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ORIGINAL ARTICLE

Content of selected biologically active compounds in tea infusions of widely used European medicinal plants

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Abstract
Herbal tea infusions are a very important source of flavonoids and other biologically active compounds in human medicine and nutrition. *Melissa officinalis, Agrimonia eupatoria, Sambucus nigra, Achillea millefolium, Filipendula ulmaria, Betula pendula* and *Glechoma hederacea* were selected as common European medicinal herbs and sources for tea infusion preparations. Quercetin, rutin, catechin, chlorogenic acid, and squalene were determined in the prepared infusions. Free quercetin was not found in any of the infusions, but tea infusions did contain rutin and other quercetin glycosides, the content of which was, after acid hydrolysis, determined as quercetin. The highest levels of total quercetin were found in infusions from *Filipendula ulmaria* and *Sambucus nigra* (120 and 108 mg L^{-1}, respectively) corresponding to the content of rutin found also in these two infusions (25.2 and 194 mg L^{-1}, respectively). The *Sambucus nigra* infusion contained the largest content of chlorogenic acid (166 mg L^{-1}), and infusions from *Melissa officinalis, Agrimonia eupatoria, Betula pendula* and *Glechoma hederacea* contained only small amounts of squalene.

Key words: medicinal plant; *Filipendula ulmaria*; *Sambucus nigra*; phenolic compounds; quercetin; rutin; HPLC; MEKC

INTRODUCTION

Extracts from either whole plants or parts of plants have been used in medicine from time immemorial. Some substances derived from plant preparations are still in use for medicinal purposes, although hundreds of synthetic medicaments are now commonly available. Many medicinal plants are used in both folk and official medicine for the cure, or at least the alleviation of the symptoms, of many diseases. There are many procedures for acquiring effective preparations from medical plants, and the most common is the preparation of water extracts in the form of herbal tea infusion or decoction, a procedure which is modified according to the stability of the biologically active substances in the processed medicinal plant.

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Fecka and Turek (2007) have evaluated the content of flavanones, flavones and caffeic acid derivatives in herbal teas from the *Lamiaceae* family using HPLC and HPTLC. Katalinić et al. (2006) have published a survey of the antioxidant capacity of substances from seventy medicinal plant infusions, measuring the total phenolic content using the Folin-Ciocalteau assay and the total antioxidant capacity using the Ferric Reducing/Antioxidant Power assay, having prepared the infusions in the same way in which teas are prepared for normal human refreshment. Atoui et al. (2005) have studied herbal infusions, their polyphenolic content, antioxidant capacity and phenolic profiles from nine different kinds of teas after ethyl acetate extraction of the water extract. Twelve types of tea infusions were analyzed by Hertog et al. (1993) to ascertain the content of quercetin, kaempferol, myricetin, luteolin and apigenin after extraction and acid hydrolysis of the flavonoid glycosides. Among other tea infusions, lemon balm has also been recently studied by Kulišić-Bilušić et al. (2008) for its antioxidant and acetylcholine esterase inhibiting activity.

One group of pharmacologically active substances occurring in medicinal plants are phenolic compounds of various chemical structures which possess important biological activities manifested in both the original plant and other organisms. Some of the phenolic compounds are effective antioxidants, which are able to quench hydroxyl and peroxide radicals. Cook and Samman (1996) have described the principle behind the antioxidant effect, and have shown that these substances are able to bind oxidation catalysing cations into chelates. The anti-oxidative effect of these substances is effective particularly on blood lipids, which are protected by these antioxidants against oxidative damage.

The properties of dietary polyphenols have been described in a review by Fresco et al. (2006) and one group of phenolic compounds – the flavonoids – are the subject of several review articles (Lampe 1999, Nijveldt et al. 2001).

The main goal of our paper was to describe the occurrence and content of quercetin and rutin and other minor biologically active compounds in seven tea infusions widely used in Europe.

