

Determination of Polyphenols in *Mentha longifolia* and *M. piperita* Field-Grown and In Vitro Plant Samples Using UPLC-TQ-MS

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Nine polyphenols in the aerial parts of *Mentha longifolia* have been separated by chromatographic techniques. Their structures have been confirmed by HPLC/electrospray ionization-MS/MS. The compounds identified included rosmarinic acid, salvianolic acid L, dedihydro-salvianolic acid, luteolin-glucuronide, luteolin-diglucuronide, luteolin-glucopyranosyl-rhamnopyranoside, and eriodictyol-glucopyranosyl-rhamnopyranoside. The extracts of *M. longifolia* and *M. piperita* field plants, in vitro plants, callus tissues, and cell suspension cultures were profiled, and their polyphenol composition was compared in different tissues and quantified using ultra-performance column liquid chromatography (UPLC)/triple-quadrupole-MS in the selected-ion recording detection mode. Determination of desired compounds was based on calibration curves obtained for standards, which were previously isolated from *M. longifolia* aerial parts. The UPLC profiles revealed considerable differences in the synthesis of secondary metabolites among samples coming from field plants, in vitro plants, callus tissues, and cell suspension cultures. Plant tissues coming from field cultivation (for both *M. piperita* and *M. longifolia*) contained several phenolic compounds (flavonoids and phenolic acids), whereas plants from in vitro conditions, callus tissues, and suspension cultures contained only a few of them. Rosmarinic acid dominated in all of these samples. These results show that under in vitro conditions, the metabolism of phenolics undergoes a fundamental change.

The genus *Mentha*, belonging to the family Lamiaceae, consists of several species and many varieties. Some sources count their number as 25–30 species. The systematics of this genus, especially at the species level, provide some difficulties, due to its long history of cultivation, high polymorphism, occurrence of hybridization in both wild and cultivated populations, and widespread occurrence. *Mentha* species are distributed all over the world and can be found in many environments. These herbs (mainly perennial) are found in damp or wet places through temperate regions of Eurasia, Australia, and South Africa (1–3). For these reasons, the number of species of the genus *Mentha* has been discussed. Recent studies, which were conducted on the basis of morphological, cytological, and chemical markers, revealed that the genus *Mentha* consists of 18 species and includes 11 named hybrids. Those hybrids are placed in four classes: *Pulegium*, *Tubulosae*, *Eriodontes*, and *Mentha* (4).

Several species of the genus *Mentha* are important agricultural crops because of their culinary and fragrance properties. *M. piperita* and *M. longifolia* leaves are used in food preparation or as a condiment in various foodstuffs, such as beverages, ice creams, candies, chewing gums, and meat dishes (5–7). In folk medicine, different *Mentha* spp. are well-known herbal remedies used to treat different diseases, especially those connected with gastrointestinal symptoms such as nausea, flatulence, vomiting, ulcerative colitis, stomach aches, and cramps (1, 3, 8).

M. longifolia and *M. piperita*, which were materials of the present research, as well as other members of this genus, produce a variety of secondary metabolites—such as terpenes, flavonoids, and phenolic acids—that have been associated with the health benefits mentioned above. These metabolites have been the subject of numerous studies, and several essential oils, flavones, and flavone glycosides have been isolated from these species. In the case of phenolics, different sources identify flavones, flavone-glycosides, and phenolic acids as well (2, 4, 5).

In vitro plant cultures, both callus and cell suspension, are an alternative system for important and valuable secondary metabolite production (especially industrial production) compared with traditional agricultural cultivation. There are

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various advantages of in vitro cultures over the conventional cultivation of whole plants. The most important benefit is the production of useful bioactive compounds under controlled conditions independent of climatic or environmental changes. Moreover, negative biological influences (microorganisms and insects) that affect secondary metabolite production in nature are eliminated. Additionally, it is possible to select cultivars with higher production of secondary metabolites to decrease costs and increase the production efficacy of secondary metabolites (9, 10).

The aim of the present study was to isolate and identify phenolics from *M. longifolia* aerial parts and to characterize the chemical composition of phenolic compounds (phenolic acids, as well as flavonoids) and determine their concentrations in extracts of *M. longifolia* and *M. piperita* plants coming from field and in vitro conditions, i.e., callus tissues and cell suspension cultures.

