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Determination of Water-Soluble Polyphenolic Compounds in Commercial Herbal Teas from Lamiaceae: Peppermint, Melissa, and Sage

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Chromatographic techniques (HPLC and HPTLC) were used for qualitative and quantitative determination of eriocitrin, luteolin 7-*O*-rutinoside, luteolin 7-*O*-β-glucuronide, lithospermic acid, rosmarinic acid, and methyl rosmarinate together with other known compounds in commercial herbal teas from the Lamiaceae family: peppermint leaf (*Menthae piperitae folium*), melissa leaf (*Melissae folium*), and sage leaf (*Salviae officinalis folium*). Contents of analyzed compounds in infusions, the most popular forms, were established using a C18 column with acetonitrile−water−formic acid as a mobile phase. The HPLC method was validated for linearity, precision, and accuracy. Luteolin 7-*O*-β-glucuronide and lithospermic acid were identified as new *Mentha* × *piperita* compounds. The investigated herbal teas delivered polyphenols in high amounts, up to 182.2 mg for the infusion of one peppermint tea bag.

KEYWORDS: Caffeate oligomers; eriocitrin; luteolin 7-*O*-β-glucuronide; lithospermic acid; methyl rosmarinate; rosmarinic acid; *Mentha* × *piperita*; *Melissa officinalis*; *Salvia officinalis*

INTRODUCTION

Lamiaceae plants are now cultivated worldwide, mainly for use as culinary and medicinal herbs. Peppermint leaf, melissa leaf (lemon balm leaf), and sage leaf are popular herbal teas and essential oil-containing drugs. The qualities of *Menthae piperitae folium*, *Melissae folium*, and *Salviae officinalis folium* are defined by volatile oil content. Melissa leaf for pharmaceutical use is also standardized to contain not less than 4.0% of total hydroxycinnamic acid derivatives expressed as rosmarinic acid. Nevertheless, all of these plants also contain other caffeate oligomers as well as flavonoid glycosides, mono-, di-, and triterpenes, etc. (*1*).

M. piperitae folium contains up to 7% of caffeic acid derivatives, flavonoids represented by lipophilic polysubstituted flavones (*O*-methylated apigenin and luteolin) and particularly by flavone and flavanone glycosides (over 17% in some clones) (*1*–*7*). Few studies (*2*–*5*) have been carried out to characterize polyphenols of peppermint extracts, and the following compounds were identified: narirutin (naringenin 7-*O*-rutinoside), eriodictyol, eriodictyol 7-*O*-β-glucoside, eriocitrin (eriodictyol 7-*O*-rutinoside), hesperidin (hesperetin 7-*O*-rutinoside), isorhoifolin (apigenin 7-*O*-rutinoside), luteolin 7-*O*-β-glucoside, luteolin 7-*O*-rutinoside, diosmin (diosmetin 7-*O*-rutinoside), rosmarinic acid, and caffeic acid as well as 4′-*O*-caffeoyl esters of apigenin glycosides (piperitoside and menthoside). Peppermint teas and extracts are used orally for symptomatic treatment of dyspepsia, flatulence, intestinal colic, and both gall bladder and biliary tract spasms. *M. piperitae folium* has carminative, spasmolytic, analgesic, antiedema, and antiallergic activity, but its medicinal uses are not supported by clinical data. It is also traditionally used to relieve nasal congestion in the common cold, as an analgesic in mouth diseases, in mouthwashes for oral hygiene, etc. The activity is predominantly, but not exclusively, due to the essential oil content (*1*).

Melissa or lemon balm leaf (from *Melissa officinalis* L.) contains caffeate oligomers (up to 11%), flavonoids (about 0.5%), and terpenoids (carnosic acid, ursolic acid, and oleanolic acid) (*1*, *8*–*12*). Two trimeric caffeate oligomers, melitric acids A and B, together with both rosmarinic and caffeic acids and their methyl esters were isolated from aerial parts of *M. officinalis* (*10*, *11*). The major flavonoid in *Melissae folium* is luteolin 3′-*O*-β-glucuronide (*12*). Lemon balm infusions and preparations are traditionally administered orally for the symptomatic treatment of gastrointestinal disturbances, functional dyspepsia, and nervous disorders in adults and children. Melissa preparations have sedative and spasmolytic actions and are used therapeutically in the form of tea infusions to treat nervous sleeping disorders and functional gastrointestinal complaints (*1*).

S. officinalis folium is rich in flavonoids (1–3%), mainly glycosides of luteolin (7-*O*-β-glucoside, 3′-*O*-β- and 7-*O*-β-glucuronide) and apigenin (6,8-di-*C*-glucosylapigenin), their methylated derivatives (genkwanin, apigenin 7,4′-dimethyl ether, luteolin 7-methyl ether), and 6-hydroxylated flavones (6-hydroxyluteolin 7-*O*-β-glucoside and 7-*O*-β-glucuronide). It contains about 2–6% labiataetannins with rosmarinic acid as the main component, as well as other caffeate oligomers such as salvianolic acid I (=melitric acid A) and its methyl ester,

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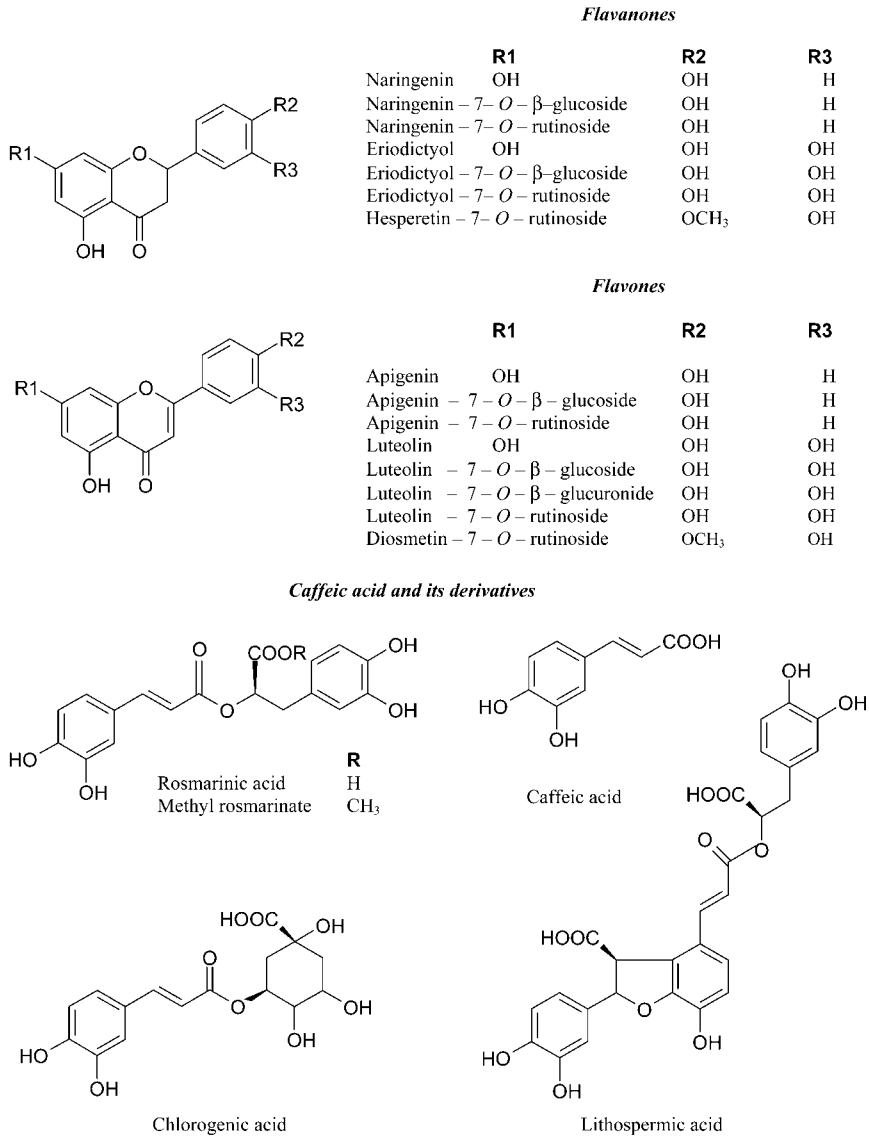

Figure 1. Structures of polyphenolic compounds analyzed in Lamiaceae.

salvianolic acids K and L, sagecoumarin, sagerinic acid, phenolic glycosides [e.g., *cis*- and *trans*-*p*-coumaric acid 4-*O*-(2′-*O*-*β*-apiosyl)-*β*-glucoside, 6-*O*-caffeoyl-*β*-fructosyl-(2→1)-α-glucoside], bitter diterpenes (carnosol, carnosic acid, methyl carnosate, rosmadial, rosmanol, epirosmanol), and triterpenes with ursane and oleanane structures (*13–20*). All caffeate oligomers showed potent antioxidant activity in three test systems several times greater than Trolox. The antioxidant activity of flavonoids was variable, and luteolin glycosides were more active than apigenin glycosides (*18*). Sage tea is used orally in case of gastrointestinal problems and for excessive perspiration. It is also recommended for inflammation of mucous membranes of the mouth and throat as a gargle (*1*).