MATERIAL AND METHODS

Plant materials

We investigated seven species of medicinal plants which are frequently used in the Czech Republic in herbal teas. The plants were identified using the botanical key of Kubát et al. (2002) and are listed in Table 1.

The selected plants were collected and processed according to the recommendations of Hubík et al. (1989) and Tomko et al. (1989), at the stage of vegetation most suitable for the preservation of the maximal content of biologically active substances The plants were air-dried to a constant weight (for approximately seven days) at room temperature of 22 °C and the dried plants were then homogenised with a laboratory mill. The powder thus obtained was used immediately for the determination of the content of phenolic compounds and for the preparation of tea infusions. All the analyses were carried out in triplicate. The results were presented in tables with standard deviations (±SD).

Reagents and material

Chemicals and polyphenol standards were obtained from the Sigma-Aldrich (St. Louis, MO, USA) and Spolana (Neratovice, Czech Republic). Solvents of chromatographic grade were from

Table 1. Review of analysed medicinal plants

Latin name	Common name	Part of plant used
Filipendula ulmaria	Meadowsweet	Herb
Melissa officinalis	Lemon balm	Herb
Betula pendula	Silver birch	Leaves
Sambucus nigra	Black elder	Inflorescence
Achillea millefolium	Bloodwort	Herb
Agrimonia eupatoria	Common agrimony	Herb
Glechoma hederacea	Ground ivy	Herb

Merck (Darmstadt, Germany). Solid phase extraction columns RP-18 were purchased from Merck and Phenomenex (STRATA-X, Torrance, CA, USA). A Sigma 2-5 centrifuge was used (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany). Samples were homogenized with Grindomix GM 200 (Retsch GmbH, Haan, Germany). The extracts were filtered through glass micro fibre filters (1.7 μm pore size) from Filpap (Štětí, Czech Republic).

Preparation of tea infusions

About 1.5 g of the plant powder was mixed with hot drinking water (150 ml, 90 °C) in a glass bottle and left to stand for 15 min. Then, the infusion was quickly cooled to 22 °C. The remnants of plant material were removed by filtration through a thick nylon textile, and the volume of water extract was measured. Part of the extract was centrifuged (1800 g for 15 min) and stored at –16 °C for the later analyses.

Preparation of methanolic extract of the total quercetin and rutin

The total content of quercetin was determined after acid hydrolysis with hydrochloric acid of methanolic extract (with 50% methanol) with subsequent concentration on SPE columns (RP-18) according to the procedure described by Dadáková et al. (2001).

For the determination of rutin content, 0.25 g of the homogenized dried material was used. It was extracted with 25 mL of 50% methanol containing 80 mg of ascorbic acid as an antioxidant. The extraction was performed at 90 °C in a water bath under reflux for 90 min. After cooling, the material was centrifuged at 1800 g for 15 min. The sediment was re-suspended with 12.5 mL methanol and 25 mL water and centrifuged under the same conditions. The combined supernatants were diluted with water to 500 mL; pH was adjusted to 3.0 with 1M HCl. The solution was then filtered through a glass fibre filter. The purification and concentration of quercetin was performed on SPE columns (RP-18 conditioned with 10 mL methanol and 10 mL water. The adsorbed substances were eluted by 1.4 mL of methanol. An internal standard (200 μg of 1 naphtylacetic acid) was added to the final eluate.

Solid phase extraction of rutin and total quercetin from infusions

Rutin: 2.5 ml of infusion was mixed with 25 ml methanol, 80 mg ascorbic acid and 150 ml water. The acidity of the solution was set to pH 3.0 and the solution was transferred to a 500 mL volumetric flask, filled in to the mark and used for SPE (RP-18). The SPE conditions were the same as described above. *Total quercetin:* a mixture consisting of 25 mL infusion, 12.5 mL methanol, 5mL distilled water and 80 mg ascorbic acid was hydrolysed for 2 h in a water bath at 90 °C under the reflux. The hydrolysate was neutralised after chilling with 2 g $NaHCO_3$ and transferred with 12.5 mL methanol and 150 mL distilled water into a 600 mL beaker. The acidity of the solution was set with saturated solution of $NaHCO_3$ to pH 3.0. The solution was poured into 500 mL volumetric flask, filled to the mark and then used for solid phase extraction. The adsorbed substances were eluted with 1.4 mL of methanol. The internal standard (200 μg 1 naphtylacetic acid) was added to the final eluate.