Experimental

Plant Material

Seeds of *M. piperita* and *M. longifolia* were received from the National Centre for Plant Genetic Resources at the Plant Breeding and Acclimatization Institute (Radzikow, Poland). Seeds were sown both in an experimental field and under in vitro conditions.

Procedures of Seed Sterilization and Sowing Under In Vitro Conditions

Seeds were washed in running tap water for approximately 15 min, sterilized in 70% ethanol for 2 min, and transferred to 10% perhydrol solution for 20 min under continuous agitation. Afterwards, they were thoroughly rinsed three times with sterile distilled water and then placed for germination on half-strength LS (Linsmaier and Skoog) basal medium (11). The medium was supplemented with 15 g/L sucrose, solidified with agar (6 g/L), and adjusted to pH 5.8, followed by autoclaving at 121°C and 0.1 MPa for 20 min. Seeds were placed on the solidified medium in Petri dishes. Seeds in Petri dishes were incubated in a growth chamber at 25°C under a 16 h light/8 h dark cycle provided by fluorescent lamps. After a few days, the sprouting plants were transferred on LS medium containing 0.2 mg/L naphthaleneacetic acid and 0.2 mg/L indole-3-acetic acid. Flasks were kept in the growth chamber under the same conditions as above.

Induction of Callus Cultures

Callus cultures were initiated using leaves coming from 3-week-old plantlets grown under in vitro conditions. Leaves were cut into small fragments and transferred to the growth media. Explants from *M. piperita* were cultured on LS medium with the addition of 2 mg/L 2iP (isopentenyladenine) and 0.5 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid). The callus cultures of *M. longifolia* were initiated on LS media containing 1 mg/L BA (6-benzylaminopurine) and 0.5 mg/L 2,4-D. Sucrose, 30 g/L, and 8 g/L agar were added to each basal medium, and pH was

adjusted to 5.8. Callus tissues were grown in Petri dishes. For each type of medium, at least 30 dishes containing 16 explants were used for callus growth. Petri dishes were incubated in a growth chamber at 25°C, with a 16 h photoperiod provided by cool white fluorescent lamps. Tissue cultures were subcultured each month.

Cell Suspension Culture Induction

Cell suspension cultures were established from callus tissue coming from leaf explants subcultured for 1 year. To establish the suspension cultures, delicate and friable calli from solid media were transferred to analogous liquid media. Fragments of callus tissue (about 200 mg) were used as an inoculum. They were suspended in 20 mL liquid medium in 100 mL Erlenmeyer flasks. In order to obtain homogeneous suspension cultures, the subculturing was performed using sterile meshes (cell dissociation kit; Sigma, St. Louis, MO) to separate large aggregates and to establish a cell culture with small clusters and single cells. Suspension cultures were incubated in an incubator shaker at 110 rpm and 25°C during the day, or 20°C at night, under illumination provided by fluorescent lamps using a 16 h photoperiod. Cell suspension cultures were subcultured to fresh media every week.

Isolation of Phenolic Standards

The aerial parts of *M. longifolia* (150 g) were finely powdered, defatted with chloroform, and extracted with 70% methanol (MeOH) overnight at room temperature. This procedure was repeated twice. The mint extracts were combined and filtered, and MeOH was removed under reduced pressure.

The crude extract was suspended in water and passed through a short column (6 × 10 cm) of 40–63 µm LiChroprep RP-18 (Merck, Darmstadt, Germany) previously preconditioned with water. The column was washed first with water to remove sugars, then with 40% MeOH to elute phenolics. This fraction was condensed nearly to dryness, dissolved in distilled water, and loaded onto a preparative column (40 × 3.5 cm) of 25–100 µm Sephadex LH-20 (Pharmacia, Stockholm, Sweden). The column was washed with MeOH. Fractions (10 mL) were collected with a fraction collector. Each fraction was monitored with TLC (cellulose; Merck), developed in 15% acetic acid, and visualized under a UV lamp. Fractions with the same profile were combined. In this way, a few fractions containing one or two phenolics were collected. They were then purified on a 25–40 µm LiChroprep RP-18 column (35 × 2 cm; Merck) using an isocratic acetonitrile (ACN)–1% H₃PO₄ system optimized for each fraction as follows, based on our long experience in the separation of natural products. Once a fraction of 1–3 compounds was obtained, isocratic separation was carried out on the semipreparative column. The proper solvent system was determined by running HPLC or ultra-performance column liquid chromatography (UPLC) with the gradient system described below and observing the concentration of ACN or MeOH in the solvent at the moment of the appearance of the peak of interest on the computer screen. Having this

