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The aromatic and polyphenolic composition of lemon balm (*Melissa officinalis* L. subsp. *officinalis*) tea

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Abstract

The chemical composition of the widely used herbal tea made from lemon balm (*Melissa officinalis* L. subsp. *officinalis*) was previously unknown. The qualitative and quantitative composition of the main aromatic and polyphenolic constituents of the infusion were examined and compared with those of the leaves before and after infusion. The dried lemon balm leaves originally contained 0.32% essential oil of which citral (neral + geranial) 0.13%, total polyphenol compounds 11.8% comprising total hydroxycinnamic compounds 11.3% (rosmarinic acid 4.1%) and total flavonoid compounds 0.5%. The tea contained 10 mg/l of essential oil (extraction yield 31%) with much more citral (74% of the essential oil). It also contained large amounts of polyphenol compounds (about 1.07 g/l) corresponding to a 93% extraction yield. © 1998 Elsevier Science B.V.

Keywords: *Melissa officinalis*; Lemon balm; Polyphenols; Essential oil

1. Introduction

Lemon balm, *Melissa officinalis* L. (*Lamiaceae*) forms two subspecies, the lemon-scented subsp. *officinalis* and the fetid subsp. *altissima* (Sibth. and Sm.) Archangeli (Tutin et al., 1972). The leaves of the subsp. *officinalis* are widely used in Europe as a herbal tea for their aromatic, digestive and antispasmodic properties in nervous disturbance of sleep and functional gastrointestinal disorders (Bisset and Wichtl, 1994). The traditional use of the tea is consistent with its generally acknowledged innocuity. Some pharmacological properties have been attributed to the principal constituents. Rosmarinic acid is antiviral and antioxidant (Koch-Heitzmann and Schultze, 1984) while the essential oil is spasmolytic and antimicrobial (Wagner and Sprinkmeyer, 1973). Enriched extracts containing rosmarinic acid are used as a virostatic against herpes viruses, alcohol extracts as sedatives and the essential oil as a digestive aid in pharmaceutical preparations (Hänsel et al., 1993).

The chemical composition of the essential oil of the lemon balm leaf (0.02–0.3% dry weight) has been studied. The major compounds were citronnellal (2–40%) and citral (neral and geranial: 10–30%), accompanied by β-caryophyllene, germacrene D, ocimene and citronellol (Tittel et al., 1982; Enjalbert et al., 1983; Nykänen and Nykänen, 1986; Mulkens and Kapetanidis, 1988; Nigam et al., 1988; Schultze et al., 1989; Sarer and Kökdil, 1991; Adzet et al., 1992; Kreis and Mosandl, 1994). The aldehydes of the citral type are absent in the essential oil of subsp. *altissima* (Dawson et al., 1988). Several monoterpene glycosides were also identified in lemon balm, e.g. geranyl or eugenyl glucosides (Mulkens et al., 1985; Baerheim Svendsen and Merckx, 1989).

The leaf also contains polyphenolic compounds: caffeic acid derivatives in large proportions, such as rosmarinic acid (ca. 2–5%) (Gracza and Ruff, 1984; Lamaison et al., 1991) and trimeric compounds (Agata et al., 1993) and

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also some flavonoids such as luteolin-7-O-glucoside (0.0002%) (Thieme and Kitze, 1973; Mulkens and Kapetanidis, 1987).

Lemon balm infusion is widely used but its aromatic and polyphenolic composition is unknown. In the present study we have evaluated the qualitative and quantitative composition of the main aromatic and polyphenolic compounds in tea made from lemon balm leaves.

2. Materials and methods

2.1. Plant material and extraction

The plant material (1 kg of dried leaves) was of commercial origin (Sicarrapam, Aubiat, Puy-de-Dôme, France), batch No. 1503. The identity of the subsp. *officinalis* was confirmed by Professor J.L. Lamaison. The plant was cultivated locally in Auvergne and the leaves were harvested just before flowering in June 1996, dried by warm air at 30–35°C for 48 h and stored in a paper bag at room temperature for 3 months. The leaves met pharmacopoeial specifications (French Pharmacopoeia X, 1996).

Infusion of the whole leaves was carried out in a covered 2 l column. Boiling demineralized water (1 l) was poured out on whole leaves at 10 g/l and the tea left to draw for 15 min. The decanted tea (1 l) had a yellow color and a pleasant lemon smell.

The quantitative analysis of polyphenol compounds was carried out with powdered dried leaves (0.5 mm). After infusion the leaves accounted for 59% w/w of the original leaves, based on the dry weight. The assay samples of the infusion and of the leaves after infusion were corrected according to their respective yields.

