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LC-MS ANALYSIS, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES FOR FIVE SPECIES OF *MENTHA* CULTIVATED IN ROMANIA

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The aim of this work was to characterize the polyphenolic composition and to evaluate antioxidant and antibacterial activities for five species of *Mentha*: *M. rotundifolia, M. spicata* subsp. *crispata*, *M. suaveolens* var. *variegata*, *M. piperita* var. *officinalis* f. *pallescens* and f. *rubescens*. The identification and quantification of major phenolic compounds was performed by a LC-MS method. The phenolic content was determined spectrophotometrically. The antioxidant activity was evaluated using the DPPH bleaching method. The antimicrobial tests were performed using the disk diffusion assay. Caftaric, gentisic, caffeic, p-coumaric, chlorogenic acids, rutin, isoquercitrin, luteolin and apigenin were present in the five extracts of *Mentha,* in different amounts. Ferulic acid was determined only in two forms of *M. piperita* and quercitrin was detected in *M. rotundifolia, M. spicata* and *M. suaveolens.* The extracts of *M. rotundifolia* and *M. piperita* f. *pallescens* showed a higher antioxidant activity and *M. spicata* extract and *M. rotundifolia* extract had a good antimicrobial activity. The results of the present investigation showed differences between five *Mentha* species cultivated in Romania that could be important for their therapeutical valorification, like antioxidant or antimicrobial agents.

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1. Introduction

The *Lamiaceae* family includes over 200 genera and more than 4000 species which are widely used for various purposes worldwide. Plants belonging to this family are rich in polyphenolic compounds and a large number of them are well known for their antioxidant properties [1, 2, 3]. Among the natural compounds, the phenolic compounds constitute one of the major groups of active principles acting as radical scavengers and antioxidants. The genus *Mentha* is an important member of this family [3, 4]. *Mentha* genus includes about 25 species and many hundreds of varieties of flowering plants distributed throughout the world. The flora of Romania comprises around 25 species and several varieties and subspecies of *Mentha* [1, 5]. Mint species are very important for their medicinal value. The leaves of *Mentha* species have been used for

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centuries as tonic, carminative, digestive, antispasmodic and anti-inflammatory agents [2, 4, 6-8]. Over the past few years, major advances have been made to investigate the antioxidant and antimicrobial properties of different *Mentha* species: *M. piperita*, *M. suaveolens* ssp. *suaveolens, M. suaveolens* ssp. *insularis*, *M. longifolia*, *M. spicata*, *M. viridis var. crispa, M. pulegium, M. aquatica, M. rotundifolia* (from Tunisia), *M. crispa* [2, 4, 9-13]. *Mentha* species are used in everyday life in various medicinal and food items. These species produce valuable secondary metabolites, with important therapeutical properties [1,2]. The aim of this work was to analyze the biologically active compounds from five *Mentha* species cultivated in Romania and to investigate their antioxidant and antimicrobial properties, for a better characterization and valorification of these natural products.

2. Experimental

2.1. Plant material and extraction procedure

The studied medicinal plants were: *Mentha x rotundifolia* (L.) Huds., apple mint (Voucher No. 1514), *Mentha x piperita* L. var. *officinalis* Sole f. *rubescens* Camus, black peppermint (Voucher No.1515), *Mentha spicata* L. subsp. *crispata,* spearmint (Voucher No. 1516), *Mentha suaveolens* Ehrh. var. *variegata,* pineapple mint (Voucher No. 1517), *Mentha x piperita* L. var. *officinalis* Sole f. *pallescens* Camus, white peppermint (Voucher No. 1518). The leaves were harvested in the phase of blooming, from experimental fields of the Fares BioVital Laboratories Orastie (Hunedoara, Romania), in July 2012. Voucher specimens were deposited in the Herbarium of the Department of Pharmaceutical Botany from the Faculty of Pharmacy, Cluj-Napoca, Romania. The plant material was reduced to a powder of a proper degree of fineness. The samples preparation: 2.0 g of the powder was extracted with 20 ml of 70% ethanol, for 30 min on a water bath, at 60°C. The samples were then cooled down and centrifuged at 4500 rpm for 15 min, and the supernatant was recovered [13-16].