The chemical composition of peppermint, lemon balm, and sage volatile oils has been widely investigated. Several HPLC and TLC-densitometry methods for analyzing caffeate oligomers and flavonoids in lamiaceous plants have been published (*2–5, 11, 21–25*). In most of the HPLC methods reversed-phase columns were used. Methanol or acetonitrile aqueous solutions were often used as elution mobile phases with a small amount of phosphoric acid, acetic acid, or formic acid added to the eluent which markedly improved separation. UV or photodiode array detections were most commonly used with typical detection wavelengths of 280 and 320–360 nm. Both caffeic and rosmarinic acids, their methyl esters, flavanones, and flavones were estimated in either aqueous or aqueous methanolic extracts of aromatic herbs, such as *Mentha* × *piperita*, *Mentha spicata*, *Melissa officinalis*, *Rosmarinus officinalis*, *Salvia officinalis*, and *Thymus vulgaris* by different HPLC methods (*2–5, 11, 22–25*). Moreover, lithospermic acid and several salvianolic acids were determined in *Salvia miltiorrhiza* using both HPLC-DAD and HPLC-MS techniques (*26, 27*).

In this work we have examined infusions of *Menthae piperitae fol.*, *Melissae fol.*, and *Salviae officinalis fol.*, in order to determine the presence and content of some investigated flavanones, flavones, and caffeate oligomers using chromatographic techniques. The aims of the present paper were to thoroughly estimate polyphenolic compound concentration in commercially available herbal teas from peppermint, melissa, and sage leaves; to evaluate the level of extracted polyphenols in three successive infusions; and to implement simple and rapid HPLC and TLC methods for polyphenolic compounds from these species.

MATERIALS AND METHODS

Solvents and Chemicals. Organic solvents and reagents used in the experimental section were of analytical grade. Acetonitrile was HPLC

Table 1. Validation Parameters of HPLC Method II

standard	r	linear range (μg/mL)	LOD (ng)	LOQ (ng)	repeatability (% CV)[a]	intermediate precision (% CV)[a]	recovery (% ± SD)[b]
			Flavanones				
naringenin	0.9999	25–150	11.9	15.1	1.5	3.0	100.3 ± 1.6
naringenin 7-*O*-β-glucoside	0.9999	10–150	12.2	16.0	2.4	3.8	100.0 ± 1.8
narirutin	0.9999	10–150	12.8	44.1	2.7	3.3	99.9 ± 1.2
eriodictyol	0.9998	10–100	11.3	16.1	2.7	4.0	100.5 ± 1.2
eriodictyol 7-*O*-β-glucoside	0.9999	10–150	23.2	26.0	1.1	2.2	100.1 ± 1.4
eriocitrin	0.9997	10–300	27.9	32.4	1.7	2.7	99.3 ± 1.5
hesperidin[c]	0.9993	10–150	7.2	12.1	2.4	3.8	99.9 ± 0.9
			Flavones				
apigenin	0.9997	12.5–125	9.0	28.0	2.5	3.0	99.7 ± 1.1
isorhoifolin	0.9997	10–100	2.4	8.0	1.2	2.1	100.1 ± 1.3
luteolin	0.9999	10–100	3.5	8.8	1.0	2.1	99.9 ± 1.0
luteolin 7-*O*-β-glucuronide[d]	0.9999	10–150	5.0	12.2	2.0	2.8	99.5 ± 0.8
luteolin 7-*O*-rutinoside	0.9998	25–200	25.7	33.6	1.8	2.8	100.1 ± 1.0
			Caffeic Acid and Its Derivatives				
caffeic acid	0.9999	10–150	3.5	5.0	1.9	2.2	100.0 ± 0.8
rosmarinic acid	0.9998	10–150	11.6	20.9	1.7	3.4	99.6 ± 1.1
methyl rosmarinate	0.9996	10–150	9.2	21.2	2.2	3.2	97.9 ± 1.7
lithospermic acid	0.9999	25–300	35.2	48.9	2.3	3.4	101.1 ± 1.1
chlorogenic acid	0.9998	25–200	11.2	17.5	2.2	3.9	99.2 ± 1.1

[a] % CV value of standard peak area ($n = 7$) for concentration 0.05 mg/mL (1000 ng per injection). [b] Recovery test ($n = 3$). [c] In this chromatographic condition hesperidin migrates together with diosmin. [d] Luteolin 7-*O*-β-glucuronide with luteolin 7-*O*-β-glucoside ($n = 6$).

gradient grade. Water used was glass-distilled and deionized. Methanol (MeOH), acetonitrile (MeCN), and 98–100% formic acid were from Merck (Germany), diisopropyl ether was from Sigma-Aldrich (USA), and glacial acetic acid, acetone, and others were from POCH (Poland).

Standards. Eriocitrin, luteolin 7-*O*-rutinoside, hesperidin, and diosmin were isolated from the peppermint leaf (*28*); luteolin 7-*O*-β-glucuronide and lithospermic acid were isolated from the wild thyme herb; methyl rosmarinate was isolated from the thyme herb (*29*). Rosmarinic acid, naringenin, naringenin 7-*O*-β-glucoside, narirutin, eriodictyol, eriodictyol 7-*O*-β-glucoside, apigenin, apigenin 7-*O*-β-glucoside, isorhoifolin, luteolin, and luteolin 7-*O*-β-glucoside were from Extrasynthese (France). Chlorogenic acid was purchased from Fluka (Switzerland) and caffeic acid from Koch-Light Laboratories (U.K.). Structures of all standards as well as isolated compounds are presented in **Figure 1**.

Stock standard solutions (1 mg/mL) were prepared by dissolving 2–5 mg of an individual compound in 2–5 mL of MeOH and filtered through a 0.45 μm membrane filter (Millipore, USA). Working standard solutions (0.01–0.3 mg/mL) were obtained by dilution with MeOH.

Plant Material and Sample Preparation. Herbal teas in the form of commercially available dried and crushed leaves were purchased from local chemists within 2001–2006 (as tea bags or a loose plant material). Leaves of peppermint (Mp), melissa (Mo), and sage (So) were produced by the Polish pharmaceutical industry and food industry: Kawon Sp.j., Phytopharm Kleka S.A., Herbapol Białystok S.A., Herbapol Wrocław S.A., Herbapol Kraków S.A., Herbapol Lublin S.A., Herbapol Łódź S.A., Herbalux S.C., Zakład Konfekcjonowania Ziół FLOS, P.P.H Ziołopex Sp.zo.o., and Kotanyi Polonia Sp.zo.o. Standards 1 and 2 of the peppermint leaf were from *Mentha* × *piperita* L. var. *officinalis* cultivated in the Medicinal Plant Garden of Wrocław Medical University. Leaves were collected from plants at a flowering stage and carefully dried.

Infusions (aqueous extracts) were obtained from 2.000 g of each sample of dried leaves. To obtain infusions of examined herbal teas, boiling distilled water (250 mL) was poured over the plant material, mixed, filtered after 15 min (Whatman No. 1), and rinsed into volumetric flasks, made up to 250 mL with water. After blending, 10 mL of each infusion for the TLC was acidified with 0.05 mL of formic acid and concentrated to 5 mL by a SPE technique with a SPE C_{18}, 6 mL, 1000 mg octadecyl solid-phase extraction microcolumn (Baker, USA). Polyphenols were then eluted two times with 2.5 mL of MeOH and combined. For HPLC analysis, 5 mL of infusion was filtered through a 0.45 μm membrane filter (Millipore, USA) and immediately used.

The detailed quantitative and qualitative analyses of Mp, Mo, and So infusions were performed with both HPLC and HPTLC methods.

Apparatus and Chromatographic Conditions. UV spectra were recorded on a Perkin-Elmer UV/vis Lambda 20 spectrometer. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and 2D NMR experiments were recorded on a Bruker 300 spectrometer using the residual solvent peaks as internal standard. HR ESI MS were recorded in MeOH on a Mariner API-TOF mass spectrometer with Mariner Data Explorer version 3.2. Thin-layer chromatography was carried out on 10 × 20 cm HPTLC NH_2 and HPTLC LiChrospher Si60 F_{254S} plates (Merck, Germany). In HPTLC methods, SPE-concentrated infusions (20–50 μL) and standards (5–20 μL) were manually applied to the plates as 10 mm bands. Chromatograms were horizontally developed in a Teflon DS chamber (Chromdes, Poland) at a distance of 9 cm from the origin. HPTLC NH_2 plates were developed with a mobile phases of acetone−formic acid (85:15 v/v) and acetone−acetic acid (85:15 v/v) and HPTLC LiChrospher Si60 F_{254S} plates with a mobile phase of diisopropyl ether−acetone−formic acid−water (55:25:10:10 v/v/v/v). Plates were then dried in a stream of warm air. Compounds were detected under UV light at 254 and 365 nm before and after spraying with a natural product polyethylene glycol reagent (NP/PEG) or 2% methanolic $AlCl_3$ and in visible light after treatment with bisdiazotized sulfanilamide.

