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Essential Oil Composition of Lemon Balm (*Melissa officinalis* L.) Leaves Grown in Hamadan Province, Iran

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ABSTRACT

This paper focuses on the analysis of the chemical composition of lemon balm essential oil (EO) cultured in Hamedan province, Iran. The EO of leaves (0.32% yield, w/v) was obtained by steam distillation with a Clevenger apparatus and analyzed by capillary GC and GC/MS. 18 substances were identified. The main components of the EO were geraniol (44.23%), citronellol (23.3%), β-caryophyllene (5.66%), citronellal (4.74%), spathulenol (3.4%), geranyl acetate (3.3%) and γ-murolene (2.13%).

Keywords: GC-MS, geranial

INTRODUCTION

Lemon balm (*Melissa officinalis* L.), a member of the Laminaceae, is a perennial plant that reaches up to 1 m. This species originates from southern Europe, Asia Minor and southern parts of North America. Lemon balm populations are distributed in all Mediterranean countries including the coastal regions of Turkey and northern Iran.

Lemon balm has several purposes, e.g. as a food additive, herb tea, an ingredient in cosmetics, ornamental plant and in medicine (Sar and Ceylan 2002). Its essential oil (EO) is currently used in medicine and pharmacology as an antimicrobial (Mimica *et al*. 2003; Mencherini *et al*. 2007), anti-tumor (de Sousa *et al*. 2004), antioxidant (Marangui *et al*. 2004; Dastmalchi *et al*. 2008), to moderate Alzheimer's disease (Khayyal *et al*. 2001), stimulate the immune system (Drozd and Anuszewska 2003) and possesses anti-HIV-1 activity (Allahverdiyev *et al*. 2004). In addition, lemon balm has traditionally been used for to its memory-enhancing properties, but it is currently widely used as a sedative or calming, spasmolytic and antibacterial agent and sleep aid (Sadraei *et al*. 2003; de Sousa *et al.* 2004; Kennedy *et al*. 2004).

The goal of the present work was to study the composition and content of the EO of lemon balm cultured in Hamedan Province, Iran. The composition of this EO may be useful in the identification of aromatic compounds of lemon oil obtained commercially by steam distillation.

MATERIALS AND METHODS

Plant material

The lemon balm plant used in the present study was prepared from Medicinal Plants Garden of Ebne–Sina at Hamadan of Iran. During summer leaves were collected from field-grown plants.

EO extraction

For the isolation of the EO, hydrodistillation with the use of a Clevenger apparatus was used. The leaves were carefully cleaned and distilled separately for 3 h. Oil samples were dried over anhydrous sodium sulfate and stored in a sealed vial at low temperature before analysis.

GC

GC analysis was performed using a Shimadzu GC -9A gas chromatograph equipped with a DB-1 fused silica column (60 m × 0.25 mm, film thickness 0.25 μm), oven temperature was held at 40°C for 5 min and then programmed to 250°C at a rate of 4°C/min; injector of 32 cm/s. Quantitative data was obtained from FID area percentage without the use of correction factors.

GC/MS

GC/MS analysis was carried out on a Varian 3400 GC/MS system equipped with a DB-1 fused silica column (as for GC). Oven temperature 50-250°C at rate of 4°C/min, transfer line temperature 260°C, carrier gas, helium with a linear velocity of 31.5 cm/s, split ratio 1/60, ionization energy 70 e V, scan time 1s, mass range 40-300 amu.

Identification of components

The components of the EO were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by compaction of their retention indices, either with those of authentic samples or with data published in the literature (Davia 1990).

RESULTS AND DISCUSSION

EO content

In lemon balm, the yield of EO is quite low (0.32%, w/v). Tınmaz *et al.* (2001) reported that the highest EO yield in lemon balm was 0.14%. Maximal EO yield was in lemon balm from 17 European regions (0.09 to 0.45%), as determined by Patora *et al.* (2003). In a previous study, we showed the yield of the EO in lemon balm flowers was 0.18% (Adinee *et al*. 2008). Significant differences among yield of the EO of different tissues of lemon balm have been reported (Patora *et al*. 2003).