Determination of rutin and total quercetin by micellar electrokinetic capillary chromatography (MEKC)

The samples containing the internal standard were analysed by capillary electrophoresis (SpectraPhoresis 2000, Thermo Separation Product, Fremont, CA, USA). Separations were performed using a fused-silica capillary (70 cm × 75 μm I.D., CElest FS75 CE column, Supelco, the effective length to the detector 67 cm). The running buffer (pH=9.2) contained 10 mM sodium tetra borate, 10 mM boric acid, 20 mM sodium dodecyl sulphate (SDS) and 15% (v/v) methanol. The analysis was performed at 25 °C, applied voltage +20 kV, hydrodynamic injection 2 s, detection of analyte at 270 nm. The analytical response is the ratio of rutin or quercetin and the internal standard peak areas. Quantification of rutin and quercetin contents was performed according to the calibration curve.

The exact concentrations of rutin and quercetin were determined for each sample by the method of standard addition (100 and 200% of analysed level). The limit of the quantification is 10 mg kg^{-1} dry matter for rutin and total quercetin in a plant material and 1 mg L^{-1} for quercetin and rutin in the infusions.

Extraction of catechin, chlorogenic acid, 3,4-dihydroxypropiophenone-3-glucoside (DHPPG) and free quercetin from dried materials

The homogenized dried material (0.25 g) was extracted with 3 mL of 90% methanol shaken for 30 min. The samples were centrifuged at 1800 g for 15 min. The sediment was washed twice with

1 mL of 90% methanol. All supernatants were pooled, their volume marked, and analysed using HPLC.

Determination of phenolic compounds by HPLC

The extracts from the dried materials and tea infusions were analyzed by liquid chromatography using HP 1050 (Hewlett-Packard, USA), DAD detector (HP 1040, Hewlett-Packard, U.S.A.) and Phenomenex Luna C18 (2), 3 μm, 2 × 150 mm column. The mobile phase A: 5% acetonitrile + 0.1% phosphoric acid. The mobile phase B: 80% acetonitrile + 0.1% phosphoric acid. The gradient of phase B increased from 0% to 53% in 55 min. Phenolic compounds were detected at wave length 220 nm (the range of scanning was between 190 nm and 600 nm).

The level of phenolic compounds was determined according to the calibration curve of corresponding standards. Only the concentration of DHPPG was determined according to the calibration curve for 4-hydroxyacetophenone. The limit of quantification was 0.1 mg kg^{-1} of dry matter in plant material.

Solid phase extraction of squalene from the infusions

25 mL of tea infusion was concentrated on an SPE column (RP 18). The SPE column was activated with 10 mL methanol and then with distilled water. After the application of the infusion, the SPE column was washed with water and then dried. Squalene was eluted with 1 mL of methanol. The sample was finally analysed using GC/MS.

Analysis of extract by GC/MS

Gas chromatography-mass spectrometry analyses were performed with a Finnigan GCQ instrument using Zebron column ZB-5, 30 m, I.D. 0.25 mm. The stationary phase thickness was 0.25 μm at the following temperature programme: initial temperature 60 °C for 1 min., then gradient 20 °C min^{-1} to 180 °C, followed by the gradient 1.5 °C min^{-1} to 275 °C. Linear inlet helium velocity was set to 40 cm sec^{-1}. Each peak in the chromatogram was evaluated using XCalibur mass spectrometry software and the obtained mass spectra were compared with the spectra from the NIST library. The concentration of squalene was determined according to its calibration curve. The limit of detection was 0.05 mg kg^{-1} of dry matter in plant material.