High-performance liquid chromatography was performed on a Knauer system (Germany) equipped with two (type 64) pumps, a sample injector, and a type 87.00 variable wavelength UV detector. A Beta Basic-18, 250 × 4.6 mm i.d., 5 μm C_{18} column (Thermo Hypersil, U.K.) and a 100 × 4.6 mm i.d. Chromolith Performance RP-18e column with a 5 × 4.5 mm i.d. Chromolith RP-18e precolumn were used. The polyphenolic profiles were recorded at a wavelength of 280 nm. Water-soluble compounds were analyzed using an acetonitrile−water gradient with formic acid (0.2% or 5%) according to solvent programs of method I, II, or III. The injection volume for all samples was 20 μL. Solvent solutions were vacuum degassed with ultrasonication prior to usage. A flow rate, composition of mobile phases, and profile of gradients for HPLC methods I−III were as follows: method I (Beta-Basic-18 column, flow rate 0.9 mL/min) solvent A, 5% formic acid in MeCN; solvent B, 5% formic acid in water; commencing with 10% A in B, rising to 40% after 25 min and then to 70% after 30 min; method II (Beta-Basic-18 column, flow rate 0.9 mL/min) solvent A, 5% formic acid in MeCN; solvent B, 5% formic acid in water; commencing with 15% A in B,

Table 2. Polyphenolic Compounds (mg/g) Delivered by *Menthae piperitae folium*[a]

no.	Er	Lr	Eg	Lgr	Nr	Ir	Ng	Hr	E	TF	CA	LA	RA	TC	TPP
1	23.9	9.7	0.2	3.0	0.2	0.8	0.7	2.1	0.6	*41.3*	0.1	3.2	5.1	*8.4*	***49.7***
2	12.6	8.4	0.2	2.9	0.1	0.9	0.5	1.5	0.3	*27.3*	0.1	2.5	2.8	*5.4*	***32.7***
3	10.7	5.7	0.2	1.9	0.0	0.7	0.6	1.6	0.2	*21.6*	0.1	1.7	4.6	*6.4*	***27.9***
4	11.0	6.1	0.2	2.7	0.0	0.3	0.6	1.2	0.4	*22.5*	0.1	1.7	3.4	*5.2*	***27.7***
5	32.2	14.1	0.3	4.1	0.5	1.0	0.9	2.2	0.3	*55.5*	0.1	3.5	7.8	*11.5*	***67.0***
6	17.9	5.5	0.3	2.5	0.2	0.7	0.8	1.9	0.1	*29.8*	0.1	2.0	5.1	*7.2*	***37.0***
7	13.2	7.7	0.2	2.9	0.0	1.1	0.8	1.5	0.1	*27.3*	0.1	2.5	5.8	*8.4*	***35.7***
8	7.9	5.2	0.2	1.7	0.1	0.7	0.2	0.9	0.4	*17.2*	0.0	1.3	1.3	*2.7*	***19.8***
9	34.0	10.7	0.3	3.2	0.3	0.7	2.2	2.2	0.6	*54.1*	0.1	4.4	8.0	*12.5*	***66.6***
10	20.0	10.7	0.3	3.8	0.3	0.7	1.4	2.5	0.3	*39.9*	0.2	6.0	11.2	*17.4*	***57.3***
11	34.4	9.4	0.3	3.7	0.0	1.8	0.7	2.3	0.9	*53.6*	0.1	4.1	6.3	*10.5*	***64.1***
12	10.9	6.7	0.2	2.5	0.0	0.7	0.6	1.2	0.2	*23.0*	0.1	1.6	3.2	*4.9*	***27.9***
13	8.9	6.4	0.2	2.3	0.2	0.6	0.4	1.0	0.4	*20.4*	0.1	1.3	2.4	*3.8*	***24.3***
14	18.1	8.1	0.1	2.4	0.9	0.2	0.9	2.6	0.4	*33.8*	0.2	3.7	12.5	*16.4*	***50.2***
15	8.3	5.2	0.2	1.8	0.0	0.6	0.5	1.0	0.5	*18.1*	0.2	1.6	3.4	*5.2*	***23.3***
16	24.0	7.5	0.2	3.5	0.1	0.5	1.7	1.9	0.2	*39.6*	0.1	4.7	4.4	*9.2*	***48.8***
17	11.2	6.1	0.3	2.3	0.2	0.2	0.5	1.3	0.5	*22.5*	0.1	1.8	3.4	*5.3*	***27.8***
18	39.9	7.6	0.4	4.3	0.2	1.5	2.3	2.1	2.2	*60.4*	0.1	5.0	9.1	*14.2*	***74.6***
19	9.5	5.7	0.2	2.3	0.0	0.8	0.4	1.0	0.3	*20.2*	0.1	1.2	3.0	*4.3*	***24.6***
20	19.4	6.7	0.2	2.6	0.1	0.7	0.4	1.9	0.4	*32.3*	0.1	2.3	3.2	*5.7*	***38.0***
21	15.4	7.4	0.2	2.9	0.3	1.3	0.8	2.2	0.4	*30.9*	0.2	3.3	5.1	*8.6*	***39.5***
22	11.1	6.0	0.2	2.0	0.1	0.8	0.7	1.9	0.4	*23.2*	0.2	1.8	4.7	*6.7*	***29.9***
23	9.5	5.2	0.1	2.3	0.2	0.4	0.9	1.2	0.4	*20.2*	0.2	1.6	3.0	*4.8*	***25.0***
24	53.3	11.3	0.2	3.8	0.0	1.9	0.9	2.0	0.9	*74.3*	0.2	3.7	7.5	*11.4*	***85.7***
25	16.2	7.0	0.1	2.5	0.0	0.8	0.7	2.0	0.5	*29.8*	0.1	1.2	4.8	*6.1*	***35.9***
26	13.6	7.8	0.2	3.2	0.1	0.7	0.7	1.5	0.4	*28.1*	0.1	1.5	5.5	*7.1*	***35.3***
27	6.4	4.1	0.1	1.5	0.1	0.1	0.5	0.9	0.2	*13.9*	0.1	0.8	1.2	*2.1*	***16.0***
28	28.3	10.1	0.1	3.6	0.0	1.1	2.2	2.0	0.6	*48.0*	0.2	3.5	7.8	*11.5*	***59.5***
29	17.0	9.0	0.2	3.4	0.3	1.3	1.2	2.2	0.1	*34.6*	0.2	5.1	9.0	*14.4*	***48.9***
30	30.9	12.2	0.2	3.7	0.3	1.1	0.8	2.1	0.8	*52.1*	0.1	3.6	6.6	*10.3*	***62.4***
31	8.7	5.9	0.2	2.2	0.0	0.7	0.4	1.0	0.3	*19.4*	0.1	1.3	2.4	*3.7*	***23.1***
32	10.7	6.6	0.2	2.3	0.0	0.8	0.6	1.2	0.2	*22.7*	0.1	1.6	3.0	*4.8*	***27.4***
33	16.6	7.0	0.2	2.3	0.1	1.6	0.8	2.3	0.8	*31.7*	0.1	2.9	11.8	*14.8*	***46.4***
34	7.9	4.7	0.1	1.7	0.0	0.3	0.4	0.8	0.6	*16.4*	0.1	1.2	3.3	*4.6*	***20.9***
35	21.8	7.9	0.2	3.4	0.0	0.6	1.6	1.7	0.5	*37.6*	0.2	4.4	4.3	*8.8*	***46.4***
36	8.3	4.7	0.3	1.8	0.1	0.4	0.5	1.0	0.3	*17.4*	0.1	1.4	2.7	*4.3*	***21.6***
37	37.1	11.7	0.2	3.9	0.2	0.9	2.0	2.0	1.5	*59.5*	0.1	4.7	8.7	*13.5*	***73.0***
38	9.6	5.5	0.2	2.2	0.0	0.8	0.4	1.1	0.2	*19.9*	0.1	1.3	3.2	*4.6*	***24.5***
39	11.6	6.2	0.2	2.3	0.0	0.5	0.6	1.8	0.3	*23.4*	0.2	1.8	3.9	*5.8*	***29.2***
40	12.0	6.7	0.2	2.5	0.0	0.7	0.5	1.5	0.4	*24.5*	0.2	1.8	3.2	*5.2*	***29.7***
41	12.5	5.9	0.2	2.1	0.1	0.8	0.7	2.0	0.2	*24.4*	0.3	2.2	4.9	*7.4*	***31.8***
42	17.4	8.8	0.2	3.0	0.2	0.8	0.8	2.0	0.2	*33.4*	0.2	2.5	6.9	*9.6*	***43.0***
43	48.2	15.5	0.2	4.4	0.2	0.9	3.4	2.3	0.6	*75.6*	1.2	6.9	7.4	*15.5*	***91.1***
44	46.2	14.4	0.2	4.1	0.2	0.8	3.8	2.1	0.8	*72.5*	1.0	7.3	8.0	*16.3*	***88.8***
45	12.5	6.6	0.2	2.0	0.1	0.7	0.7	1.9	0.3	*24.9*	0.2	2.2	4.0	*6.4*	***31.2***
St 1	149.6	25.7	0.7	5.0	0.2	1.7	8.9	3.7	0.7	*196.1*	0.4	18.1	16.1	*34.6*	***230.7***
St 2	149.0	21.9	1.0	4.5	0.3	3.0	8.1	3.2	0.6	*191.0*	0.2	15.5	12.7	*28.3*	***219.3***
mean 1–45	18.9	7.8	0.2	2.8	0.1	0.8	1.0	1.7	0.5	*33.7*	0.2	2.8	5.3	*8.3*	***42.0***
SD	12.0	2.7	0.1	0.8	0.2	0.4	0.8	0.5	0.4	*16.5*	0.2	1.6	2.7	*4.1*	***19.9***
min	6.4	4.1	0.1	1.5	0.0	0.1	0.2	0.8	0.1	*13.9*	0.0	0.8	1.2	*2.1*	***16.0***
max	53.3	15.5	0.4	4.4	0.9	1.9	3.8	2.6	2.2	*75.6*	1.2	7.3	12.5	*17.4*	***91.1***
median 1–45	13.6	7.0	0.2	2.5	0.1	0.7	0.7	1.9	0.4	*28.1*	0.1	2.2	4.7	*7.1*	***35.7***

