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ORIGINAL ARTICLE

Comparative analysis of the aroma chemicals of *Melissa officinalis* using hydrodistillation and HS-SPME techniques

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Abstract Headspace solid-phase micro extraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) has been used for the chemical analysis of *Melissa officinalis* (leaves) cultivated in Institute Germplasm. The HS-SPME analysis led to the identification of 22 components constituting 99.1% of the total volatile constituents present in the leaves whereas its hydrodistillate led to the identification of 24 volatile constituents constituting 98.1% of the volatile material. The chemical composition of the SPME and hydrodistilled extract of *M. officinalis* leaves comprised mainly of oxygenated monoterpenes (78.5% and 57.8% respectively) and sesquiterpene hydrocarbons (14.9% and 29.7% respectively). The major components identified in the HS-SPME extract were citronellal (31.1%), citronellol (18.3%), β-caryophyllene (12.0%), (*E*)-citral (11.9%), (*Z*)-citral (9.6%), geraniol (3.6%), (*Z*)-β-ocimene (3.1%) and 1-octen-3-ol (2.0%) whereas hydrodistilled essential oil was rich in (*Z*)-citral (19.6%), β-caryophyllene (13.2%), (*E*)-citral (11.2%), citronellal (10.2%), germacrene-D (8.3%), δ-3-carene (5.0%), 6-methyl-5-hepten-2-one (3.7%) and citronellyl acetate (3.7%). The comparative analysis of volatile constituents of *M. officinalis* leaf extract using HS-SPME and hydrodistillation techniques shows both qualitative as well as quantitative differences. The current study is the first report involving rapid analysis of volatile components of *M. officinalis* by HS-SPME.

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1. Introduction

Different methods that can be used for the extraction of volatile compounds from plants include steam distillation, soxhlet extraction, simultaneous distillation extraction (SDE), supercritical fluid extraction (SFE) and headspace techniques such

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as static-headspace sampling (SHS), purge and trap (P & T) and solid-phase micro extraction (SPME) (Sides et al., 2000). Monoterpenes may be susceptible to chemical changes under steam distillation and even the conventional solvent extraction during the removal of solvent by distillation. On the other hand static headspace (SHS) and Purge and Trap (P & T) techniques are simpler, faster and solvent free. SHS has been widely applied for the analysis of volatile compounds because the extracting phase (air, helium or nitrogen) is compatible with gas chromatography. However, SHS has some limitations like low sensitivity and risk of cross contamination (Mazza and Cottrell, 1999). An alternative to traditional headspace sampling is SPME which is a rapid, simple, inexpensive and solvent-free technique very stable for the analysis of volatile and semi volatile compounds in different types of samples. SPME has been successfully utilized for the qualitative analysis of various food substances and flavors. Extraction efficiency and reproducibility by SPME technique are affected by modifying the matrix of the fiber, extraction and desorption time, incubation temperature, sample volume, and other sample treatments (Bazemore et al., 1999; Roberts et al., 2000; Bicchi et al., 2000; Kusakabe et al., 2001). Headspace SPME enables many possibilities in aroma analysis. It has been successfully used for the chemical analysis of different plant materials like spices (Rohlofl, 1999; Sostaric et al., 2000), fruits (Ibanez et al., 1998; Augusto et al., 2000), flowers (An et al., 2001; Fernando and Grun, 2001), coffee (Roberts et al., 2000), organic pollutants in environmental samples (Potter and Pawliszyn, 1992; Pefialver et al., 1999) and pharmaceutical products (Ligor and Buszewski, 1999).

Lemon balm, *Melissa officinalis* L. (Lamiaceae), a native of the northern Mediterranean region and western Asia, is cultivated as a medicinal herb (Schultze et al., 1993). Lemon balm holds the prime position among a diversity of aromatic plants being cultivated throughout the world. It was used in ancient Greece and Rome as a topical treatment for wounds. In the middle ages it was used as a sedative and in the 17th century, English herbalist Culpeper claimed it could improve mood and stimulate clear thinking (Braun and Cohen, 2007). It is listed in a number of European Pharmacopoeia for its carminative, digestive, diaphoretic and stimulant activities (Gbolade and Lockwood, 1989), calmative, antiseptic (Baytop, 1984), antimicrobial (Larrando et al., 1995), antioxidative (Ribeiro et al., 2001), and also possesses antiviral activity (Herrmann and Kucera, 1967; Dimitrova et al., 1993). It also inhibits thyroid activity (Bown, 1995). Lemon balm has been found to exhibit CNS acetylcholine receptor activity, with both nicotinic and muscarinic binding properties (Wake et al., 2000). *In vitro* testing has identified its anti-HIV activity against HIV-1 reverse transcriptase (Yamasaki et al., 1998) and antitumour activity (Galasinski, 1996). Externally, it is used to treat herpes, sores, gout, insect bites and as an insect repellent (Bown, 1995).