2.2. LC–MS analysis

An Agilent 1100 HPLC Series system (Agilent, USA) was used equipped with a degasser, binary gradient pump, column thermostat, autosampler, and UV detector. The HPLC system was coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL). For the separation, a reverse-phase analytical column was employed (Zorbax SB-C18, 100 x 3.0 mm i.d., 3.5 µm particle); the work temperature was 48°C. The detection of the compounds was performed on both UV and MS mode. The UV detector was set at 330 nm until 17.5 min, then at 370 nm. The MS system operated using an electrospray ion source in negative mode. The chromatographic data were processed using ChemStation and DataAnalysis software from Agilent, USA. The mobile phase was a binary gradient: methanol and acetic acid 0.1% (v/v). The elution started with a linear gradient, beginning with 5% methanol and ending at 42% methanol, for 35 minutes; then 42% methanol for the next 3 minutes. The flow rate was 1 $mLmin^{-1}$ and the injection volume was 5 µL [13,17,18]. The MS signal was used only for qualitative analysis based on specific mass spectra of each polyphenol. The MS spectra obtained from a standard solution of polyphenols were integrated in a mass spectra library. Later, the MS traces/spectra of the analyzed samples were compared to spectra from library, which allows positive identification of compounds, based on spectral mach. The UV trace was used for quantification of identified compounds from MS detection. Using the chromatographic conditions described above, the polyphenols eluted in less than 35 minutes (Table 1). Four polyphenols could be quantified in current chromatographic conditions due overlapping (caftaric acid with gentisic acid and caffeic acid with chlorogenic acid). However, all four compounds can be selectively identified in MS detection (qualitative analysis) based on differences among their molecular mass and MS spectra. The detection limits were calculated as minimal concentration producing a reproductive peak with a signal-to-noise ratio greater than three. 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 point plot were used to determine the concentration of polyphenols in plant samples [13,17,18].

2.3. Determination of polyphenolic compounds content (flavonoids, caffeic acid derivatives and total polyphenols)

The spectrophotometric aluminum chloride method was used for the flavonoids determination. Each extract (5 mL) was mixed with sodium acetate (5.0 mL, 100 $g \cdot L^{-1}$), aluminum chloride (3.0 mL, 25 $g \cdot L^{-1}$), and filled up to 25 mL by methanol in a calibrated flask. The absorbance was measured at 430 nm [16]. The total flavonoids content values was determined using an equation obtained from calibration curve of the 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 [16]. Caffeic acid derivatives were determined with hydrochloric acid (1 mL, 0.5 N), Arnow's reagent (1 mL) and sodium hydroxide solution (1 mL, 1 N). The absorbance was determined spectrophotometrically at 500 nm. The percentage of phenolic acids, expressed as caffeic acid equivalent on dry weight, was calculated using an equation that was obtained from calibration curve of caffeic acid graph ($R^2= 0.9941$).

The total phenolic content (TPC) of the extracts was determined by the Folin-Ciocalteau method with some modifications [14,15]. 2 mL of ethanolic extracts diluted 25 times were mixed with 1.0 mL of Folin-Ciocalteu reagent, 10.0 mL of distilled water and diluted to 25.0 mL with a 290 g/L solution of sodium carbonate. The samples were incubated in the dark for 30 min. The absorbance was measured at 760 nm. Gallic acid was used as standard for the calibration curve and was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL gallic acid that was prepared in methanol: water (70:30, v/v). TPC values were determined using an equation that was obtained from calibration curve of gallic acid graph ($R^2=0.9990$).