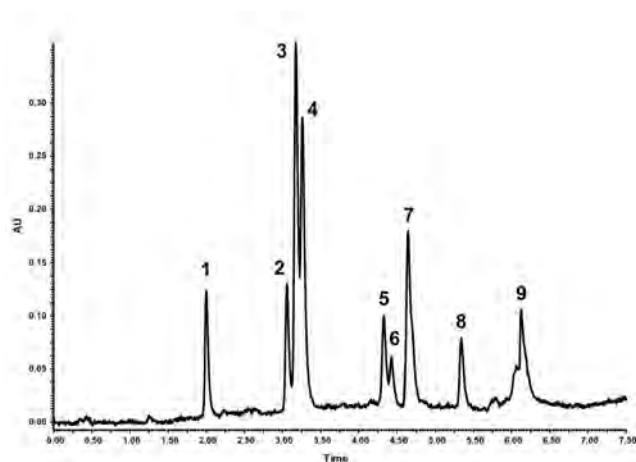


Figure 1. UPLC profile of *M. longifolia* phenolic extract (peak numbers correspond to the numbering of flavonoids in Table 1); x-axis = min.

ratio, the water concentration was increased by 5%; in 99% of cases, this was the best mobile phase for isocratic separation. This procedure optimizes for the compound to be purified, not necessarily for all compounds in the fraction. Nine individual compounds were isolated.

Preparation of Samples for Determination of Phenolics

Samples used for phenolic determination were derived from *M. piperita* and *M. longifolia* field plants, in vitro plants, callus tissues, and suspension cultures. Suspension cultures were filtered through filter paper (Whatman No. 1; Florham Park, NJ) to collect biomasses and culture filtrates. Field plants were air-dried, while in vitro plants, callus tissues, and filtered cells were lyophilized. All samples were powdered mechanically and used for extraction and purification.

Extraction and Purification

The powdered samples were extracted using an automated accelerated solvent extractor, ASE 200 (Dionex, Sunnyvale, CA). Extraction was performed with 70% MeOH at 1500 psi solvent pressure, 100°C cell temperature, flush 150%, and two static cycles for 2 min each. Extracts (25 mL) were collected in vials. The solvent was evaporated under reduced pressure at 40°C. The dried extracts were dissolved in Milli-Q water (Millipore Corp., Billerica, MA) and purified by SPE using C18 Sep-Pak cartridges (1 cm³, 360 mg; Waters Corp., Milford, MA) preconditioned with water. Cartridges were washed with water to remove carbohydrates; then to elute phenolics they were washed with 1 mL 40% (v/v) MeOH. The phenolic fractions were evaporated and then dissolved in 1 mL 40% MeOH. All analyses were performed in triplicate for three independent samples and stored in a freezer at -20°C before analysis.

Table 1. Isolated phenolics identified by HPLC/ESI-MS/MS in an extract of *M. longifolia* aerial parts

