [1,2,10,14]. TPC values, expressed as gallic acid equivalent (GAE), were determined using an equation that was obtained from calibration curve of gallic acid (R^2=0.9990). The spectrophotometric aluminum chloride method was used for flavonoids determination [15]. Total flavonoid content, expressed as rutin equivalent (RE), values were determined using an equation that was obtained from calibration curve of rutin graph (R^2 = 0.9996). The total phenolic acids content in the plant material was determined using the spectrophotometric method with Arnow's reagent [15]. The percentage of phenolic acids, expressed as caffeic acid equivalent (CAE) on dry

weight, was determined using an equation that was obtained from calibration curve of caffeic acid (R^2: 0.994083).

DPPH radical-scavenging activity. An aliquot of 1 mL extract was added, at an equal volume, to ethanolic solution of DPPH ($0.1gL^{-1}$). The absorbance was recorded at 517 nm. Butylated hydroxy toluene (BHT) was used as a positive control. The capability of samples to scavenge DPPH was expressed as percentage values: DPPH radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$, where $Abs_{control}$ is the absorbance of DPPH radical + ethanol (containing all reagents, except the sample) and Abs_{sample} is the absorbance of DPPH radical + sample extract or standard [10,12].

All the samples were analyzed in duplicate or triplicate; the average and the relative SD were calculated using the Excel software package.

Results and Discussion

HPLC-MS results

HPLC method has been developed for the determination of 18 phenolic compounds (seven phenolic acids, four quercetol glycosides, and seven flavonol and flavone aglycones) from plant material. The method allows a simultaneous analysis of different classes of polyphenols by a single pass column (the separation of all examined compounds was carried out in 35min). The concentrations of identified polyphenolic compounds in both analysed samples are presented in order of their retention time in Table I.

Table I

The polyphenolic compounds in *Mentha sp*.

Polyphenolic compounds	**Rt±SD (min)**	***M. viridis var. crispa* (mg/100 g dry mass)**	***M. longifolia* (mg/100 g dry mass)**
caftaric acid	2.10±0.06	<0.2	<0.2
caffeic acid	5.60±0.04	<0.2	<0.2
chlorogenic acid	5.62±0.05	<0.2	<0.2
p-coumaric acid	8.7±0.08	15.240	2,687
ferulic acid	12.2±0.10	27.325	<0.2
sinapic acid	14.3±0.10	6.601	NF
isoquercitrin	19.60±0.10	NF	15.803
rutin	20.20±0.15	NF	0,822
luteolin	29.10±0.19	4.682	1.764
apigenin	33.10±0.15	NF	<0.2

Note: NF - not found, below limit of detection.

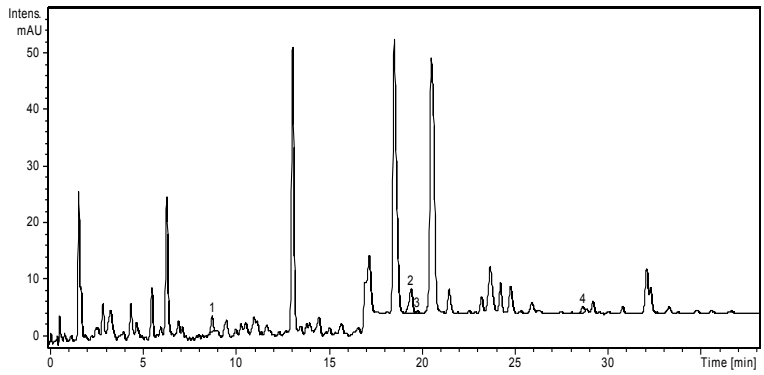

Figure 1
HPLC chromatogram of *M. longifolia* extract

In the extract of *M. viridis var. crispa,* ferulic acid was determined in the highest concentration (27.32 mg/100 g dried plant) followed by p-coumaric acid (15.24 mg/100 g dried plant) and sinapic acid (6.60 mg/100 g dried plant). Caftaric acid, caffeic acid and chlorogenic acid were also identified, but they were found in low quantities. Luteolin was identified and quantified (4.68 mg/100 g dried plant) in this ethanolic extract (Table I).