2.2. Chemicals

The terpenoids, rosmarinic acid and luteolin-7-O-glucoside of standard quality were purchased from Extrasynthese, Genay, France. The reagents and the solvents used were of analytical or HPLC grade, 2-aminoethyldiphenylborinate from Fluka, Buchs, Switzerland, the others from Merck, Darmstadt, Germany.

2.3. Essential oil hydrodistillation

The infusion (500 ml) was promptly distilled (2 h, 2–3 ml/min) to determine its essential oil content, operating without xylene addition. The original whole leaves were treated according to the method of the French Pharmacopoeia (without xylene). The decanted leaves after infusion were used wet and promptly distilled in the same conditions. The averaged results from 3 assays (v/w) are given relative to the weight of the original dried leaves.

2.4. Gas chromatography (GC) of the essential oil

The essential oil composition of the infusion and of the leaves before and after infusion were determined by GC. GC/MS analysis was performed using a Delsi 700 gas chromatograph coupled with a Nermag R10-10C mass spectrometer, i.e. 70 eV as ionization current. The column was a 60 m × 0.32 mm DB Wax (J&W) fused silica capillary column. The temperature program is as follows: 60–240°C at 5°C/min, carrier gas helium 2.5 ml/min. The injection consisted of 1.0 μl of distilled oil diluted to 5% v/v with isooctane. Components were identified by both retention times and MS spectra. Quantitation was performed by area percent, FID-response factor = 1. The retention times of the principal constituent standards were approximately: citronellal 15.0 min, neral 21.2 min, geranial 23.6 min.

2.5. High-performance liquid chromatography (HPLC) of the polyphenolic compounds

The polyphenolic composition was analysed by qualitative and quantitative HPLC-DAAD with a two pump 510, solvent programmer 680 and photodiode array detector 991 (Waters). Column: Lichrocart 125-4 Superspher RP 8-E, 4 μm (Merck). Gradient elution: two solvents were used: (A) H_2O–H_3PO_4 85% (100:0.3) and (B) MeCN–H_2O–H_3PO_4 85% (80:20:0.3). Elution profile (quadriconcave gradient): 0–5 min, 12–15% B in A (gradient 10 waters); 5–30 min, 15–30% B in A (gradient 10); 30–35 min, 30–50% B in A (gradient 10); 35–40 min, 50–70% B in A (gradient 10) and 40–45 min, 70% B in A (isocratic). The flow rate was 2 ml/min and UV detection was at 330 nm. The injection consisted of 25 μl of water–ethanol (1:1) solution, standard solution 0.5 mg/ml, extractive solution 0.200 g/200 ml (as 2.6) concentrated to 20 ml.

The retention times of the standards were approximately: rosmarinic acid, 30 min and luteolin-7-O-glucoside, 18 min. Quantitatively, the major flavonoid compound was expressed as luteolin-7-O-glucoside. The correlation coefficient was above 0.99 for both compounds (5 points; 3 assays).

2.6. Total hydroxycinnamic acid content

The total hydroxycinnamic acid content was determined by a spectrophotometric method with the Arnow reagent. The results are expressed in rosmarinic acid (French Pharmacopoeia X, 1996).

2.7. Total flavonoid content

The total flavonoid content was determined by a spectrophotometric method with boric acid–oxalic acid reagent (Glasl, 1985), with luteolin-7-O-glucoside as standard at 405 nm. The correlation coefficient was above 0.99 (5 points; 3 assays).

3. Results and discussion

3.1. Qualitative composition of the essential oil

The chemical composition of the essential oil of the leaf infusion of *Melissa officinalis* subsp. *officinalis* was compared with those of the leaves before and after infusion. The aromatic composition of 94% of the infusion was established. The percentages of hydrocarbons (Hc), ketones (Ke), oxides (Ox), aldehydes (Al), alcohols (Ol) and esters (Es) were accurately calculated (Table 1).

The composition of the leaf essential oil agreed with the data of the previous authors. Citral (neral + geranial) represented 48% of the essential oil. The other main constituents were citronellal, 40% and β-caryophyllene, 2%.

The infusion essential oil was markedly enriched in aldehydes (90%) with a citral level near 74% but was poorer in citronellal (16%). A typical GC profile shows the predominance of these compounds (Fig. 1). The contents of the other classes of terpenoids were reduced, such as non-polar hydrocarbons or weakly polar esters and oxides. The infusate contained alcohols and ketones in percentages analogous to those in the leaf. Importantly, large amounts of the more volatile compounds (monoterpenes, aldehydes) were lost during the various operations. In the residual leaves after infusion the main constituent of the essential oil was citronellal (43%) but the citral level (36%) was reduced. Increased amounts of non- or weakly polar minor compounds such as β-caryophyllene (6%) were also observed.