Peak No.	RT, min ^a	UV absorption peak(s), nm	[M-H] ⁻ , m/z ^b	Fragments, m/z (relative abundance)	Cone voltage ^c	Identification	Ref.
1	2.02	251.4, 338.8	637.4	461 (12), 351 (100), 285 (32)	50	Luteolin-diglucuronide	12, 13
2	3.08	283.3	595.4	449 (100), 287 (20)	50	Eriodictyol-glycopyranosyl-rhamnopyranoside	14, 15
3	3.20	252.6, 345.7	461.3	285 (100)	50	Luteolin-glucuronide	12, 13
4	3.28	253.8, 345.7	593.5	447 (30), 285 (100)	50	Luteolin-glycopyranosyl-rhamnopyranoside	16
5	4.35	284.5, 337.5	717.3	519 (25), 339 (100), 321 (25)	50	Salvianolic acid L	17
6	4.45	327.6	358.9	223 (20), 197 (25), 179 (30), 161 (100), 133 (5), 123 (5)	50	Rosmarinic acid	17, 18
7	4.66	267.3, 332.6	461.2	285 (100)	50	Luteolin-glucuronide	12, 13
8	5.36	283.3, 344.4	715.3	517 (20), 337 (100), 321 (10)	50	Dedihydro-salvianolic acid	19
9	6.20	258.7, 316.5	475.5	460 (15), 285 (100)	50	Luteolin-glucuronide-methyl	12, 13

^a RT = Retention time.

^b M = Molecular ion.

^c Cone voltage for the determination of phenolics with the TQ detector.

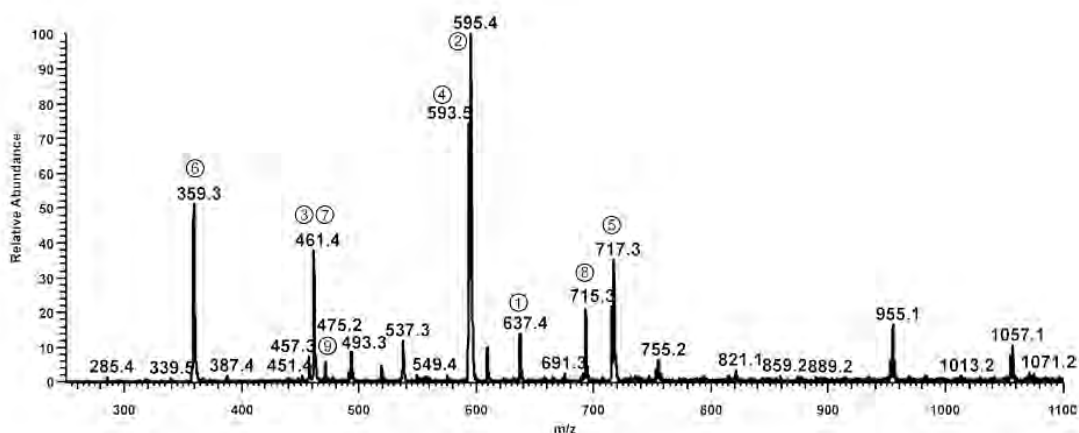


Figure 2. Direct injection ESI-MS of *M. longifolia* extract (peak numbers correspond to the numbering of flavonoids in Table 1).

UPLC and MS Analysis

A Waters ACQUITY UPLC system equipped with a binary pump system and coupled to a Waters ACQUITY TQD quadrupole tandem mass spectrometer with an electrospray ionization (ESI) source was used for quantitative analyses. All data were acquired and processed using Waters MassLynx 4.1 software with a QuanLynx program. A Waters ACQUITY UPLC BEH C18 column (50 × 2.1 mm, 1.7 μm) was used to separate the analytes. A gradient elution program was conducted for chromatographic separation with mobile phase A (water containing 0.1% formic acid) and mobile phase B [water–ACN (60 + 40) containing 0.1% formic acid] as follows: 0 (80% A); 5.1 min (50% A); 6.0 min (0%, A); 6.5 min (0% A); 7.0 min (80% A); and finished at 7.5 min. The flow rate was 0.4 mL/min, and column temperature was 50°C. Injection wash solvents were methanol–water (5 + 95, v/v) and methanol–water (50 + 50, v/v) for weak and strong wash, respectively. For MS detection, negative ESI was used as the ionization mode. Nitrogen was used as the desolvation and cone gas with a flow rate of 800 and 100 L/h, respectively.

The optimal MS parameters were as follows: capillary, 3 kV; source temperature, 120°C; desolvation temperature, 350°C; and cone voltage, 50. Quantification was performed using the selected-ion recording (SIR) mode.