p-Coumaric acid (2.68 mg/100 g dried plant) was quantified in the ethanolic extract of *M. longifolia* (Table I). Caftaric, caffeic, chlorogenic, ferulic acids were also identified in the aerial parts extract, but they were in too low concentration to be quantified. Two flavonoid glycosides, isoquercitrin and rutin were quantified considering the used flavonoids standards (Table I, Figure 1). Two free aglycons, flavones (luteolin, apigenin) were detected in the extract of aerial parts from *M. longifolia*. Considering the 18 standard compounds used in this study, some other peaks were not identified.

In conclusion, in *M. viridis var. crispa* aerial parts extract, the phenolic profile showed the presence of the phenolic acids (p-coumaric, ferulic, sinapic caftaric, caffeic, chlorogenic acids), while in the *M. longifolia* aerial parts extract, the flavonoids predominated and they were represented especially by the glycosides (isoquercitrin) and the aglycones (luteolin).

Determination of polyphenolic compounds content: total polyphenols, flavonoids and caffeic acid derivatives

The total polyphenolic content (TPC), the flavonoidic content and the phenolic acids content values were summarized in Table II. The highest amount of the total polyphenols (TPC) was determined in the extract of *M.*

viridis var. crispa extract (246.7±0.47 mgGAE/g dry mass) followed by *M. longifolia* (219.2±0.97 mgGAE/g dry mass). The extract of *M. viridis var. crispa* (9.41±0.08 mgRE/g dry mass) was richer in flavonoids than *M. longifolia* (6.75±0.09 mgRE/g dry mass). The highest amount of the phenolic acids was determined in the extract of *M. viridis var. crispa* extract (43.80±1.39).

The ethanolic extract of *M. viridis var. crispa* extract contains a larger amount of polyphenols (total polyphenols, flavonoids and caffeic acid derivatives) compounds than the extract of M. *longifolia* (Table II).

Table II

Total phenolic content in the extracts of *Mentha sp.* and DPPH (%) values

Samples	TPC (mg GAE/g dry mass)	Flavonoids (mg RE/g dry mass)	Caffeic acid derivatives (mg CAE/g dry mass)	DPPH (%)
M.viridis var. crispa	246.7±0.47	9.41±0.08	43.80±1.39	18.34±2.2
M. longifolia	219.2±0.97	6.75±0.09	20.38±1.10	25.31±0.6

Each value was obtained by calculating average of three experiments with ą standard deviation

TPC – total phenolic content, GAE – gallic acid equivalent, RE – Rutin equivalent, CAE – Caffeic acid equivalent, DPPH – 2,2-diphenyl-1-picrylhydrazyl.

Antioxidant activity

The radical scavenging effect of *M. longifolia* at a concentration of 0.4 mg plant product/mL extract was 25.31%, followed by the extract of *M. viridis var. crispa* (18.34 %) at the same concentration (Table II). The highest radical scavenging activity was showed by BHT, a synthetic antioxidant (94.77%±0.64) at the same concentration (0.4 mg mL^{-1}). The results showed that the extract of *M. longifolia* had superior antioxidant capacity compared to the extract of *M. viridis var. crispa*, even if the polyphenolic compounds content of *M. longifolia* aerial parts was slightly lower than that of *M. viridis var. crispa* aerial parts. Thus, two species of mint contained a considerable amount of polyphenols, there is no simple correlation between phenolic content and their antioxidant capacity.

Conclusions

After HPLC-MS analysis, 18 phenolic compounds were found in this study. In *M. viridis var. crispa* aerial parts extract, the phenolic profile showed the presence of the phenolic acids, while in the *M. longifolia* aerial parts extract, the flavonoids predominated. The extracts of *M. viridis var.*

crispa and M. longifolia contain a large amount of phenolic compounds (polyphenols, flavonoids, caffeic acid derivatives), and show moderate antioxidant activity. The utilisation of the aerial parts in traditional medicine could be justified through the pharmacological activities of phenolic compounds that were identified.

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