RESULTS AND DISCUSSIONS

Many studies deal with teas from *Camelia sinensis* regarding catechins and their derivatives, and the total phenolics content in teas is presented by Lachman et al. (2003). In this present work, the content of selected phenolic compounds was determined in typical European medicinal plants.

Quercetin occurs in plants mostly in the form of its glycosides, very rarely in its free form. Quercetin and rutin were analysed by MEKC according to the method described by Dadáková et al. (2001), which is a method similar to that described by other authors; for example Sun et al. (2003). The contents of rutin, total quercetin and other substances present in infusions from the plants studied are shown in Table 2. Free quercetin was determined only in infusions of meadowsweet (1.7 mg L^{-1}), where the highest content of total quercetin was found. A very high content of total quercetin was also found in black elder and silver birch. The highest content of rutin was found in black elder (149 mg L^{-1}), but in spite of the high rutin content in black elder, the infusions from lemon balm and silver birch did not contain any rutin. One of the dominant phenolic compounds in black elder is chlorogenic acid and a small amount of this substance was also found in silver birch and bloodwort. The content of catechin was not high and it was found only in three of the herbal infusions. It is interesting that squalene (a non-polar compound) was also found in the infusions. This substance was determined by GC/MS after its extraction on the SPE column. In addition to quercetin, which was present only in the form of glycosides, the dominant compound in silver birch was 3,4-dihydroxypropiophenone-3-glucoside (DHPPG).

We determined also the content of the biologically active compounds in the original dried material of the studied medicinal plants extracted with methanol for comparison with tea infusions. The results are presented in Table 3. These data revealed that methanolic extracts particularly contain rutin, which is the major flavonoid present in the flowers of black elder. Relatively high levels of various quercetin glycosides were present in silver birch (more than 10 g kg^{-1}), but the rutin content was below the detection detectable limit. The dominant quercetin derivative in birch leaves is, according to Keinänen and Julkunen-Tiitto (1998), quercetin-3-galactoside. Meadowsweet contains also a high amount of quercetin glycosides (about 14.8 g kg^{-1}) while the content of rutin was at

only an average level, Krasnov et al. (2006) has found hyperoside and quercetin-3-O-glucuronide in addition. A fast method of quantification of flavonoids in meadowsweet using the HPLC/ESI-MS technique was published recently by Pemp et al. (2007).

Rutin was not found in lemon balm and also the content of total quercetin and chlorogenic acid was low. The dominant substances in lemon balm are rosmarinic acid and its derivatives together with derivatives of luteolin; compounds which possess anti-depressive and anti-allergic activity (Fecka and Turek 2007). However, experiments on rats have revealed that rosmarinic acid has genotoxic effects, the doses of pure rosmarinic acid in experiments ranging from 1 to 8 mg kg^{-1} (Pereira et al. 2005). According to Fecka and Turek (2007), one tea bag of lemon balm (weight 2 g) contains about 65 mg of all polyphenols.

Methanolic extracts of black elder and bloodwort were rich in chlorogenic acid (about 28.7 g kg^{-1}). DHPPG was found only in silver birch. Table 4 shows the yield (extractability) of the investigated biologically active compounds from medicinal plants by hot water during the preparation of tea infusions, in comparison with extraction by methanol. The content of the compounds in the methanolic extract is assumed to be 100%. A very good yield for rutin and total quercetin was achieved for almost all plants (lower in meadowsweet) with the exception of ground ivy. The extractability of these substances from ground ivy was extremely low, which may be associated with availability of these substances for water in the plant matrix.