The qualitative analyses for structure confirmation and positioning of phenolics in the profile were performed using a Surveyor HPLC pump, a Surveyor autosampler, a Surveyor photodiode array (PDA) detector, and an LCQ Advantage ion-trap mass spectrometer (Thermo Finnigan, San Jose, CA) equipped with Xcalibur 1.3 software.

MWs were verified by ESI-MS/MS analysis on the LCQ system. Infusion (direct injection) into the ion-trap mass spectrometer was performed using a flow of standard compounds (1 mg/mL) coming from an integrated syringe pump at a flow rate of 5 μL/min for tuning the mass spectrometer and optimizing the ESI source. The ESI source and MS/MS parameters were automatically optimized and saved in a tune file. Spray needle voltage was set at 4.2 kV for all samples, automatic gain control was on, maximum isolation time was 200 ms, and three microscans/scan were

Table 2. LOD, LOQ, linearity range, and R^2 data

Compound No.	Identification	LOD, ng	LOQ, ng	Linearity, ng	R^2
1	Luteoline diglucuronide	5.4	16.1	20–350	0.95543
2	Eriodictyol-glc-rha	0.8	2.3	2.75–750	0.97081
3	Luteoline glucuronide	2.1	6.2	10–300	0.92006
4	Luteoline-glc-rha	0.8	2.5	5–400	0.96968
5	Salvianolic acid	7.2	21.8	25–500	0.97302
6	Rosmarinic acid	6.9	20.8	50–750	0.97748
7	Luteoline glucuronide	2.5	7.6	11–300	0.92106
8	Didehydro-salvianolic acid	8.3	24.8	30–500	0.96948
9	Luteoline glucuronide-Met	4.6	14	15–300	0.90471

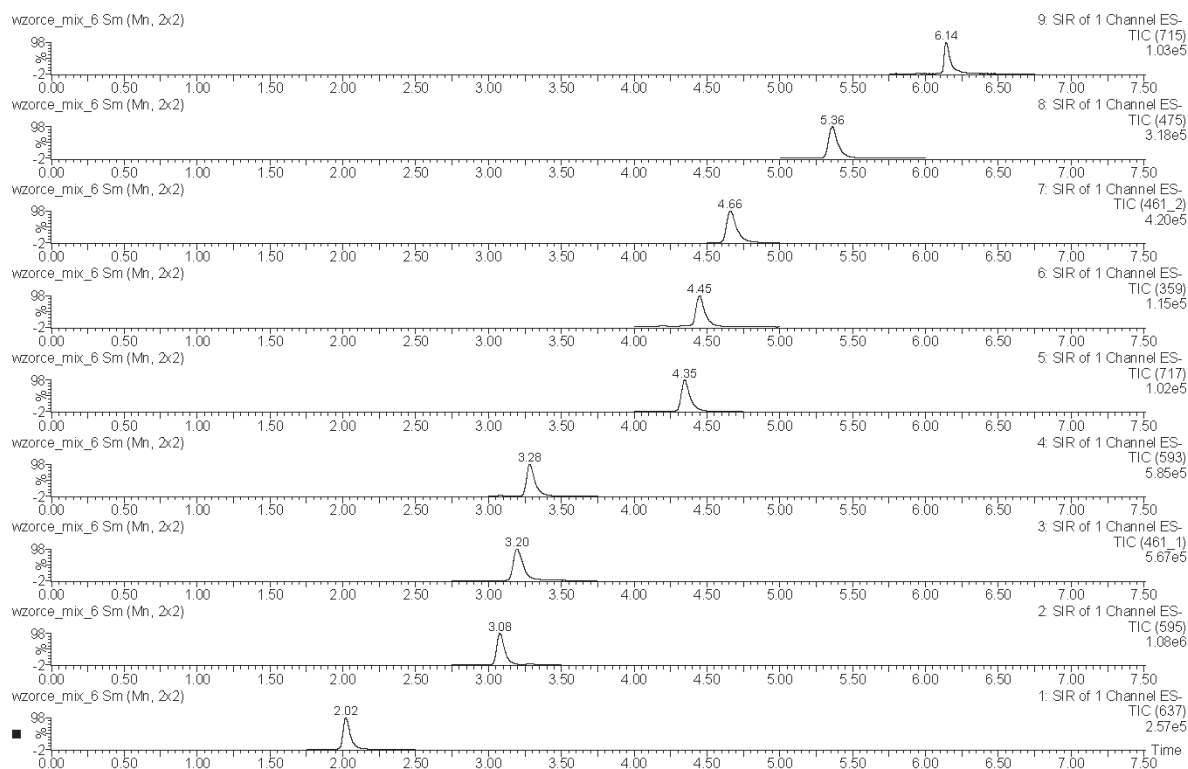


Figure 3. SIR chromatograms of mint phenolics.