Table 1 Essential oil composition of *Melissa officinalis*.

S. No.	Compound	RI[a,b]	Peak area % (HS-SPME)	Peak area % (HD)	Methods of identification
1	β-Pinene	974	0.2	0.2	MS, RI
2	Artemiseole	976	1.0	1.0	MS, RI
3	1-Octen-3-ol	979	2.0	0.9	MS, RI
4	3-Octanol	991	–	0.2	MS, RI
5	6-Methyl-5-hepten-2-one	995	0.4	3.7	MS, RI
6	δ-3-Carene	1011	–	5.0	MS, RI
7	(*Z*)-β-Ocimene	1032	3.1	0.6	MS, RI, Std
8	Linalool	1095	0.5	2.7	MS, RI
9	Cis-rose oxide	1106	1.2	0.5	MS, RI
10	Trans-rose oxide	1125	0.6	–	MS, RI
11	Cis-verbenol	1141	–	0.7	MS, RI
12	Limonene oxide	1142	0.7	–	MS, RI
13	Citronellal	1148	31.1	10.2	MS, RI, Std
14	Isopulegone	1149	0.3	–	MS, RI
15	Myrtenol	1195	–	2.8	MS, RI
16	Citronellol	1225	18.3	–	MS, RI, Std
17	Geraniol	1252	3.6	1.9	MS, RI, Std
18	(*Z*)-Citral	1316	9.6	19.6	MS, RI, Std
19	Methyl geranate	1324	0.3	1.3	MS, RI
20	(*E*)-Citral	1338	11.2	11.2	MS, RI, Std
21	Citronellyl acetate	1352	0.1	3.7	MS, RI
22	α-Copaene	1376	–	1.4	MS, RI
23	Geranyl acetate	1379	–	2.2	MS, RI
24	β-Caryophyllene	1417	12.0	13.2	MS, RI, Std
25	α-Bergamotene	1434	0.4	–	MS, RI, Std
26	α-Humulene	1452	0.9	1.9	MS, RI, Std
27	Germacrene-D	1484	1.4	8.3	MS, RI, Std
29	α-Farnesene	1505	0.2	2.3	MS, RI, Std
30	δ-Cadinene	1523	–	2.6	MS, RI
	Total (%)		99.1	98.1	

RI, retention index.
[a] As identified by GC–MS software; names according to NIST mass spectral library, and by comparing their Kovats retention indices.
[b] Kovats retention indices of each component were collected from the literature for column RTX-5.

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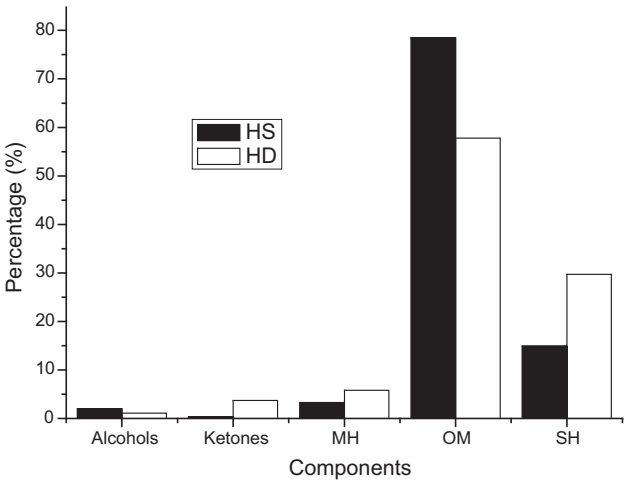

Figure 1 Compound class distribution in the HS-SPME (HS) and Hydrodistilled (HD) essential oil of *Melissa officinalis* leaf.