Table 2. The content of selected compounds in infusions of medicinal plants [mg L^{-1}]

Plant	Catechin	Chlorogenic acid	DHPPG	Rutin	Total quercetin	Squalene
Meadowsweet	61.8±2.5	ND	ND	25.2±1.3	120±5.8	ND
Lemon balm	ND	ND	ND	ND	2.6±0.1	0.152±0.008
Silver birch	8.2±0.4	25.2±1.2	47.8±1.9	ND	78.9±3.9	0.171±0.008
Black elder	ND	166±8.3	ND	194±7.8	108±5.4	ND
Bloodwort	ND	74.2±3.7	ND	17.4±0.9	20.8±1	ND
Common agrimony	65.4±2.6	ND	ND	7.2±0.4	43.5±2.2	0.068±0.003
Ground ivy	ND	ND	ND	5.1±0.3	5.1±0.3	0.002±0.001

DHPPG = 3,4-dihydroxypropiophenone-3-glucoside
ND = under detection limit

Table 3. The content of selected compounds in methanolic extract of medicinal plants [mg kg^{-1} dry matter]

Plant	Catechin	Chlorogenic acid	DHPPG	Rutin	Total quercetin
Meadowsweet	6470±260	ND	ND	2960±148	14800±740
Lemon balm	ND	160±8	ND	ND	250±12.5
Silver birch	1510±61	1650±82	5860±293	ND	1080±53
Black elder	ND	18100±899	ND	17600±880	9770±480
Bloodwort	ND	28700±1430	ND	1620±81	2160±108
Common agrimony	ND	7950±397	ND	693±34.6	4110±200
Ground ivy	ND	8930±446	ND	5710±28.5	3880±194

DHPPG = 3,4-dihydroxypropiophenone-3-glucoside
ND = under detection limit

Table 4. Hot water (tea infusion) extraction efficiency for catechin, chlorogenic acid, rutin and total quercetin (calculated as a content of these compounds in tea infusion to the content in methanolic extract = 100%)

Plant	Volume of tea infusion	[a]Content of catechin	Catechin extraction efficiency	[a]Content of chlorogenic acid	Chlorogenic acid extraction efficiency	[a]Content of rutin	Rutin extraction efficiency	[a]Content of total quercetin	Total quercetin extraction efficiency
	[L]	[mg.g^{-1}]	[%]	[mg.g^{-1}]	[%]	[mg.g^{-1}]	[%]	[mg.g^{-1}]	[%]
Meadowsweet	0.127	5.23	80.84			2.13	71.96	10.16	68.55
Lemon balm	0.130							0.23	92.00
Silver birch	0.124	0.68	45.03	2.08	126.06			6.52	60.37
Black elder	0.129			14.28	78.90	16.68	94.77	9.28	94.99
Bloodwort	0.126			6.23	21.70	1.46	90.12	1.75	81.02
Common agrimony	0.132	5.76	72.45			0.63	91.30	3.83	93.19
Ground ivy	0.134					0.45	7.88	0.45	11.60

[a] = calculation based on the content of the compound in tea infusion

The higher intake of phenolic substances contributes to the prevention and cure of heart and blood circulation diseases (Hertog 1996). Flavonoids like quercetin and catechin also have effects on the blood platelets function similar to stilbene resveratrol (Pignatelli et al. 2000). Phenolic acids are effective antioxidants due to their chemical structure (hydroxyl group in their molecules).

Squalene represents an important precursor of sterols. It is an important product in the biosynthesis of phytosterols. Nevertheless, in some plants only a proportion of squalene is changed into sterols, and a significant part is deposited in various parts of the plant. Some authors (Sotiroudis et al. 2003, Waterman and Lockwood 2007) suppose claim that squalene has chemo-protective effects.

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REFERENCES

Atoui AK, Mansouri A, Boskou G, Kefalas P (2005): Tea and herbal infusions: Their antioxidant activity and phenolic profile. Food Chem 89: 27–36.

Cook NC, Saman S (1996): Flavonoids-chemistry, metabolism, cardioprotective effect and dietary sources. J Nutr Biochem 7: 66–76.

Dadáková E, Procházková E, Křížek M (2001): Application of micellar electrokinetic chromatography for quantitative analysis of quercetin in plant material. Electrophoresis 22: 1573–1578.