acquired. Voltages on the capillary and tube lens were -10V . These were set by automatic optimization using the LCQ autotune program on the mass spectrometer. Nitrogen was used as the sheath and auxiliary gas. Helium was used as the collision gas in the ion trap. The sheath gas flow rate was set at 40 units (on a scale of arbitrary units in the 0–100 range defined in the LCQ system), the auxiliary gas was turned off, and capillary temperature was 240°C .

Results and Discussion

Analysis of Phenolics

The extraction of *M. longifolia* aerial parts with 70% MeOH yielded an extract rich in phenolics. The extract after solid-phase fractionation provided the phenolic fraction with an efficiency of 6.5% of plant dry matter. A chromatographic profile of this extract showed the presence of several phenolic compounds (Figure 1). Purification of this extract on a preparative C18 column led to recovery of nine individual phenolic compounds. Their structures were elucidated by comparison of their fragmentation patterns obtained by HPLC/ESI-MS/MS and their UV spectra with the data presented in literature. Under optimized conditions, as a result of HPLC/MS/MS analyses, two luteolin–glucuronides (**3**, **7**), methylated luteolin–glucuronide (**9**), luteolin–diglucuronide (**1**), luteolin–glycopyranosyl–rhamnopyranoside (**4**), eriodictyol–glycopyranosyl–rhamnopyranoside (**2**), rosmarinic acid (**6**), salvianolic acid L (**5**), and dedihydro–salvianolic acid (**8**)

were identified. Their chromatographic and spectral characteristics and literature references are summarized in Table 1. The two compounds showing identical UV and fragmentation patterns characteristic for luteolin–glucuronide were assumed to be the glycosides with glucuronic acid attached at two different positions of the luteolin molecule. The luteolin was among the flavone aglycones identified previously in *Mentha* spp., including *M. longifolia* and *M. piperita* (20).

In the studied plant material, we were not able to separate and identify 5,7,4'-trihydroxy-6,2',3'-trimethoxyflavone (**21**), naringenin 4'-methyl ether 7-O-fucoparanosyl-(1→6)-glucoside (**2**), tricetin 7-O-methylether 3'-O-glucoside 5'-O-rhamnoside, tricetin 3'-O-glucoside 5'-O-rhamnoside, and tricetin 3'-O-rhamnosyl-(1→4)-rhamnoside (**22**), reported previously in *M. longifolia*. The characteristic molecular ions of these compounds could not be confirmed in the molecular profile obtained using direct-injection MS (Figure 2).

In Vitro Plant Material

For biosynthesis of secondary metabolites in some cases in vitro cultures are a good option, but in some instances development of cultures may be difficult. For *Mentha* spp., this was not the case; calli and cell cultures could be easily developed. The addition of 2 mg/L 2iP and 0.5 mg/L 2,4-D in the case of *M. piperita*, and media enriched with 1.0 mg/L BA and 0.5 mg/L 2,4-D in the case of *M. longifolia* gave satisfactory results for callus tissue induction, which started

Table 3. Concentration of total phenolics (mg/g d.w.) in analyzed extracts of *M. longifolia* and *M. piperita*