Table 1
Composition of the essential oil of the leaf, of the liquid infusion and of the leaf remaining after infusion of lemon balm (% of the essential oil)

Constituents	Leaf	Infusion	Leaf after infusion
Hc α-pinene	0.11	—	—
Hc *cis*-β-ocimene	0.10	—	—
Hc *trans*-β-ocimene	0.30	—	0.42
Ke 6-methyl-5-heptene-2-one	0.35	0.27	0.45
Ox *cis*-linalool oxide	0.39	0.39	0.11
Al citronellal	39.47	16.81	42.67
Ol linalool	0.56	0.43	0.29
Hc β-caryophyllene	2.37	1.38	6.34
Al neral	20.40	30.15	15.80
Al geranial	27.84	43.53	20.70
Es geranyl acetate	0.58	0.18	1.07
Ol citronellol	0.63	0.70	0.57
Ol geraniol	0.18	0.32	0.15
Ox caryophyllene oxide	0.67	0.24	1.99
Hc hydrocarbons	2.88	1.38	6.76
Ke ketones	0.35	0.27	0.45
Ox oxides	1.06	0.63	2.10
Al aldehydes	87.71	90.49	79.17
Ol alcohols	1.37	1.45	1.01
Es esters	0.58	0.18	1.07
Total	93.95	94.40	90.56

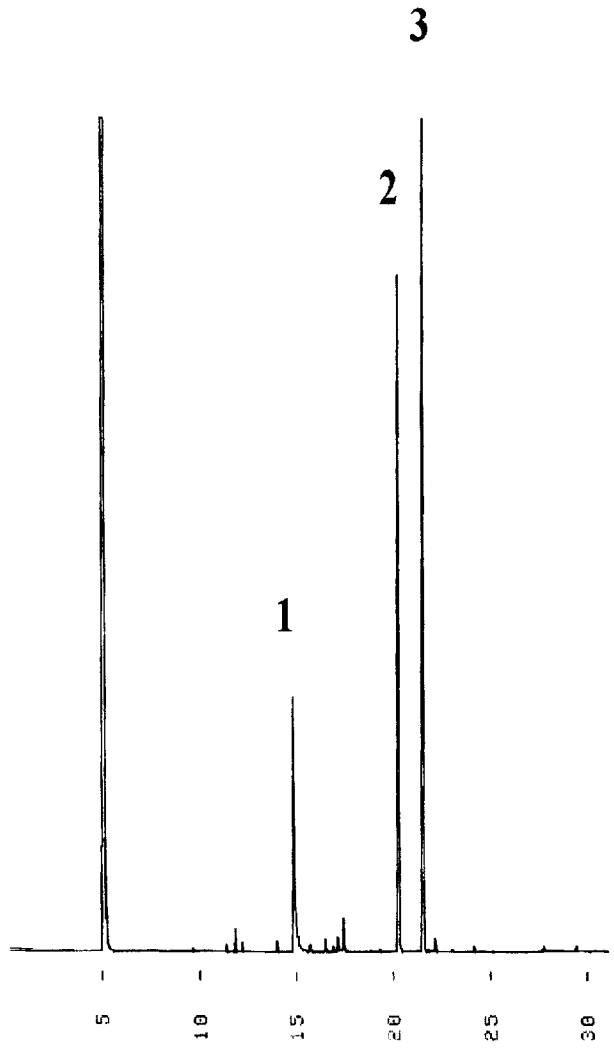

Fig. 1. Typical GC chromatogram of the essential oil of lemon balm infusion: (1) citronellal, (2) neral and (3) geranial.

3.2. Qualitative composition of the polyphenolic compounds

In both the infusion and the leaf the main polyphenolic compound was rosmarinic acid (t_R: 30 min) among the hydroxycinnamic derivatives. Another peak (t_R: 31 min) corresponding to the main flavonoid compound was a luteolin-derivative (spectra superimposable on that of luteolin-7-O-glucoside by HPLC-DAAD: λ_{max}, 254 and 347 nm). The sugar moiety of this compound detected here from the batch of *Melissa officinalis* studied is unknown. Luteolin-7-O-glucoside (standard t_R: 18 min) previously isolated in very small quantities from lemon balm leaf was not detected here by a direct method without purification. The HPLC profile of the lemon balm tea shows the prevalence of the two constituents, rosmarinic acid and the luteolin derivative. Other minor peaks correspond to other unidentified hydroxycinnamic derivatives (Fig. 2).