Fecka I, Turek S (2007): Determination of water-soluble polyphenolic compounds in commercial herbal teas from Lamiaceae: Peppermint, melissa, and sage. J Agric Food Chem 55: 10908–10917.

Fresco P, Borges F, Diniz C, Marques MPM (2006): New insights on the anticancer properties of dietary polyphenols. Med Res Rev 26: 747–766.

Hertog MGL, Hollman PCH, Putte van de B (1993): Content of potentially anticarcinogenic flavonoids of tea infusions, wines, and fruit juices. J Agric Food Chem 41: 1243–1246.

Hertog MGL (1996): Epidemiological evidence on potential health properties of flavonoids. Proc Nutr Soc 55: 385–397.

Hubík J, Dušek J, Spilková J, Šícha J (1989): General pharmacognosis II: Secondary compounds. SPN Praha (in Czech).

Katalinić V, Miloš M, Kulisić T, Jukić M (2006): Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem 94: 550–557.

Keinänen M, Julkunen-Tiitto R (1998): High-performance liquid chromatographic determination of flavonoids in *Betula pendula* and *Betula pubescens* leaves. J Chromatogr A 793: 370–377.

Krasnov EA, Raldugin VA, Shilova IV, Avdeeva EYu (2006): Phenolic compounds from *Filipendula ulmaria*. Chem Nat Compd 42: 148–151.

Kubát K, Hrouda L, Chrtek J, Jr., Kaplan Z, Kirschner J, Štěpánek J (2002): Key to the Flora of the Czech Republic. Academia Praha (in Czech).

Kulišić-Bilušić T, Katalinić V, Dragović-Uzelac V, Ljubenkov I, Kriško A, Dejanović B, Jukić M, Politeo O, Pifat G, Miloš M (2008): Antioxidant and acetylcholinesterase inhibiting activity of several aqueous tea infusions *in vitro*. Food Technol Biotechnol 46: 368–375.

Lachman J, Orság M, Pivec V, Dudjak J, Krym O (2003): Polyphenol content in green, black and oolong tea (*Camelia sinensis* /L./Kuntze) infusions in different times of tea maceration. Sci Agric Bohem 34: 22–28.

Lampe JW (1999): Health effects of vegetables and fruit: assessing mechanisms of action in human experimental studies. Am J Clin Nutr 70: 475–490.

Nijveldt RJ, van Nood E, van Hoorn DEC, Boelens PG, van Norren K, van Leeuwen PAM (2001): Flavonoids: a review of probable mechanisms of action and potential applications. Am J Clin Nutr 74: 418–425.

Pemp E, Reznicek G, Krenn L (2007): Fast quantification of flavonoids in *Filipendulae ulmariae* flos by HPLC/ESI-MS using a nonporous stationary phase. J Anal Chem 62: 669–673.

Pereira P, Tysca D, Oliveria P, da Silva Brum FL, Picada JN, Ardenghi P (2005): Neurobehavioral and genotoxic aspects of rosmarinic acid. Pharmacol Res 52: 199–203.

Pignatelli P, Pulcinelli FM, Celestini A, Lenti L, Ghiselli A, Gazzaniga PP, Violi F (2000): The flavonoids quercetin and catechin synergistically inhibit platelet function by antagonizing the intracellular production of hydrogen peroxide. Am J Clin Nutr 72: 1150–1155.

Sotiroudis TG, Kyrtopoulos SA, Xenakis A, Sotiroudis GT (2003): Chemopreventive potential of minor components of olive oil against cancer. Ital J Food Sci 15: 169–185.

Sun Y, Guo T, Sui Y, Li F (2003): Quantitative determination of rutin, quercetin, and adenosine in Flos Carthami by capillary electrophoresis. J Separ Sci 26: 1203–1206.

Tomko J, Kresánek J, Hubík J, Suchý V, Felklová M, Sikita B, Libický A (1989): Pharmacognosis. Osveta Martin (in Slovak).

Waterman E, Lockwood B (2007): Active components and clinical applications of olive oil. Altern Med Rev 18: 331–342.