2.4. DPPH radical-scavenging activity

The free radical scavenging activity of the ethanolic extracts of the five species of *Mentha* was measured in terms of hydrogen donating or radical scavenging ability using the stable DPPH radical method. The DPPH solution (0.1 gL^{-1}) in ethanol was prepared and 4.0 ml of this solution was added to 4.0 ml of extract solution (or standard) in ethanol at different concentrations (10-50 µg/mL). After 30 minutes of incubation at 40°C in a thermostatic bath, the decrease in the absorbance (n =3) was measured at 517 nm. The percent DPPH scavenging ability was calculated as: DPPH scavenging ability = $(A_{control} - A_{sample}/A_{control}) \times 100$, 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. Afterwards, a curve of % DPPH scavenging capacity *versus* concentration was plotted and IC_{50} values were calculated. IC_{50} denotes the concentration of sample required to scavenge 50% of DPPH free radicals. Trolox was used as a positive control [13, 15, 17-19].

2.5. Antibacterial activity test

The ethanolic extracts of *Mentha* species were tested for the antimicrobial activity against two Gram-positive bacterial strains: *Staphylococcus aureus* (ATCC 49444), *Listeria monocytogenes* (ATCC 13076), against two Gram-negative bacterial strains: *Escherichia coli* (ATCC 25922), *Salmonella typhymurium* (ATCC 14028) and one fungal strain: *Candida albicans* (ATCC10231) by a previously described disc diffusion method, in Petri dishes [20,21]. Each microorganism was suspended in Mueller Hinton (MH) broth and diluted approximately to 10E6 colony forming unit (cfu)/mL. They were "flood-inoculated" onto the surface of MH agar and MH Dextroxe Agar (MDA) and then dried. Six-millimetre diameter wells were cut from the agar using a sterile cork-borer, and 60 µL of each extract were delivered into the wells. The plates were incubated at 37°C and the diameters of the growth inhibition zones were measured after 24 h. Gentamicin (10 µg/well) and Fluconazole (25 µg/well) were used as standard drugs. The controls were performed with only sterile broth and with only overnight culture and 10 µL of 70% ethanol. All tests were performed in triplicate, and clear halos greater than 10 mm were considered as positive results [20, 21].

2.6. Statistical analysis

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

3. Results and discussions

3.1. LC-MS results

A liquid chromatographic method has been developed for the determination of nineteen phenolic compounds (eight phenolic acids, four quercetin glycosides, and seven flavonol and flavone aglycones) from natural products. The simultaneous analysis of different classes of polyphenols was performed by a single column pass, and the separation of all examined compounds was carried out in 35 min. The concentrations of identified polyphenolic compounds in all the five analysed samples are presented in Table 1. The HPLC chromatograms for all mint extracts are shown in Figures 1-5. The quantitative determination was performed using the external standard method.

Caftaric acid, gentisic acid, caffeic acid and chlorogenic acid were identified in all analyzed extracts of *Mentha* species, but they were found in low quantities (<0.2mg/100 g plant material). The p-coumaric acid was determined in all the samples, in different amounts. Larger amounts of p-coumaric acid were found in *M. x rotundifolia* (3.69 mg/100 g plant material), followed by *M. piperita x* var. *officinalis* f. *pallescens* (2.30 mg/100 g plant material) and *M. suaveolens* var. *variegata* (2.12mg/100 g plant material). Ferulic acid was determined only in two subspecies of *M. x piperita* var. *officinalis* (f. *rubrescens*: 0.35 mg/100 g plant material and f. *pallescens:* 0.45 mg/100 g plant material). Three flavonoid glycosides were identified in the five extracts. Isoquercitrin was quantified in large quantities in: *M. suaveolens* var. *variegata* (31.63 mg/100 g plant material), *M. spicata* subsp. *crispata* (16.06 mg/100 g plant material), *M. x piperita* var. *officinalis* f. *pallescens* (15.60 mg/100 g plant material) and *M. piperita x* var. *officinalis* f. *rubrescens* (9.44 mg/100 g plant material). Isoquercitrin was identified in the ethanolic extract of *M. rotundifolia*, but its concentration was too low to be quantified. In both extracts of *M. piperita*, the rutin was determined in quantities of 12.43 mg/100 g plant material (f. *rubrescens*) and 14.66 mg/100 g plant material, respectively (f. *pallescens*). Rutin was found in traces in the other samples. Quercitrin was detected only in *M. x rotundifolia* (4.66 mg/100 g plant material), *M. spicata* subsp. *crispata* (2.23 mg/100 g plant material) and *M. suaveolens* var. *variegata* (3.54 mg/100 g plant material). The flavonoid aglycones (luteolin and apigenin) were identified and determined in low quantities. *M. x rotundifolia* was the richest in luteolin (3.37mg/100 g plant material) and apigenin (4.99mg/100 g plant material) (Table 1). Considering the 19 standard compounds used in this study, some other peaks were not identified.