Moreover, the leaves are used in traditional medicine to prepare a tea for its nerve calming and spasmolytic effects (Wheatley, 2005; Kennedy et al., 2006). In clinical practice, lemon balm is often prescribed in combination with other herbal medicines thereby making it difficult to determine the efficacy of this herb individually (Braun and Cohen, 2007).

Phytochemical investigation of the plant has revealed the presence of flavonoids (cynaroside, cosmosin, rhamnocitrin, isoquercitrin, luteolin, luteolin 7-*O*-β-D-glucuronopyranoside, apigenin 7-*O*-β-D-glucopyranoside, luteolin 7-*O*-β-D-glucuronopyranoside, luteolin 3′-*O*-β-D-glucuronopyranoside and luteolin 7-*O*-β-D-glucopyranoside-3′-*O*-β-D-glucuronopyranoside), terpenes and triterpene acids (ursolic and oleanolic acid) (Patora et al., 2003), caffeic acid and rosmarinic acid (Herodez et al., 2003), mono- and sesquiterpenes (Mikus et al., 2000), phenolic substances and tannins (Hohmann et al., 1999).

Here we report the essential oil composition of *M. officinalis* (leaf) using the HS-SPME technique and the chemical analysis results obtained have been compared with those obtained through conventional hydrodistillation.

2. Experimental

2.1. Plant material

The plant material of *M. officinalis* was collected from Gene Bank of IIIM Srinagar, where it is cultivated as a medicinal herb. After proper identification, a voucher specimen (No. 1455/97) was deposited in the Herbarium of the Indian Institute of Integrative Medicine, Srinagar. Fresh leaves of *M. officinalis* were subjected to hydrodistillation for 3 h, using a modified Clevenger-type apparatus.

2.2. Head space-solid phase microextraction (HS-SPME)

The fresh leaves of *M. officinalis* were subjected to chemoprofiling using head space solid phase microextraction (HS-SPME) coupled with gas chromatograph–mass spectrometer (GC–MS). The sample (2 g) was placed in a 30 ml vial which was sealed with a screw-capped top containing a Teflon-lined septum. The 100 μm SPME fiber coated with carboxen-PDMS was exposed to the headspace of the sample at 60 °C for 20 min. The fiber was then retracted and inserted into the injector of the gas chromatograph coupled to mass spectrometer. Desorption was performed for 5 min, with the injector at 250 °C and in splitless mode.

2.3. Gas chromatography–mass spectrometry (GC–MS)

GC–MS analysis was carried out on a Varian Gas Chromatograph series 3800 fitted with a VF-5 ms fused silica capillary column (60 m × 0.25 mm, film thickness 0.25 μm) coupled with a 4000 series mass detector under the following conditions: injection volume 0.5 μl with split ratio 1:60, helium as carrier gas at 1.0 ml/min constant flow rate, injector temperature 230 °C, oven temperature 60–280 °C at 3 °C/min. Mass spectra: electron impact (EI +) mode, 70 eV and ion source temperature 250 °C. Mass spectra were recorded over 50–500 a.m.u range.

Identification of the essential oil constituents was done on the basis of Retention Indices (RI, determined with respect to homologous series of n-alkanes (C_9-C_{24}, Polyscience Corp., Niles IL) under the same experimental conditions), co-injection with standards (Sigma Aldrich and standard isolates), MS Library search (NIST 98 and WILEY), by comparing with the MS literature data (An et al., 2001; Fernando and Grun, 2001). The relative percentages of the individual components were calculated based on GC peak area (FID response) without using correction factors.

2.4. Gas chromatography (GC–FID)

Gas chromatography was carried out on Perkin Elmer auto system XL Gas Chromatograph 8500 series equipped with flame ionization detector (FID) and head space analyzer using a fused silica capillary column (30 m × 0.32 mm, film thickness 0.25 μm) coated with dimethyl polysiloxane ($RT_{X\text{-}5}$). Oven temperature was programmed from 60 to 280 °C with injector temperature 230 °C and detector temperature 250 °C. Injection volume was 1 μl, hydrogen was used as a carrier gas (1.0 ml/min).