<i>M. longifolia</i>				<i>M. piperita</i>			
Field plant	In vitro plant	Callus tissue	Suspension culture (cell biomass)	Field plant	In vitro plant	Callus tissue	Suspension culture (cell biomass)
17.44	5.74	21.73	12.78	41.74	10.76	37.60	61.41

after 15 days. Callus received on this media was light green (*M. piperita*) or creamy (*M. longifolia*) and friable. Small necrosis on explant surfaces were observed; however, roots or shoots were not formed. Obtained tissues were successively subcultured. Cell suspension cultures have been obtained from 1-year-old callus cultures. These cultures were evaluated by the color, growth characteristics, cell shape, and aggregate size distribution. Suspension cultures of *M. piperita* were green, and those of *M. longifolia* yellowish. After successive subculturing using meshes (cell dissociation kit), only single cells and small aggregates were observed. Cells were small and rounded, with small vacuoles. Growth characteristics included fresh weight and dry weight determinations, as well as packed cell volume. The biggest biomass of mint cells was observed 8 days after inoculation of cells into fresh medium. Similarly, the highest concentration of rosmarinic acid (RA) was also noticed during this (stationary) phase.

Phytochemical Analysis

The phenolics were produced in all examined samples (i.e., plant coming from the field, plant obtained under in vitro conditions, and callus tissue and suspension cultures) of *M. longifolia* and *M. piperita*. The individual phenolics, isolated by preparative column chromatography, were used as a standards for their positioning in the UPLC profile (Figure 1) and for quantitative determination in the mint

extracts. These determinations were carried out using the external standard method and external calibration curves. Some validation data are given in Table 2. The application of UPLC for determination of phenolics allowed us to separate these compounds in quite a short time of 7 min. However, some of the peaks in UPLC profiles were partially overlapping when the PDA detector was used (Figure 1). This created problems with proper integration of incompletely separated peaks and in the correctness of the determination. For this reason, the triple-quadrupole mass detector and the SIR mode were used for determination. Selected-ion chromatograms recorded for pseudomolecular ions produced single-peak profiles that could be easily integrated (Figure 3).

The total phenolic yields are summarized in Table 3. Their content ranged from 5.74 (*M. longifolia* in vitro plant) to 61.4 mg/g dry weight (d.w.) (*M. piperita* suspension culture cell biomass), as reported in Table 4.

The chromatographic profiles of phenolics of different samples of *M. longifolia* and *M. piperita* showed substantial differences in secondary metabolite quality and content. The chromatograms of the extracts of two field-grown mint species showed a high level of similarity; both species included all the phenolics described above. The dominant compound was flavonoid **2**, the content of which ranged from 7.60 mg/g d.w. in *M. longifolia* to 18.08 mg/g d.w. in *M. piperita*. Also, the flavone **4** was present in high amounts: 3.57 mg/g d.w. in *M. longifolia*,

Table 4. The concentration of individual phenolics (mg/g d.w.) in extracts of *M. longifolia* and *M. piperita*

Compound	<i>M. longifolia</i>				<i>M. piperita</i>			
	Field plant	In vitro plant	Callus tissue	Suspension cultures (cell biomass)	Field plant	In vitro plant	Callus tissue	Suspension cultures (cell biomass)
1	1.432	ND ^a	ND	ND	2.039	ND	ND	ND
2	7.604	0.135	0.062	0.006	18.083	0.009	ND	0.006
3	2.237	0.007	ND	Traces	3.088	Traces	ND	Traces
4	3.576	1.134	0.018	0.013	8.883	0.124	0.012	0.020
5	0.084	ND	ND	ND	0.389	ND	ND	ND
6	1.933	4.445	21.576	12.768	7.107	10.469	37.492	60.214
7	0.285	0.005	0.074	ND	1.153	0.026	0.011	0.081
8	0.282	Traces	ND	ND	0.994	0.118	0.087	1.089
9	0.007	0.013	ND	ND	0.009	0.019	ND	ND

^a ND = Not detected.

Table 5. Concentration of RA (% of total phenolics) in different samples of *M. longifolia* and *M. piperita*