For the species of *Mentha*, the phenolic profile showed the presence of phenolic acid derivatives (caftaric, gentisic, caffeic, p-coumaric, chlorogenic and ferulic acids), three flavonoid glycosides (rutin, isoquercitrin and quercitrin) and two free flavonoids (luteolin, apigenin), in different concentrations (Table 1, fig.1-5).

Table 1. Polyphenolic compounds content in Mentha species (mg/100 g plant material).

Polyphenolic compounds	Rt±SD (min)	*M. x rotundifolia*	*M. spicata* subsp. *crispata*	*M. suaveolens* var. *variegata*	*M. x piperita* var. *officinalis* f. *rubrescens*	*M. x piperita* var. *officinalis* f. *pallescens*
caftaric acid	2.10±0.06	<0.2	<0.2	<0.2	<0.2	<0.2
gentisic acid	2.15±0.07	<0.2	<0.2	<0.2	<0.2	<0.2
caffeic acid	5.60±0.04	<0.2	<0.2	<0.2	<0.2	<0.2
chlorogenic acid	5.62±0.05	<0.2	<0.2	<0.2	<0.2	<0.2
p-coumaric acid	8.70±0.08	3.69±0.10	0.44±0.02	2.12±0.08	0.62±0.05	2.30±0.05
ferulic acid	12.20±0.10	NF	NF	NF	0.35±0.02	0.45±0.01
isoquercitrin	19.60±0.10	<0.2	16.06±0.44	31.63±0.87	9.44±0.56	15.60±0.6
rutin	20.20±0.15	<0.2	<0.2	<0.2	12.43±0.77	14.66±0.22
quercitrin	26.80±0.15	4.66±0.19	2.23±0.07	3.54±0.11	NF	NF
luteolin	29.10±0.19	3.37±0.08	2.23±0.05	2.54±0.08	2.12±0.12	1.91±0.09
apigenin	33.10±0.15	4.99±0.10	<0.2	1.16±0.04	2.73±0.03	2.83±0.18

Note: NF - not found, below limit of detection. Values are the mean ± SD (n = 3).