3. Results and discussion

The chemical analysis results obtained by both hydrodistillation and HS-SPME methods are given in Table 1, where the compounds are listed in order of their elution from the RTX-5 column. The oil yield calculated on fresh weight basis was 0.03% (v/w) for the leaf part. HS-SPME analysis led to the identification of 22 components from the leaf and GC–MS analysis led to the identification of 24 components from its essential oil.

HS-SPME analysis led to the identification of 22 volatile components accounting for 99.1% of the total identified components. The chemical composition of *M. officinalis* (leaf) using SPME technique shows oxygenated monoterpenes (78.5%) and sesquiterpenoids (14.9%) (Fig. 1; Table 2). The major components identified in the HS-SPME extract were citronellal (31.1%), citronellol (18.3%), β-caryophyllene (12.0%), (*E*)-citral (11.9%), (*Z*)-citral (9.6%), geraniol (3.6%), (*Z*)-β-ocimene (3.1%) and 1-octen-3-ol (2.0%) (Fig. 2). The steam distilled essential oil led to the identification of 24 volatile constituents constituting 98.1% of the total identified compounds. The chemical composition of the essential

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Table 2 Compound class composition in the HS-SPME (HS) and Hydrodistilled (HD) essential oil of *Melissa officinalis* leaf.

S. No.	Class of compounds	Peak area % (HS-SPME)	Peak area% (HD)
1	Alcohols	2.0	1.1
2	Ketones	0.4	3.7
3	Monoterpene hydrocarbons	3.3	5.8
4	Oxygenated monoterpenes	78.5	57.8
5	Sesquiterpene hydrocarbons	14.9	29.7
6	Total (%)	99.1	98.1

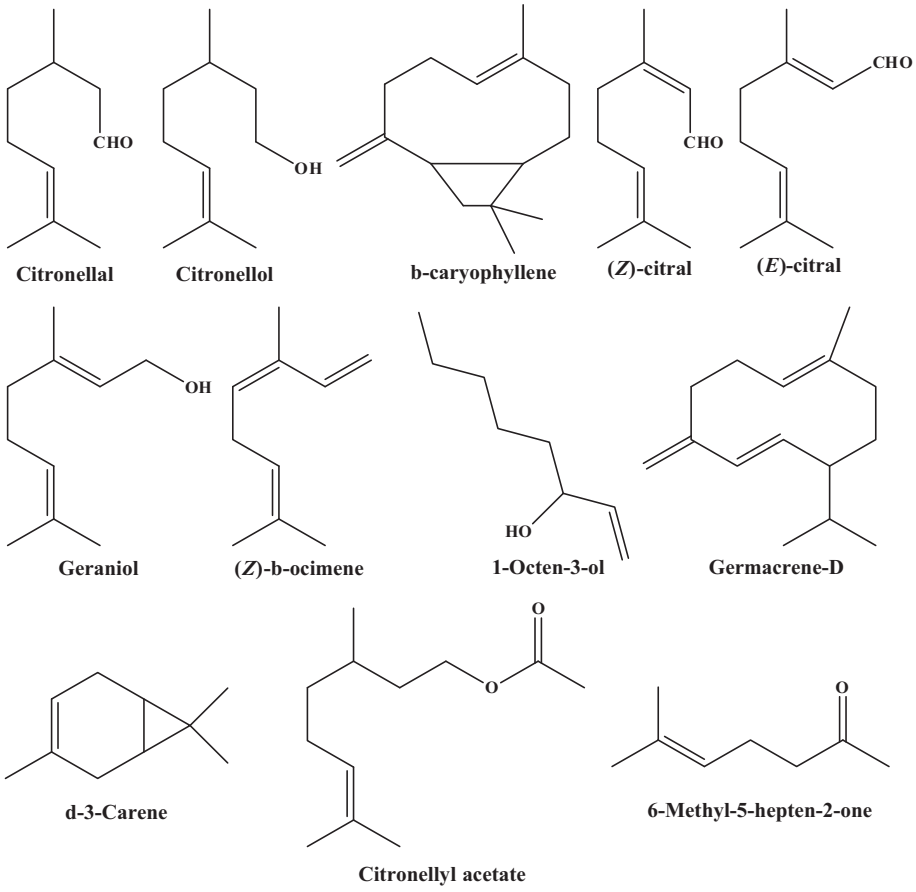

Figure 2 Structures of the major essential oil constituents.

oil comprised mainly of oxygenated monoterpenes (57.8%) and sesquiterpene hydrocarbons (29.7%) (Fig. 1). The major constituents of the essential oil are (*Z*)-citral (19.6%), β-caryophyllene (13.2%), (*E*)-citral (11.2%), citronellal (10.2%), germacrene-D (8.3%), δ-3-carene (5.0%), 6-methyl-5-hepten-2-one (3.7%) and citronellyl acetate (3.7%).