[a] Key: no., number of commercially available samples evaluated; *TF*, total flavonoids; *TC*, total caffeic acid derivatives; *TPP*, total polyphenols; Er, eriocitrin; Lr, luteolin 7-*O*-rutinoside; Eg, eriodictyol 7-*O*-β-glucoside; Lgr, luteolin 7-*O*-β-glucuronide; Nr, narirutin; Ng, naringenin 7-*O*-β-glucoside; Hr, hesperidin; E, eriodictyol; L, luteolin; N, naringenin; CA, caffeic acid; RA, rosmarinic acid; St 1, peppermint standard 1; St 2, peppermint standard 2.

rising to 35% after 25 min and then to 70% after 27 min; method III (Chromolith Performance RP-18e column, flow rate 1.5 mL/min) solvent C, 0.2% formic acid in MeCN; solvent D, 0.2% formic acid in water; commencing with 10% C in D, rising to 25% after 15 min and to 35% after 17 min and then to 70% after 19 min. All chromatographic experiments were performed at 20 °C.

Method Validation. Calibration plots of analyzed compounds were constructed on the basis of peak areas (y) using six concentration solutions (x) to determine a linearity of HPLC methods. All plots were linear in the examined range (0.01–0.3 mg/mL). Linear ranges have been shown as an amount of standard (nanograms) applied with a single injection. The limits of detection (LOD) and quantification (LOQ) were calculated from calibration equations using a signal-to-noise ratio (respectively S/N $\geq$ 3:1 and S/N $\geq$ 10:1) and expressed as an amount (nanograms) of examined polyphenol per injection. Precision was expressed as the coefficient of variation (% CV) of multiple independent determinations. Repeatability (injection precision) and intermediate precision were performed using mixtures of standards solutions (Mix17) at a concentration of 0.05 mg/mL (1000 ng per injection) to measure peak areas. Luteolin 7-*O*-β-glucoside was validated individually. To measure repeatability, the same sample was independently analyzed seven times according to the same HPLC procedure. Intermediate precision was examined using seven samples which were prepared with the same sample preparation procedure and analyzed with the same chromatographic conditions within three different days (interday precision). Accuracy of the method was evaluated with the recovery test. Samples of infusions were prepared to contain an analyzed compound in amounts between 0.05 and 0.1 mg/mL. An equal volume

Table 3. Polyphenolic Compounds (mg/g) Delivered by *Melissae folium*[a]

no.	Lg/TF	CA	RA	MeR	Cd1/RA	Cd2/RA	Cd3/RA	TC	TPP
1	0.9	0.2	19.5	0.9	1.3	0.9	5.0	*27.8*	***28.7***
2	0.8	0.2	20.3	1.0	1.0	0.9	3.6	*27.0*	***27.9***
3	0.3	0.4	24.6	0.7	1.2	1.2	5.4	*33.4*	***33.7***
4	0.4	0.2	11.0	0.0	0.9	0.7	4.1	*17.0*	***17.4***
5	0.2	0.2	26.1	3.3	1.8	2.5	9.9	*43.7*	***43.9***
6	0.9	0.2	19.8	0.7	1.4	1.1	6.0	*29.3*	***30.1***
7	0.9	0.2	19.4	1.2	1.0	0.8	4.4	*26.9*	***27.7***
8	1.2	0.3	22.1	0.8	1.0	1.1	4.9	*30.1*	***31.4***
9	0.5	0.2	9.1	0.1	0.5	0.6	1.9	*12.4*	***12.9***
10	1.8	0.2	23.6	4.9	1.7	2.4	7.5	*40.3*	***42.1***
11	0.5	0.3	19.7	2.4	1.1	0.1	5.6	*29.3*	***29.8***
12	0.2	0.1	5.2	0.5	1.6	0.3	1.6	*9.3*	***9.5***
13	1.5	0.4	20.5	2.2	1.2	1.3	5.0	*30.5*	***31.9***
14	0.8	0.3	29.6	3.7	1.6	1.3	4.3	*40.6*	***41.4***
15	0.7	0.3	29.6	2.9	1.1	1.2	3.3	*38.5*	***39.2***
16	1.3	0.2	28.5	2.8	1.0	1.0	7.4	*40.9*	***42.1***
17	1.3	0.2	14.8	2.3	0.8	0.8	6.5	*25.4*	***26.7***
18	0.9	0.1	12.8	2.9	0.8	1.8	4.0	*22.5*	***23.3***
19	0.9	0.1	24.6	2.6	1.0	1.9	3.7	*33.8*	***34.7***
20	0.3	0.5	28.1	5.5	1.2	1.1	6.4	*42.6*	***43.0***
21	0.4	0.5	25.3	6.2	1.3	1.7	2.1	*37.0*	***37.4***
22	0.3	0.3	20.6	0.1	1.2	1.4	5.1	*28.6*	***28.9***
23	0.4	0.4	20.1	0.1	1.1	1.2	4.6	*27.4*	***27.8***
24	0.4	0.2	19.7	0.1	1.1	1.2	5.2	*27.5*	***27.8***
25	0.7	0.3	31.6	2.6	2.4	1.4	8.6	*46.9*	***47.6***
27	0.6	0.3	27.3	0.8	1.0	0.9	3.8	*34.0*	***34.6***
28	0.8	0.3	27.2	6.6	1.6	1.9	6.5	*44.0*	***44.8***
29	0.6	0.3	32.6	5.1	2.6	1.6	10.8	*52.9*	***53.5***
mean	0.7	0.3	21.9	2.3	1.3	1.2	5.3	*32.1*	***32.9***
SD	0.4	0.1	6.8	2.0	0.4	0.5	2.2	*10.1*	***10.2***
min	0.2	0.1	5.2	0.0	0.5	0.1	1.6	*9.3*	***9.5***
max	1.8	0.5	32.6	6.6	2.6	2.5	10.8	*52.9*	***53.5***
median	0.7	0.3	21.4	2.2	1.1	1.2	5.0	*30.3*	***31.6***

[a] Key: no., number of commercially available samples evaluated; TF, total flavonoids; TC, total caffeic acid derivatives; TPP, total polyphenols; Lg, luteolin 7-*O*-β-glucoside; CA, caffeic acid; RA, rosmarinic acid; MeR, methyl rosmarinate; Cd1/RA−Cd3/RA, caffeic acid derivatives calculated as RA.