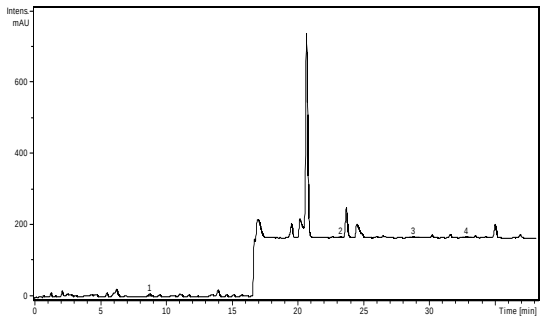

Fig.1. HPLC chromatogram of M. x rotundifolia

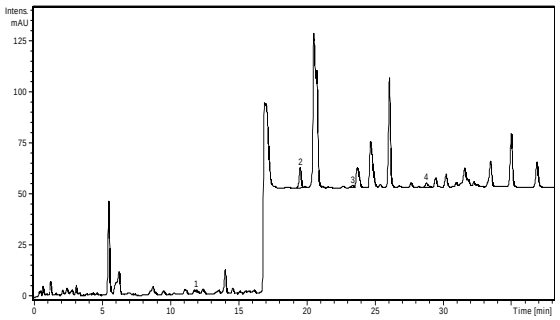

Fig.2 HPLC chromatogram of M. spicata

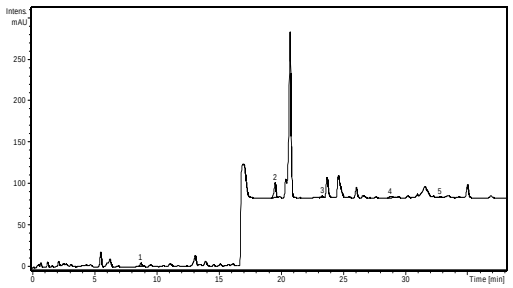

Fig.3. HPLC chromatogram of M. suaveolens

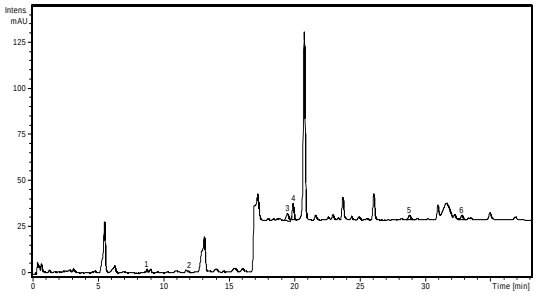

Fig.4. HPLC chromatogram of M. x piperita f. *rubrescens*

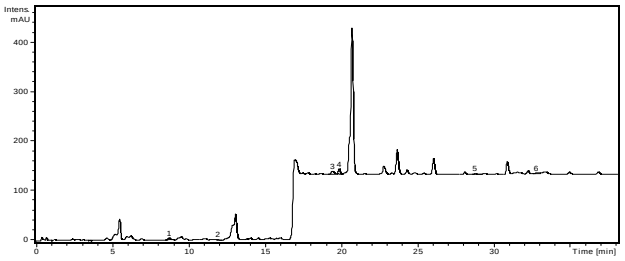

Fig.5. HPLC chromatogram of M. x piperita f. *pallescens*

3.2. Determination of polyphenolic compounds content

Phenolic compounds are secondary metabolites, one of the most widely occurring groups of phytochemicals, with considerable physiological and morphological importance in plants. Polyphenolic compounds also function as reducing agents, free radical scavengers, and quenchers of singlet oxygen. In addition, flavonoids and phenolic acids components play important roles in the control of cancer and other human diseases, as antioxidants. Due to their importance in plants and human health, it would be useful to know the concentration of the polyphenolic compounds that could indicate their potentials as therapeutic agents, but also for predicting and controlling the quality of medicinal herbs. The total phenolic content (TPC) values summarized in Table 2 were quantified based on the linear equation obtained from gallic acid standard calibration curve. Thus, TPC values were expressed as gallic acid equivalent (g GAE/100 g s plant material). The highest amount of the polyphenols was determined in the extracts of *M. x rotundifolia* (9.78g GAE/100 g plant material) and *M. x piperita* var. *officinalis* f. *pallescens* (9.67g GAE/100 g plant material), followed by the extracts of *M. spicata* subsp. *crispata* (8.62g GAE/100 g plant material) and *M. suaveolens* var. *variegata* (7.60g GAE/100 g plant material). The lowest level of polyphenols was detected in *M. x piperita* var. *officinalis* f. *rubrescens* (5.14g GAE/100g plant material). Concerning the content of flavonoids, the extracts of *M. x rotundifolia* (0.49 mg RE/g plant material), *M. suaveolens* var. *variegata* (0.40 g RE/g plant material) and *M. x piperita* var. *officinalis* f. *pallescens* (0.31 g RE/g plant material) were richer in flavonoids than the extract of *M. spicata* subsp. *crispata* (0.13 g RE/g plant material) and *M. x piperita* var. *officinalis* f.