Table 2
Levels of the main constituents of the leaf, of the liquid infusion and of the leaf remaining after infusion from lemon balm (% based on the dry weight of the leaf)

Constituents	Leaf	Infusion	Leaf after infusion
Essential oil	0.32	0.10	0.12
Citronellal	0.13	0.02	0.05
β-Caryophyllene	0.01	—	0.01
Neral	0.07	0.03	0.02
Geranial	0.09	0.04	0.02
Total flavonoid compounds	0.51	0.44	0.11
Luteolin derivative	0.54	0.51	0.07
Total hydroxycinnamic derivatives	11.29	10.49	1.86
Rosmarinic acid	4.05	3.99	0.33

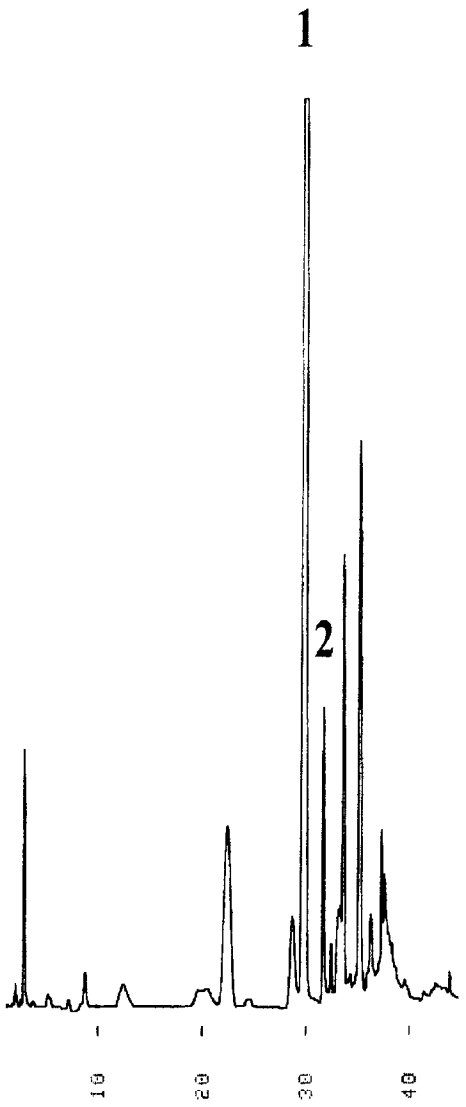

Fig. 2. Typical HPLC chromatogram of polyphenol compounds of lemon balm infusion: (1) rosmarinic acid and (2) luteolin glycoside (unknown).

3.3. Quantitative composition of aromatic and polyphenolic compounds

The levels of the main aromatic and polyphenolic constituents of the lemon balm infusion were compared with those of the leaves before and after infusion. The percentages of essential oil (total aromatic constituents), total flavonoid compounds, total hydroxycinnamic derivatives and main individual aromatic and polyphenolic compounds are based on the dry weight (v/w or w/w) of the original leaves (Table 2).

The lemon balm tea contains 31% (0.10/0.32%) of the initial essential oil. This proportion was largely due to the high level of aldehydes and citral in the infusion. The quantities corresponded in the case of the leaf infusion (10 g/l) to about 10 mg/l of essential oil and 9 mg/l of total aldehydic compounds of the citral type. The leaf after infusion contained 0.12% of essential oil depleted in citral.

Large amounts of polyphenolic compounds (near 12% of which about 11% of hydroxycinnamic derivatives and 0.5% of flavonoid compounds) were contained in the lemon balm leaf. The data for quantitative analysis by HPLC confirm the predominance of a luteolin derivative (0.5%) among the flavonoids and rosmarinic acid (4.1%) among the hydroxycinnamic derivatives. In the infusion the extraction yield of the polyphenolic compounds was very high (93%) for an infusion carried out from whole leaves at 10 g/l for only 15 min.

Herbal tea from lemon balm is principally employed as an antispasmodic in nervous and digestive disorders. An infusion made with 1.5 g of leaf in 150 ml of water contains about 150 mg of polyphenolic compounds and 1.5 mg/l of essential oil. Melissae folium doses specified in the German national requirements for registration of phytotherapeutics and preparation of teas (Commission E) are 1.5 to 4.5 g/150 ml (Hänsel et al., 1993), several times a day. Thus, one cup (150 ml) can contain about 150 to 450 mg of polyphenolic compounds (of which 60 to 180 mg of rosmarinic acid) and 1.5 to 4.5 mg of essential oil (principally citral). The essential oil concentrations seem to account only for the aromatic character of the lemon balm tea. These quantities are well below the recommended doses for a spasmolytic activity (0.05 to 0.2 ml). On the other hand, the phenolic constituents are probably involved in the activity of the tea. Extraction solvents with low alcohol titers, like boiling water, extract the phenolics almost quantitatively. A water–ethanol (30% w/w) extract (1:3.5) at 10 ml/l, corresponding to 3 g of leaf/l, exhibited a weak spasmolytic activity in vitro (Forster et al., 1980). A more concentrated balm infusion (10 to 30 g/l) should be a more effective pharmacological agent. The conclusion of the authors is that it is conceivable that the small antispasmodic effects of these plant extracts may be sufficient for the treatment of minor spasms, can be applied to the lemon balm tea.

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