Table 4. Polyphenolic Compounds (mg/g) Delivered by *Salviae officinalis folium*[a]

no.	Er	Lr	Eg	Lgr	TF	CA	RA	Cd4/RA	TC	TPP
1	0.3	0.4	0.2	4.0	*4.9*	0.6	7.2	1.8	*9.6*	***14.5***
2	0.3	0.4	0.2	6.5	*7.3*	0.5	11.1	2.2	*13.8*	***21.2***
3	0.3	0.5	0.5	10.6	*11.8*	0.3	15.7	6.3	*22.2*	***34.0***
4	0.5	0.4	0.3	5.4	*6.5*	0.2	21.9	8.0	*30.1*	***36.6***
5	0.4	0.7	0.3	8.5	*9.9*	0.4	11.5	2.7	*14.6*	***24.5***
6	0.4	0.4	0.3	5.5	*6.6*	0.3	19.2	6.0	*25.5*	***32.1***
7	0.3	0.4	0.2	8.4	*9.3*	0.3	2.9	0.3	*3.5*	***12.8***
8	0.4	0.5	0.2	5.6	*6.7*	0.4	3.9	0.9	*5.2*	***11.9***
9	0.5	0.4	0.6	8.0	*9.4*	0.4	10.3	3.6	*14.3*	***23.7***
10	0.3	0.5	0.4	6.9	*8.1*	0.2	4.3	1.3	*5.8*	***13.8***
11	0.3	0.3	0.3	10.5	*11.3*	0.3	18.4	1.4	*20.1*	***31.4***
12	0.3	0.4	0.3	5.6	*6.5*	0.2	5.3	1.4	*6.9*	***13.4***
13	0.5	0.4	0.3	8.3	*9.5*	0.5	5.8	1.0	*7.3*	***16.8***
14	0.5	0.3	0.4	7.3	*8.6*	0.3	5.3	1.9	*7.5*	***16.0***
15	0.5	0.3	0.3	6.0	*7.0*	0.7	8.4	2.3	*11.4*	***18.4***
16	0.3	0.4	0.5	6.6	*7.7*	0.3	9.9	1.6	*11.9*	***19.6***
17	0.4	0.4	0.3	5.4	*6.4*	0.3	17.0	6.3	*23.6*	***29.9***
18	0.3	0.5	0.3	7.3	*8.4*	0.3	5.0	1.5	*6.9*	***15.2***
19	0.3	0.4	0.3	5.8	*6.7*	0.2	5.3	1.5	*6.9*	***13.7***
20	0.3	0.5	0.2	7.4	*8.3*	0.4	5.2	1.3	*6.9*	***15.2***
21	0.5	0.4	0.3	8.5	*9.7*	0.4	10.3	3.7	*14.4*	***24.1***
22	0.3	0.4	0.4	10.2	*11.3*	0.3	15.4	1.9	*17.6*	***29.0***
23	0.4	0.3	0.3	9.1	*10.1*	0.5	11.1	2.8	*14.4*	***24.5***
24	0.3	0.4	0.4	5.3	*6.3*	0.2	22.1	8.3	*30.6*	***36.9***
25	0.3	0.4	0.2	8.4	*9.3*	0.3	3.0	0.4	*3.7*	***13.0***
26	0.3	0.4	0.3	5.6	*6.6*	0.2	3.9	1.1	*5.1*	***11.8***
mean	0.4	0.4	0.3	7.2	*8.2*	0.3	10.0	2.8	*13.1*	***21.3***
SD	0.1	0.1	0.1	1.8	*1.8*	0.1	6.0	2.3	*8.0*	***8.3***
min	0.3	0.3	0.2	4.0	*4.9*	0.2	2.9	0.3	*3.5*	***11.8***
max	0.5	0.7	0.6	10.6	*11.8*	0.7	22.1	8.3	*30.6*	***36.9***
median	0.3	0.4	0.3	7.1	*8.2*	0.3	9.2	1.8	*11.6*	***19.0***

[a] Key: no., number of commercially available samples evaluated; TF, total flavonoids; TC, total caffeic acid derivatives; TPP, total polyphenols; Er, eriocitrin; Lr, luteolin 7-*O*-rutinoside; Eg, eriodictyol 7-*O*-β-glucoside; Lgr, luteolin 7-*O*-β-glucuronide; CA, caffeic acid; RA, rosmarinic acid; Cd4/RA, the caffeic acid derivative calculated as RA.

of a standard solution with the same amount of this compound was than added. Results for validation of HPLC method II are presented in **Table 1**. HPLC method I was validated similarly (*29*).

Content Measurement. Quantitative determination was carried out using external standards for calibration. A concentration of analyzed compounds was measured in duplicate as the mean of independent solutions using either HPLC method I (Mo and So infusions) or HPLC method II (Mp infusions) and solvent system with the 5% formic acid addition. We calculated an amount (milligrams per gram) of individual polyphenols delivered by 1 g of a dried herbal product (Mp, Mo, So) used to prepare the infusion. A total polyphenol content (TPP) was calculated as the sum of all detected polyphenolic compounds which were present in analyzed species from their average values. Total contents of flavonoids (TF) and caffeic acid derivatives (TC) were calculated similarly. The mean values, standard deviations (SD), medians, and both minimum and maximum amounts of all achieved results were also determined (**Tables 2**−**4**).

RESULTS AND DISCUSSION

Chromatographic Conditions. To optimize the HPLC separation conditions, we studied the effect of acetonitrile and formic acid concentration on the resolution of the octadecyl columns used. Optimal chromatographic conditions determined first for a mixture of examined polyphenols were then applied to the analysis of infusions. Both columns used in our experiment separated lithospermic acid from rosmarinic acid, but on the Beta Basic column lithospermic acid migrated before rosmarinic acid, contrary to results observed for the Chromolith column where we recorded picks inversion (**Figure 2**). Flavonoids were better separated by the Beta Basic column, which enabled us to separate narirutin from isorhoifolin with method II. The detection was carried out at 280 nm, the UV absorption maximum of flavanones. Therefore, for a peppermint polyphenol analysis we chose method II. Since water-soluble polyphenols from lemon balm and sage contain more caffeate derivatives than flavonoids for these samples, we decided to choose method I, which provided separation RA from undefined compounds Cd1 and Cd4 (**Figure 3**). The linear gradients described allowed good separation of all components, yielding well-resolved peaks within a short time (35 min). The chromatograms of reference compound mixtures are shown in **Figure 2**. Typical HPLC chromatograms from the analysis of peppermint, lemon balm, and sage infusions are presented in **Figure 3**.

Sample Extraction. The average volume of cups and glasses used by consumers is 200–300 mL. We accepted 250 mL as the most representative volume of infusions. Herbal tea bags contain about 1.5–2.0 g of a crushed plant source. We performed repeated extraction to determine how many polyphenolic compounds were normally extracted using boiling water, i.e., analysis of extraction efficiency. Seventeen different herbal teas from Lamiaceae were three times infused using the same procedure (2 g, 3 × 250 mL, 15 min). Since analyzed polyphenols are also the main constituents of both thyme and wild thyme herbs (*29*), we used them additionally in this experiment. **Table 5** shows the percentage of caffeic acid derivatives and flavonoids in different herbal tea infusions. It was observed that the highest content of both caffeate oligomers and flavonoids