rubrescens (0.10 g RE/g plant material). The calculation of total flavonoid content of plant extracts was carried out using the standard curve of rutin and presented as rutin equivalents (g RE/g plant material) (Table 2). The phenolic acids contents were expressed as caffeic acid equivalent (g CAE/100 g plant material). The results are presented in Table 2. The highest amount of phenolic acids was determined in the extracts of *M. x rotundifolia* (2.09 g CAE/100 g plant material), *M. spicata* subsp. *crispata* (1.97 g CAE/100 g plant material) and *M. suaveolens* var. *variegata* (1.88 g CAE/100 g plant material). For the extracts of *M. x piperita*, the obtained values were between 0.73 and 1.10 g CAE/100 g plant material.

Table 2. The content of total polyphenols, flavonoids and caffeic acid derivates in Mentha species extracts

Plant samples	TPC (g GAE/100 g plant material)	Flavonoids (g RE/100 g plant material)	Caffeic acid derivatives (g CAE/ 100 g plant material)
M. x rotundifolia	9.78±0.46	0.49±0.01	2.09±0.26
M. spicata subsp. *crispata*	8.62±0.27	0.13±0.09	1.97±0.25
M. suaveolens var. *variegata*	7.60±0.10	0.40±0.04	1.88±0.23
M. x piperita var. *officinalis* f. *rubrescens*	5.14±0.35	0.10±0.09	0.73±0.10
M. x piperita var. *officinalis* f. *pallescens*	9.67±0.62	0.31±0.02	1.10±0.10

Each value is the mean ± SD of three independent measurements. GAE: Gallic acid equivalents; RE: rutin equivalents; CAE: caffeic acid equivalents.

The extract of *M. x rotundifolia* contained highest amount of polyphenolic, flavonoidic compounds and caffeic acids derivates, while the extract of *M. x piperita* var. *officinalis* f. *rubrescens* was the poorest in these active principles.

3.3. Determination of the free radical scavenging activity of plant extracts

The antioxidant activity of the ethanolic extracts of *Mentha* species was further assessed by the DPPH radical bleaching method, Trolox was used as standard control (Table 3). The highest radical scavenging activity was observed for *M. x rotundifolia* (IC_{50} = 104.74±1.76 μgmL^{-1}), while lowest for *M. x piperita* var. *officinalis* f. *rubrescens* (284.61±1.03 μgmL^{-1}). The $IC_{50(DPPH\bullet)}$ values of the extracts increased in the following order: *M. x rotundifolia* < *M. x piperita* var. *officinalis* f. *pallescens* < *M. spicata* subsp. *crispata* < *M. suaveolens* var. *variegata* < *M. x piperita* var. *officinalis* f. *rubrescens.*This is in good agreement with the TPC values listed in Table 2. Therefore, it is likely that the phenolic constituents present in the *Mentha* species are responsible for the antioxidant and free radical scavenging activities. Compared to the reference compound, Trolox (IC_{50} = 12±0.54 μgmL^{-1}), the ethanol extracts of *Mentha* species showed lower antioxidant capacity, at the same concentration (25 µg samples/mL^{-1}). The literature data on the antioxidant activities of *Mentha* species are often difficult to compare because of the differences in the methodologies. Some of our results were generally similar to those obtained for *M. rotundifolia* or *M. spicata* species [4,11]. The result of the present study suggests that these plant materials especially *M. x rotundifolia* and *M. x piperita* var. *officinalis* f. *pallescens* can be used as a natural source of antioxidants.