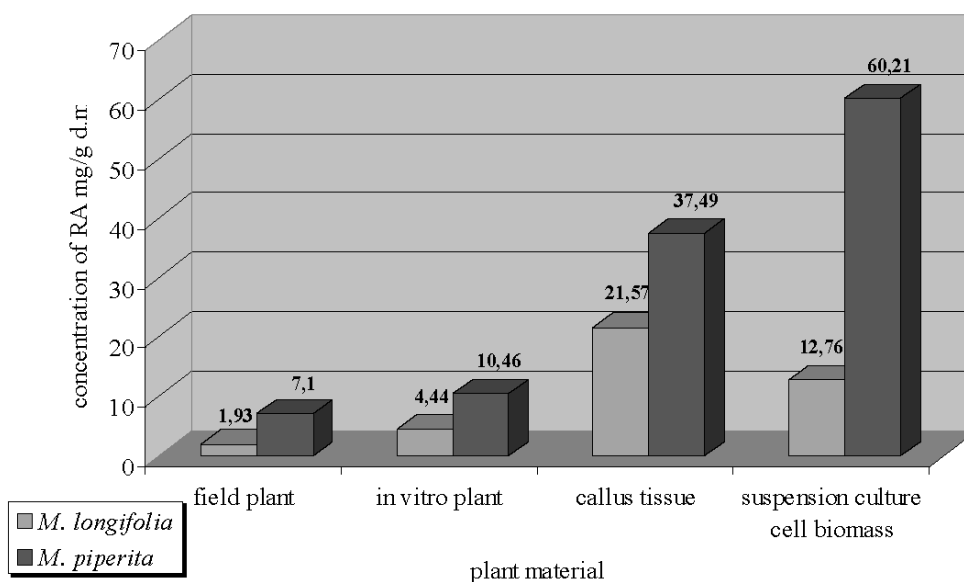
<i>M. longifolia</i>				<i>M. piperita</i>			
Field plant	In vitro plant	Callus tissue	Suspension culture (cell biomass)	Field plant	In vitro plant	Callus tissue	Suspension culture (cell biomass)
11.08	77.43	99.29	99.90	17.02	97.29	99.71	98.05

and 8.88 mg/g d.w. in *M. piperita*. Among the other secondary metabolites, RA (an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid) was identified. Its concentration was rather low in *M. longifolia* (1.93 mg/g d.w.) and slightly higher in *M. piperita* (7.10 mg/g d.w.). This compound was reported to possess several biological properties, including antiviral, antibacterial, anti-inflammatory, and antioxidant (23) activities; this is why its occurrence in plant extract is highly desired. The remaining identified phenolics were found in smaller amounts.

The shape of the phenolic profile in the in vitro plants, callus tissue, and suspension cultures of both species was substantially different from those of the field-grown plants, with RA being the dominant phenolic constituent. The highest concentration of this compound was found in *M. piperita* suspension cultures (60.21 mg/g d.w.), and it was detected during the stationary phase of the cell growth. RA was found in the cell biomass, and not detected in the culture media (Table 5). This is in good agreement with the published data, which showed that RA is readily accumulated in undifferentiated plant cell cultures, in some cases in concentrations much higher than in the parent plant itself. For example, suspension cultures of *Coleus blumei* were found to accumulate RA in concentrations of up to 21% d.w. Similarly,

suspension cultures of *Salvia officinalis* were characterized by a high concentration of RA, approaching 36% of cell d.w. (23). In the present study, RA concentrations ranged from 0.19 to 6.02% of d.w. The lowest concentrations were found in field-grown plants (0.19% for *M. longifolia* and 0.71% of d.w. in the case of *M. piperita*). In the in vitro plantlets, the content of the RA was at a slightly higher level than in field-grown plants, 0.42 and 0.98% of d.w. for *M. longifolia* and *M. piperita*, respectively. In the callus tissues and suspension cultures, concentrations were even higher; the highest contents were found in callus tissue (3.74% of d.w.) and suspension cultures (6.02% of d.w.) of *M. piperita*.

All the samples of *M. piperita* had higher concentrations of RA than did *M. longifolia* (Figure 4). In some in vitro samples, RA constituted 99.8–99.9% of total phenolics (Table 5), which shows that under in vitro conditions, plant secondary metabolism is simplified to just a few compounds. Thus, the callus and suspension cultures of mint species—especially of *M. piperita*, in which RA is present in high amounts—could be used for biotechnological production of this molecule. Application of appropriate elicitors and further optimization of the growth medium could greatly improve the efficacy of RA production in *M. piperita* cell suspension cultures.

**Figure 4. Concentration of RA (mg/g d.w.) in different samples of *M. longifolia* and *M. piperita*.**

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