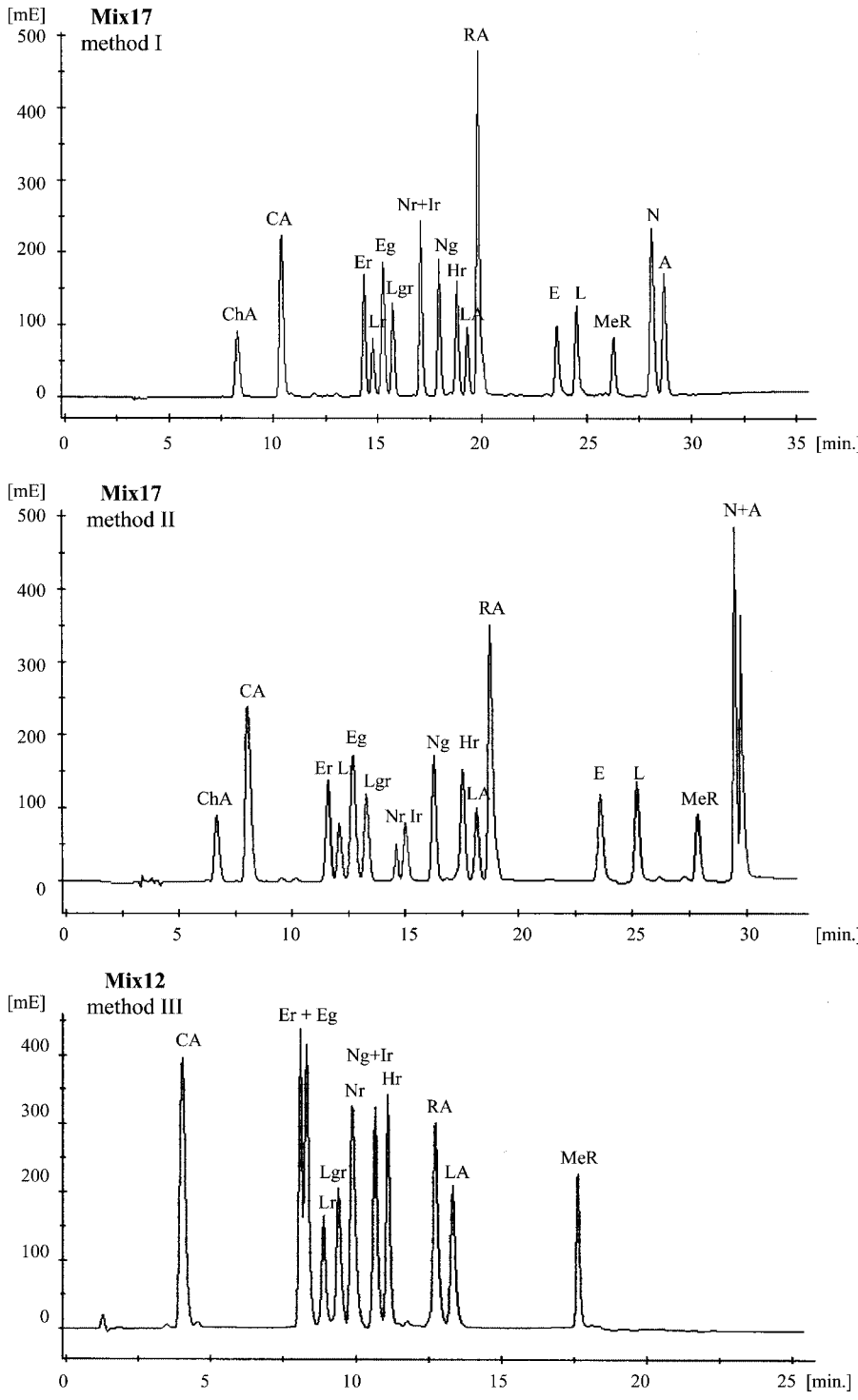

Figure 2. HPLC RP18 chromatograms of standard mixtures (UV, $\lambda = 280$ nm) achieved for methods I−III. Key: CA, caffeic acid; Er, eriocitrin; Lr, luteolin 7-*O*-rutinoside; Eg, eriodictyol 7-*O*-β-glucoside; Lgr, luteolin 7-*O*-β-glucuronide; Ir, isorhoifolin; Nr, narirutin; Ng, naringenin 7-*O*-β-glucoside; Hr, hesperidin; LA, lithospermic acid; RA, rosmarinic acid; E, eriodictyol; L, luteolin; MeR, methyl rosmarinate; N, naringenin; A, apigenin.

was in the first infusion (rosmarinic acid up to 95.2%, lithospermic acid 93.5%, eriocitrin 88.6%, luteolin 7-*O*-rutinoside 84.4%, luteolin 7-*O*-β-glucoside 95.5%, and luteolin 7-*O*-β-glucuronide 88.3%). The content of polyphenols substantially decreased with the later infusions; however, the infusing rate for the flavonoids was relatively slower. Since these data directly resulted from brewing herbal tea, it can be used to evaluate the intake of polyphenols from drinking infusion.

Method Validation. The external standard method was used to obtain the regression equations. The calculated results for method II are given in **Table 1**. All of the standard compounds showed good linearity ($r \geq 0.9993$) in the concentration range between 0.01 and 0.3 mg/mL. LOD and LOQ were expressed as nanograms per injection in the range from 2.4 ng for isorhoifolin to 35.2 ng for lithospermic acid (LOD) and from 5.0 to 48.9 ng (LOQ) for caffeic acid and lithospermic acid, respectively (**Table 1**). In our experiment repeatability and intermediate precision were about 1.0−2.7% CV and 2.1−4.0% CV, respectively. Recoveries were between 97.9% and 101.1%. Therefore, the precision and recovery of suggested HPLC method II for the determination of water-soluble polyphenolic compounds in herbal teas from peppermint are sufficient.

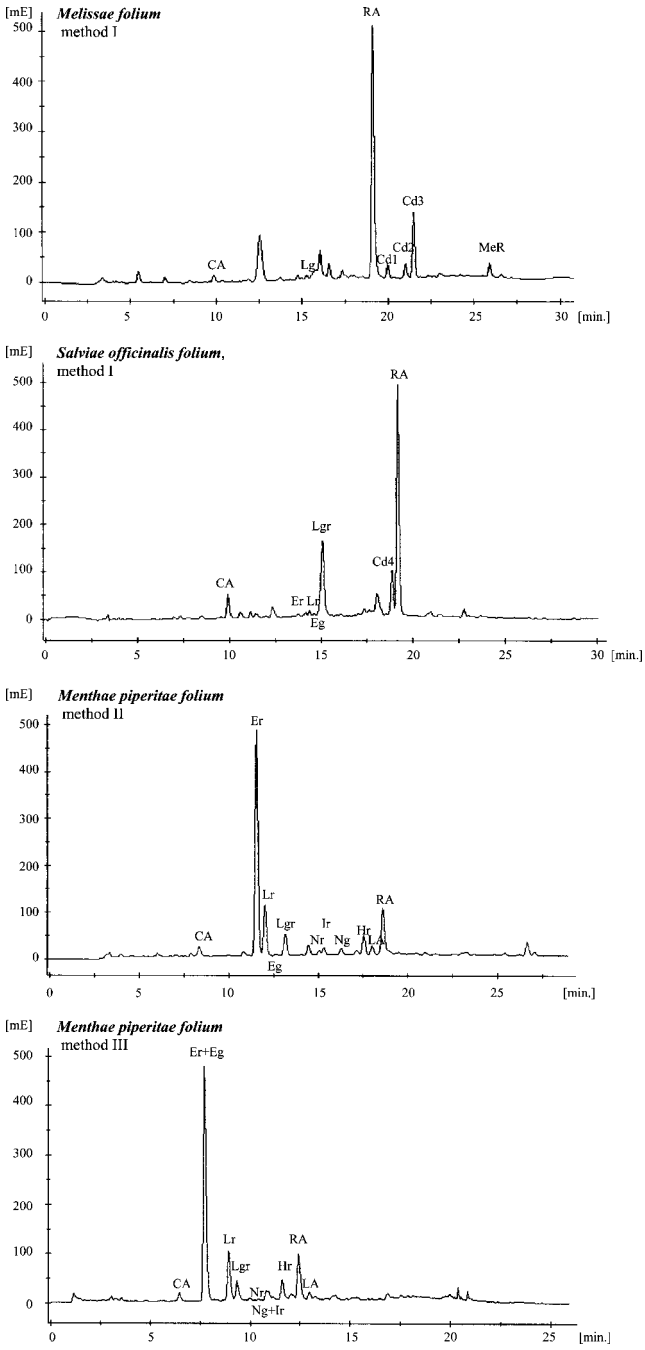

Figure 3. HPLC RP18 chromatograms of typical infusions (UV, $\lambda = 280$ nm). Key: CA, caffeic acid; Er, eriocitrin; Lr, luteolin 7-*O*-rutinoside; Eg, eriodictyol 7-*O*-β-glucoside; Lgr, luteolin 7-*O*-β-glucuronide; Lg, luteolin 7-*O*-β-glucoside; Nr, narirutin; Ng, naringenin 7-*O*-β-glucoside; Hr, hesperidin; LA, lithospermic acid; RA, rosmarinic acid; E, eriodictyol; MeR, methyl rosmarinate.

Validation parameters of HPLC method I were described previously for analysis of polyphenols from *Thymus vulgaris*, *Thymus serpyllum*, and *Majorana hortensis* (*29*).

Isolation, Chromatographic Analysis, and Content Assay. We performed the isolation and identification of eriocitrin, luteolin 7-*O*-rutinoside, hesperidin, and diosmin from the 50% aqueous acetone extract of peppermint. Luteolin 7-*O*-β-glucuronide and lithospermic acid were separated from wild thyme, while methyl rosmarinate was from thyme (*29*). Structures of isolated polyphenols were elucidated from their UV, ^{1}H and ^{13}C NMR, HMQC, and HR ESI MS spectra compared with literature data (*17, 28–33*).