Table 3. Results of DPPH free radical scavenging

Samples (25 µg samples/ mL^{-1})	**DPPH Radical Scavenging Activity (%)**	**IC_{50} (µg/ml)**
M. x rotundifolia	24.41±0.59	104.74±1.76
M. spicata subsp. *crispata*	14.49±1.01	151.05±1.95
M. suaveolens var. *variegata*	9.86±0.14	169.86±0.14
M. x piperita var. *officinalis* f. *rubrescens*	13.57±0.38	284.61±1.03
M. x piperita var. *officinalis* f. *pallescens*	14.88±0.37	109.86±1.14
Trolox	90.60±1.4	12±0.54

3.4. Antimicrobial activity

The antimicrobial tests of *Mentha* extracts were performed *in vitro* using the disk diffusion method in Petri dishes (Table 4).The antibacterial activity is ranked from no activity (inhibition diameter < 10 mm), low (inhibition diameter between 10 and 15 mm), moderate (inhibition diameter between 15 and 20 mm) and high activity (diameter inhibition ≥ 20 mm) [21].

Table 4. Antimicrobial activity (inhibition zone expressed in mm)[a] of Mentha species extracts

Samples	**Inhibition zone in diameter (mm)**				
	Staphylococcus aureus	***Listeria monocytogenes***	***Escherichia coli***	***Salmonella typhymurium***	***Candida albicans***
M. spicata subsp. *crispata*	18.0±0.05	26.0±0.1	10.0±0.05	12.0±0.00	22±0.1
M. x rotundifolia	18.0±0.07	28.0±0.00	8.0±0.05	12.0±0.02	20±0.05
M. suaveolens var. *variegata*	10.0±0.05	26.0±0.07	8.0±0.05	16.0±0.00	8±0.00
M. x piperita var. *off.* f. *rubrescens*	10.0±0.05	26.0±0.1	8.0±0.05	12.0±0.00	8±0.05
M. x piperita var. *off.* f. *pallescens*	18.0±0.05	24.0±0.1	10.0±0.05	14.0±0.00	8±0.07
Gentamicin	19±0.05	18±0.1	22±0.00	18±0.05	-
Fluconazole	-	-	-	-	25±0.00

Notes: [a]The values represent the average of three determinations ± standard deviations. Gentamicin (10µg/disk) and Fluconazole (25 µg/well) were used as a positive control.

The majority of the studied extracts of *Mentha* were active against at least one or two microorganisms. All the five extracts of *Mentha* species showed a high antibacterial activity towards *Listeria monocytogenes* (diameter inhibition 24-28 mm), stronger compared to Gentamicin use as reference antibiotic. The extracts of *M. spicata* subsp. *crispata, M. x rotundifolia,* and *M. x piperita* var. *officinalis* f. *pallescens* showed a moderate antibacterial activity against *S. aureus* (inhibition diameters 18 mm), comparable to Gentamicin, and low antibacterial effect on *S. typhymurium* (inhibition diameter 12 - 14 mm). All extracts were inactive on *E. coli* (inhibition diameters 8-10 mm). *M. suaveolens* var. *variegata* showed a moderate activity towards *S. typhymurium* (inhibition diameter 16 mm). The extracts of *M. spicata* subsp. *crispata* and *M. x rotundifolia* showed intensive antifungal activity against *Candida albicans* (inhibition diameter 20 - 22 mm), but the other extracts were inactive against the fungal strain.

The results of the present investigation suggest that *M. spicata* subsp. *crispata* and *M. x rotundifolia* have an important antibacterial and antifungal activity and all investigated *Mentha* species are very active on *L. monocytogenes*.