Though hydrodistillation is the most popular, widely used and cost effective method for the extraction of essential oils it has certain serious drawbacks as well. Essential oils with high solubility in water and susceptible to decomposition under temperature cannot be distilled. Some of the fragile and thermosensitive constituents may get decomposed resulting into artefacts due to heating. There is also a likelihood of it getting charred and thus imparting an unwanted odor (burning note) to the essential oil. Prolonged action of hot water can cause hydrolysis of some important chemical entities of the essential oil. One of the major drawbacks of hydrodistillation is that it is comparatively a time-consuming method and large amounts of plant material are needed.

Since the essential oil is rich in monoterpenes which are vulnerable to chemical changes under steam distillation conditions, even conventional solvent extraction is likely to involve losses of more volatile compounds during removal of the solvent (Presti et al., 2005). There is direct evidence of loss of some major and pharmacologically important minor chemical constituents from the hydrodistilled essential oil when compared to HS-SPME. On comparative analysis of *M. officinalis* (leaf) essential oil using HS-SPME and hydrodistillation, it is evident that the two chemoprofiles show qualitative as well as quantitative differences. Unlike steam distilled essential oil sample of *M. officinalis*, HS-SPME sample was rich in citronellol (18.3%) in addition to minor constituents like limonene

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oxide, trans-rose oxide, α-bergamotene and Isopulegone (Table 1). On the other hand the components like δ-3-carene, 3-Octanol, Cis-verbenol, Myrtenol, geranyl acetate, δ-cadinene and α-copaene were found in steam distilled sample only (Table 1).

Previous reports show that the essential oil of *M. officinalis* is composed of some important compounds like (*E*)-caryophyllene and caryophyllene oxide in addition to major constituents such as citronellal, neral and geranial (Sorensen, 2000; van de Berg et al., 1997; Holla et al., 1997; Shalaby et al., 1995). Literature reveals that the essential oil of *M. officinalis* subsp. *officinalis* contains significant amounts of citral and/or citronellal, whereas *M. officinalis* subsp. *Altissima* contains only traces (van de Berg et al., 1997; Wolf et al., 1999; Dawson et al., 1988). Van de Berg et al. (1997) have identified the main components of the leaf oils of cultivated *M. officinalis* subsp. *altissima* of Greek origin such as β-caryophyllene (7.27–12.66%), germacrene-D (34.79–51.50%), sabinene (0.91–14.68%) and β-pinene (0.53–8.03%). These compounds have also been detected as the main constituents in the present studies except sabinene.

The current study is the first report involving rapid analysis of volatile components of *M. officinalis* using HS-SPME technique.

In conclusion this report on chemical composition of the essential oil of *M. officinalis* was determined using two different techniques i.e., hydrodistillation and HS-SPME. From the two techniques (hydrodistillation and HS-SPME) it is concluded that the volatile constituents of *M. officinalis* show both qualitative as well as quantitative differences. On one hand hydrodistillation led to the identification of 24 volatile constituents and on the other hand only 22 constituents were identified using the HS-SPME technique. The variation in chemical composition may be attributed to the formation of artefacts during hydrodistillation/HS-SPME technique (Vazquez-Araujo et al., 2013).

4. Conclusions

In conclusion, the present report of the chemical profile of the essential oil of *M. officinalis* provides further indepth information about the chemo diversity in the chemical composition of the essential oil of the genus *Melissa*. Also the HS-SPME is a rapid, simple and eco-friendly method for the essential oil screening of aromatic plants. This novel process can produce essential oil in concentrate form, free from any residual solvents, contaminants, or artefacts. A study of the application of this new method for the quantitative determination of volatile constituents from food, cosmetics and medicine is under way.

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