Table 5. Efficiency of Extraction

compound	mean (% ± SD) first infusion	second infusion	third infusion
Menthae piperitae folium, $n = 8$			
eriocitrin	88.6 ± 3.4	10.1 ± 3.5	1.4 ± 0.5
luteolin 7-*O*-rutinoside	84.4 ± 3.5	13.3 ± 3.3	2.6 ± 0.5
luteolin 7-*O*-β-glucuronide	82.1 ± 3.2	13.0 ± 2.9	5.3 ± 0.8
hesperidin	89.3 ± 2.8	10.5 ± 3.0	0.0
lithospermic acid	92.0 ± 1.7	8.0 ± 1.7	0.0
rosmarinic acid	93.6 ± 2.6	4.8 ± 2.0	1.0 ± 0.1
total polyphenols, TPP	88.3 ± 2.6	8.9 ± 2.1	1.7 ± 0.4
Melissae folium, $n = 4$			
rosmarinic acid	95.2 ± 1.7	4.1 ± 1.6	0.8 ± 0.1
luteolin 7-*O*-β-glucoside	95.5 ± 1.6	3.9 ± 1.7	0.6 ± 0.1
total polyphenols, TPP	95.3 ± 1.7	4.0 ± 1.7	0.7 ± 0.1
Thymi herba and *Serpylli herba*, $n = 5$			
luteolin 7-*O*-β-glucuronide	88.3 ± 3.5	10.2 ± 3.5	1.5 ± 0.2
lithospermic acid	93.5 ± 4.2	7.1 ± 0.6	0.0
rosmarinic acid	92.1 ± 2.7	7.0 ± 2.7	0.9 ± 0.1
total polyphenols, TPP	90.7 ± 2.7	7.8 ± 2.8	1.1 ± 0.1

In this work we evaluated the amount of flavanones, flavones, and caffeic acid derivatives from herbal teas containing *Menthae piperitae fol.*, *Melissae fol.*, and *Salviae officinalis fol.* by HPLC methods I and II (**Figures 2** and **3**). Compounds detected have been expressed as milligrams per 1 g of dried herbs, which are reported in **Tables 2**−**4**. We calculated the content of polyphenols in infusions that are the most popular form of preparations. The presence of flavanones, flavones, caffeic acid, and caffeate esters in the commercial herbal teas was additionally confirmed by planar chromatography using HPTLC LiChrospher Si60 and HPTLC NH_2 plates (**Figures 4** and **5**).

The predominant identified compounds in the peppermint leaf infusions were glycosides of flavanones and flavones. Melissa leaf infusions contained mainly caffeate oligomers; sage leaves delivered caffeate oligomers and glycosides of flavones. The principal caffeic acid derivative in all analyzed species was the caffeate dimer, rosmarinic acid. Two compounds were in common with all species: caffeic acid and rosmarinic acid. Lithospermic acid (caffeate trimer) was identified for the first time in the genus *Mentha*, while luteolin 7-*O*-β-glucuronide has already been described only in *Mentha longifolia* L. by Bourwieg (*30*). To confirm the presence of lithospermic acid in peppermint samples analyzed by HPLC methods II and III, a detailed high-performance thin-layer chromatography comparison was carried out with the corresponding reference substance (**Figures 4** and **5**).

The main compound of peppermint leaves was eriocitrin that appeared in an amount up to 15% calculated with reference to the dried material (standards 1 and 2) (**Table 2**). The content of eriocitrin was comparable with the results of Guédon (*2*), who studied *Mentha* × *piperita* clones (6.6–15% of dried leaves). It is noteworthy that commercial products yielded eriocitrin in the lower range varying from 6.4 to 53.3 mg per 1 g (0.6–5.3%). Another eriodictyol derivative, eriodictyol 7-*O*-β-glucoside, was found in a concentration below 0.4 mg/g. Free eriodictyol had a mean value of 0.5 mg/g. Two rutinosides, hesperidin (mean 1.7 mg/g) and narirutin (mean 0.1 mg/g), and one 7-*O*-β-glucoside of naringenin (mean 1.0 mg/g) were also present among flavanones. Flavones were represented by apigenin, luteolin, and diosmetin glycosides. Luteolin 7-*O*-rutinoside was the second most abundant compound in peppermint leaf infusions (mean 7.8 mg/g), which also agrees with Guédon (*2*). This compound was found in all commercially available peppermint teas studied and reached the concentration

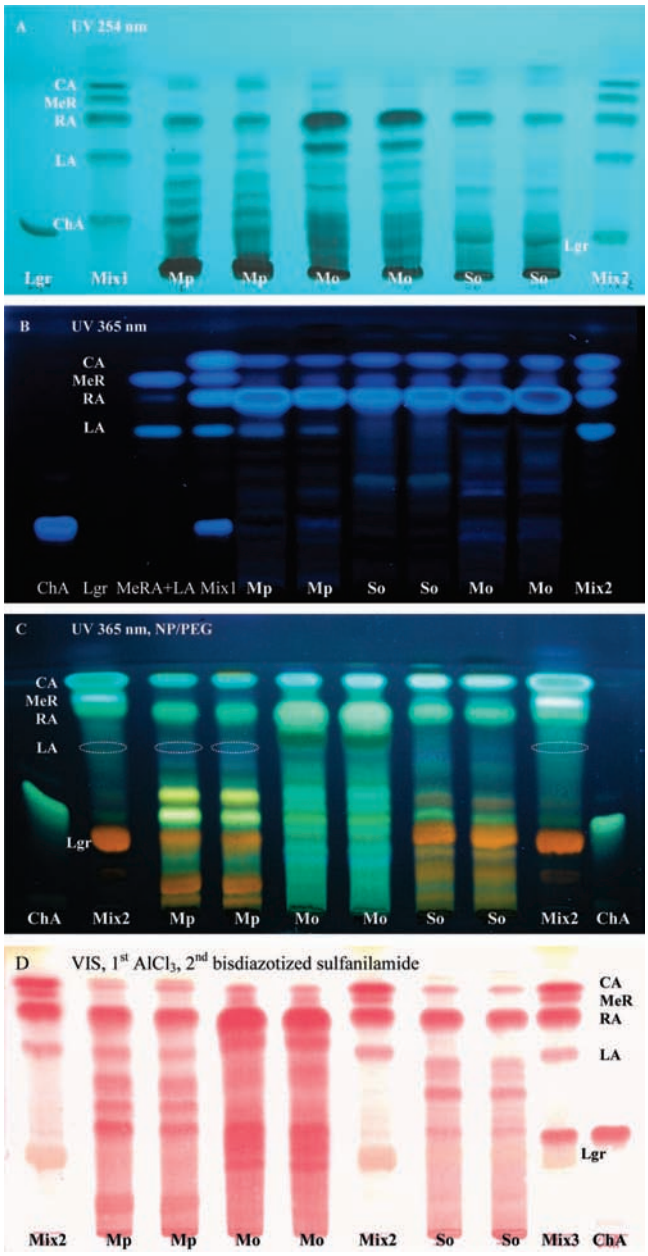

Figure 4. HPTLC Si60 Lichrospher chromatograms of analyzed infusions and standards developed with diisopropyl ether−acetone−water−formic acid (55:25:10:10 v/v). Compounds were detected under UV light at 254 nm **(A)**, 365 nm before **(B)** and after **(C)** treatment with NP/PEG, and in visible light with $AlCl_3$ and bisdiazotized sulfanilamide **(D)**. Tracks: Lgr, luteolin 7-*O*-β-glucuronide; CA, caffeic acid; ChA, chlorogenic acid; LA, lithospermic acid; MeR, methyl rosmarinate; RA, rosmarinic acid; Mix1, ChA + LA + RA + MeR + CA; Mix2, Lgr + LA + RA + MeR + CA; Mix3, Lgr + ChA + LA + RA + MeR + CA; Mp, *Mentha* × *piperita* leaf; Mo, *M. officinalis* leaf; So, *S. officinalis* leaf.

of 0.4–1.6% (dried leaves), which was lower than in leaves of *Mentha* × *piperita* clones (1.0–2.3%). Other flavone rutinosides, isorhoifolin and diosmin, were recorded in amounts below 1.9 mg/g. The highest content of luteolin 7-*O*-β-glucuronide was observed in *S. officinalis folium*, between 4.0 and 10.6 mg/g (mean 7.2 mg/g) (**Table 4**). *M. pipperitae folium* delivered 1.5−4.4 mg/g (mean 2.8 mg/g) of this flavonoid. Sage leaf infusions also delivered luteolin 7-*O*-rutinoside (mean 0.4 mg/g), eriocitrin (mean 0.4 mg/g), and eriodictyol 7-*O*-β-glucoside (mean 0.3 mg/g). Luteolin was present in infusions from both sage and peppermint leaves, but its concentration was below