4. Conclusions

We have determined the polyphenolic composition, the antioxidant and antimicrobial activities for five species of *Mentha* cultivated in Romania, for better pharmacognostical knowledge of the mint. The antioxidant activity evaluated using the DPPH bleaching method indicated that *M. x rotundifolia* and *M. x piperita* var. *officinalis* f. *pallescens* extracts were the most powerful antioxidant, related with the polyphenolic total content. The antimicrobial tests underlined an important activity against *Listeria monocytogenes* for all *Mentha* species. The phytochemical comparative study showed qualitative and quantitative differences between the five species; *M. x rotundifolia* was the richest one concerning poliphenolic compounds. Our results confirm that *Mentha* species may be considered a favourable source of the polyphenols with antioxidant and antimicrobial properties.

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References

[1] S. Erum, M. Naeemullah, S. Masood, Pakistan J. Agric. Res, **25**(1):55 (2012).
[2] M. Grzeszczuk, D. Jadczak, E Herba Pol. **55**(3):193-199 (2009).
[3] M. Tamas, Ed. Med. Univ. "Iuliu Hatieganu" Cluj-Napoca: 215-219 (1999).
[4] B. Nickavar, A. Alinaghi, M. Kamalinejad, IJPR **7**(3):203-209 (2008).
[5] V. Ciocârlan, Ed. Ceres, Bucureşti 667-670 (2009).
[6] P. Md Hajjaj Yousuf, N.Y. Noba, M. Shohel, R. Bhattacherjee, B. Kumar, BJPR, **3**(4): 854-864 (2013).
[7] L. Riahi, M. Elferchichi, H. Ghazghazi, J. Jebali, S. Ziadi, C. Aouadhi, H. Chograni, Y. Zaouali, N. Zoghlami, A. Mliki, IND CROP PROD. **49**:883–889 (2013).
[8] F. Leal, A.C. Coelho, T. Soriano, C. Alves, M. Matos, Med Food. **16**(4):273 (2013).
[9] F. Nedel, K. Begnini, P.H. Carvalho, R.G. Lund, F.T. Beira, F.A. Del Pino, J Med Food, **15**(11):955-8 (2012).
[10] N. Ahmad, H. Fazal, I. Ahmad, B.H. Abbasi, Toxicol Ind Health. **28**(1):83-9 (2012).
[11] B. Mandana, A.R. Russly, G. Ali, S.T. Farah, International Food Research Journal *IFRJ* **18**:543-547 (2011).
[12] M.A. Ebrahimzadeh, S.F. Nabavi, S.M. Nabavi, B. Eslami, Pharmacologyonline **1**:744-752 (2010).
[13] D. Benedec, L. Vlase, I. Oniga, A.C. Mot, R. Silaghi-Dumitrescu, D. Hanganu, B. Tiperciuc, G. Crişan, **61**(2):261-267 (2013).
[14] x x x European Pharmacopoeia, 5th ed. Strasbourg: 221 (2005).
[15] K. Slinkard, V.L. Singleton, Am. J. Enol. Vitic. **28**: 49-55 (1977).
[16] x x x Farmacopeea Română, ed. a X-a, Ed. Medicală, Bucureşti (1993).
[17] A.M. Anton, A.M. Pintea, D.O. Rugină, Z.M. Sconţa, D. Hanganu, L. Vlase , D. Benedec, Dig J Nanomater Bios. **8**(3), 2013, p. 973 – 980
[18] D. Benedec, L. Vlase, D. Hanganu, I, Oniga, Dig J Nanomater Bios. **7**(3):1263-1270 (2012).
[19] J.D.D. Tamokou, J.R. Chouna, E. Fischer-Fodor, G. Chereches, O. Barbos, G. Damian, D. Benedec, M. Duma, P.A. Nkeng Efouet, H.K. Wabo, J.R. Kuiate, A.C. Mot, R. Silaghi-Dumitrescu, PloS One, **8**(2):e55880 (2013).
[20] D.S. Mi Reeves, L.O. White, 3rd Edition, Blackwel, Oxford, 140-162 (1983).
[21] H. Mi Zbakh, H. Chiheb, H. Bouziane, V. Motilva Sánchez, H. Riadi, JMBFS, **1**:219-228 (2012).