Components identification and quantification of EO

In **Fig. 1** the chromatogram of lemon balm leaves EO is

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Short Communication

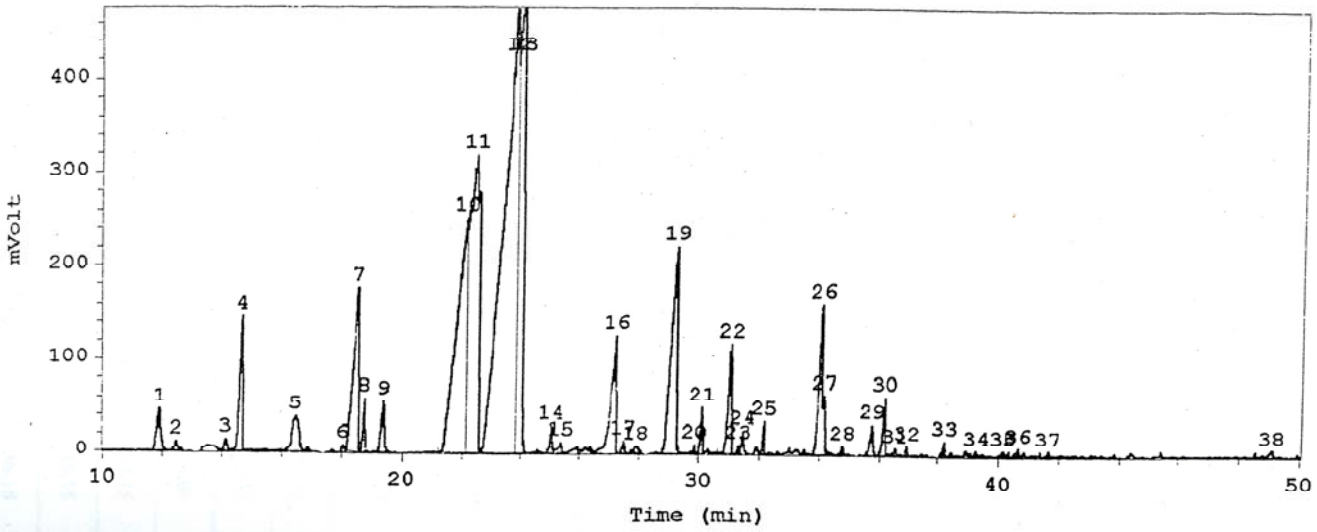

Fig. 1 Chromatogram of lemon leaf EO in Hamedan province of Iran.

Table 1 Percentage of lemon balm leaf essential oil components in Hamadan province of Iran.

Components	Peak number	Kovats retention index	Relative percentage
1-octen-3-ol	2	970	0.13
Trans-ocimene	4	1028	1.9
Linalool	5	1072	1.18
Citronellal	7	1126	4.74
Trans-verbenol	8	1132	0.52
Lavandulol	9	1150	0.78
Citronellol	10	1223	12.3
	11	1231	15.0
Geraniol	12	1266	32.78
	13	1271	11.46
Geranyl acetate	16	1356	3.33
β-Bourbonene	18	1375	0.12
β-Caryophyllen	19	1412	5.66
α-Humulene	21	1438	0.43
γ- Murolol	22	1468	2.13
δ-Cadinene	25	1500	0.25
Spathulenol	26	1572	3.04
Caryophyllen oxide	27	1624	0.16
α-Cadinol	29	1637	0.44
Trans-Murolol	30	1645	0.72
			97.07

presented. 18 substances were identified while 20 were not. The principal components of EO of the leaves are presented in **Table 1**: geraniol (44.23%), citronellol (23.3%), β-caryophyllene (5.66%), citronellal (4.74%), spathulenol (3.4%), geranyl acetate (3.3%) and γ-murolene (2.13%). Citronellal, citronellol, linalool and geranial as the major components of the EO of lemon balm have previously been reported (Sar and Ceylan 2002).

Many researchers have reported that the main component of lemon balm is geranial. However, there were significant differences among the rates of those reported components. The geranial level in the EO was reported as 31.6% by Werker *et al.* (1985) and 35-85% by Sar and Ceylan (2002). These differences may be due to different environmental conditions and/or the use of different genetic material. Genetic variations among lemon balm populations for the rate and components of the oil and the significant effect of the environment on these two are known (Patora *et al*. 2003; Kennedy *et al*. 2006; Farahani *et al*. 2009). However, geranial contributes significantly to the quality of lemon balm flavor and aroma, and concentration is by far the most important factor in determining the commercial value of lemon balm oil (Gramshaw and Sharpe 1980; Sar and Ceylan 2002).

Lemon balm has traditionally been used due to its memory-enhancing properties, but is currently more widely used as a sedative (Kennedy *et al*. 2003; Sadraei *et al*. 2003; Kennedy *et al*. 2004; de Sousa *et al*. 2004; Dastmalchi *et al*. 2008).

In conclusion, we found that geraniol and citronellol are the principal components of EO in the leaves of *M. officinalis*. This is of interest since both compounds have great commercial value and are mainly utilized as fragrance fixatives in the flavor industry (Hener *et al*. 1995).

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