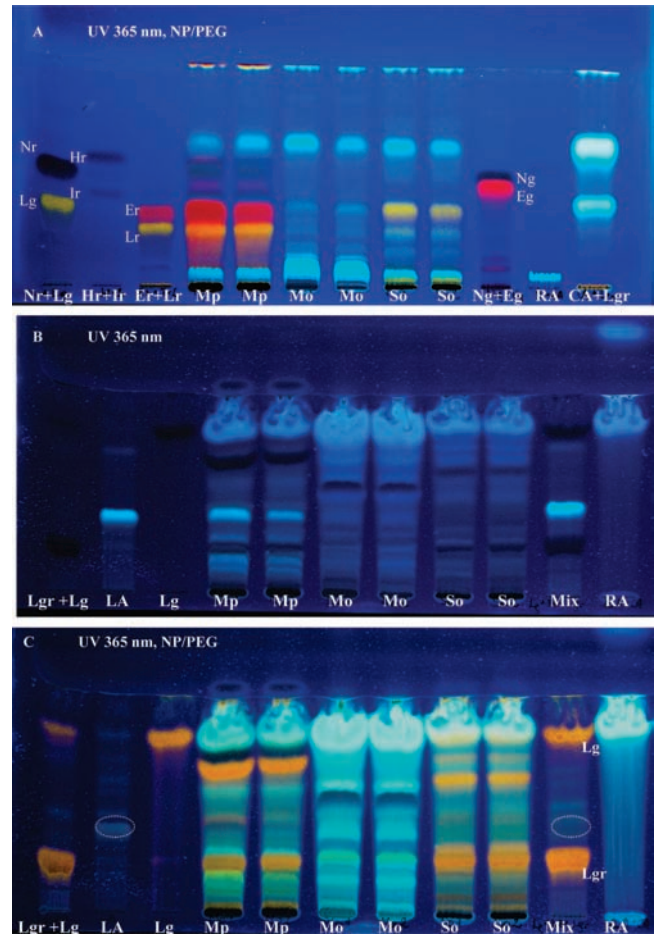

Figure 5. HPTLC NH_2 chromatograms of analyzed infusions and standards developed with acetone−acetic acid (85:15 v/v) **(A)** and acetone−formic acid (85:15 v/v) **(B, C)**. Tracks: Ng, naringenin 7-*O*-β-glucoside; Eg, eriodictyol 7-*O*-β-glucoside; Er, eriocitrin; Hr, hesperidin; Ir, isorhoifolin; Lg, luteolin 7-*O*-β-glucoside; Lgr, luteolin 7-*O*-β-glucuronide; CA, caffeic acid; LA, lithospermic acid; RA, rosmarinic acid; Mix, Lgr + LA + Lg; Mp, *Mentha* × *piperita* leaf; Mo, *M. officinalis* leaf; So, *S. officinalis* leaf.

the LOQ. Since luteolin 7-*O*-β-glucoside migrated together with luteolin 7-*O*-β-glucuronide in the HPLC methods used, it was identified in sage only by thin-layer chromatography with HPTLC NH_2 plates (**Figure 5**), except for lemon balm leaves where luteolin 7-*O*-β-glucuronide was absent (0.2–1.8 mg/g). No traces of chlorogenic acid, apigenin, and its 7-*O*-β-glucoside were detected in analyzed infusions.

Herbal teas containing melissa leaf supplied 5.2–32.6 mg/g (mean 21.9 mg/g) of rosmarinic acid (**Table 3**). Sage and peppermint leaves delivered rosmarinic acid from 2.9 to 22.1 mg/g (mean 10.0 mg/g) and from 1.2 to 12.5 mg/g (mean 5.3 mg/g), respectively. The rosmarinic acid contents were found to be comparable with results of others (*2*, *3*, *5*, *8*, *22–24*, *31*). Peppermint also contained lithospermic acid up to 7.3 mg/g (mean 2.8 mg/g), but in lemon balm and sage this compound was not observed. Methyl rosmarinate occurred only in *Melissae fol.* in amounts below 6.6 mg/g (mean 2.3 mg/g). Free caffeic acid was estimated in all herbal teas in low concentration (up to 1.2 mg/g). Among analyzed polyphenols we also detected other caffeate oligomers named Cd1−Cd4 (**Figure 3**) which were probably melitric acids (*Melissa officinalis*) or salvianolic acids (*Salviae officinalis*). They appeared in studied species in a variable quantity, and their content was expressed as rosmarinic acid (**Tables 3** and **4**). These undefined caffeate

oligomers were also observed as blue, dark brown, or red bands on HPTLC chromatograms (**Figures 4** and **5**).

The total content of polyphenols (TPP) represented by derivatives of caffeic acid (TC) and flavonoids (TF) and determined by the use of HPLC methods I and II ranged from 9.5 to 91.1 mg per 1 g of dried leaves, which was equal to or higher than described essential oil contents. In sage the average TPP value was 50% lower than that calculated for peppermint. The high concentration of caffeic acid derivatives up to 52.9 mg/g was observed in lemon balm (mean TC, 32.1 mg/g). Leaves of sage and peppermint delivered caffeic acid derivatives up to 30.6 mg/g and 17.4 mg/g (mean TC, 13.1 and 8.3 mg/g), respectively. Peppermint provided an exceptionally great amount of flavanone and flavone glycosides up to 75.6 mg/g (mean TF, 33.7 mg/g). Sage was also rich in flavone glycosides up to 11.8 mg/g (mean TF, 8.2 mg/g). According to Guédon (*2*) results eriocitrin accounted for about 80% of the total flavonoid glycosides identified in peppermint clones. In our research eriocitrin comprised 77% of TF and 66% of TPP calculated for average values of peppermint standards 1 and 2. In commercial teas the amount of eriocitrin was found to be 56% of TF and 45% of TPP, but the estimated TF and TPP values included also luteolin 7-*O*-β-glucuronide, naringenin 7-*O*-β-glucoside, free eriodictyol, and lithospermic acid, which had not been signified by Guédon (*2*). The predominant compound of melissa teas was rosmarinic acid (68% of TC, 67% of TPP). Sage delivered especially rosmarinic acid (76% of TC and 47% of TPP) and luteolin 7-*O*-β-glucuronide (88% of TF and 34% of TPP).

Polyphenolic compounds are plant secondary metabolites with a broad spectrum of bioactivity. Caffeate oligomers exhibit various properties such as antioxidative, antimutagenic, antiinflamatory, hepatoprotective, and antimicrobial. Rosmarinic acid shows antithrombotic, antiallergic, and antiviral activity against herpes simplex and human immunodeficiency virus (*19, 31, 35*). Lithospermic acid also has radical scavenging and anti-HIV properties. Moreover, rosmarinic and lithospermic acids inhibit gonadotrophin release and xanthine oxidase and adenylate cyclase activities. They prevent lipid peroxidation and platelet aggregation (*18, 19, 35, 36*). Luteolin 7-*O*-β-glucuronide reveals antioxidant (*18, 31*), antiallergic (*34*), and antigonadotropic effects (*37*). Luteolin 7-*O*-rutinoside also exhibits an antiallergic effect, and it may be clinically useful in alleviating nasal symptoms of allergic rhinitis (*7*). Eriocitrin and other peppermint polyphenols are powerful antioxidants and free radical scavengers (*38, 39*).

Although processing methods and species characteristics vary, which might be responsible for the observed variance in the flavonoids and caffeic acid derivative content, most herbal teas are good dietary sources of antioxidants from these phytochemical classes. Despite the fact that utilization of herbal teas in different pharmaceutical forms is becoming of interest to many people, making a cup of herbal tea by brewing the plant material directly is still the most common form of intake. The level of extracted polyphenolic compounds in the infusion is very high, with the first infusion delivering from 88% to 95% of total polyphenols (**Table 5**), which agrees with the findings of Carnat and co-workers (*8*). A typical herbal tea bag contains 2 g of crushed plant material. Therefore, if one tea bag (2 g) of peppermint leaves is consumed, about 16.6 mg of caffeate oligomers and 67.4 mg of flavonoids would be ingested (mean 84.0 mg of polyphenols). Similarly, one melissa tea bag (2 g) gives about 64.2 mg of caffeate oligomers (mean 65.8 mg of polyphenols), but the same sage tea bag gives only 26.2 mg of caffeate oligomers and 16.4 mg of flavonoids (mean 42.6 mg of polyphenols). It is commonly known that tea beverages are one of the main sources of dietary polyphenols (*25, 40*). Based on the USDA Database for the Flavonoid Content of Selected Foods (*40*) one cup of infusion prepared from 2g of green tea leaves (NBD No. 99070) delivers an average of 11.1 mg of flavonols and flavones determined as aglycons, which is less than herbal teas studied in this work. However, the content of polyphenols is several times higher (mean 266.7 mg) because catechins and epicatechins mainly as gallic acid esters are the predominant compounds (mean 255.6 mg). The investigated commercial herbal teas provide eriocitrin, luteolin 7-*O*-rutinoside, luteolin 7-*O*-β-glucuronide, hesperidin, rosmarinic acid, lithospermic acid, and other water-soluble polyphenols in great amounts. Therefore, we can conclude that the detected polyphenolic compounds together with essential oil might be considered as potential active constituents of infusions studied in this work. The proposed HPLC and HPTLC methods enable successful separation of estimated polyphenols and can be used for rapid analysis of commercial herbal drugs and herbal teas.

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