The Influence of Drying Treatments on the Essential Oil Content and Composition of *Melissa officinalis* L. Compared with the Fresh Sample

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Abstract

Background: In the recent decade, artificial drying has been one of the most important needs of the pharmaceutical industries. In addition, different drying methods have different effects on the quantity and quality of the essential oils produced from medicinal plants.

Objective: The main objective of this study was to evaluate the effects of different drying methods (shade and oven drying at 35 °C and 55 °C) in comparison with the fresh sample on the essential oil yield and volatile composition of *M. officinalis*.

Methods: This experiment was conducted in completely randomized design with three replicates. The essential oil samples were isolated by hydrodistillation in a Clevenger type apparatus and analyzed using GC and GC–MS methods.

Results: Different drying treatments had a significant effect on the content of *M. officinalis* essential oil (ranging from 0.08 to 0.3 % v/w; overall average of 0.22% v/w). The main components of the essential oil of shade dried, oven-dried at 35 °C and oven-dried at 55 °C samples were β-caryophyllene, geranial and γ-cadinene, respectively. Oven drying at 55°C resulted in disappearance of neral, geranial and neryl acetate. Sesquiterpene hydrocarbons constituted the principal fraction of all samples, followed by oxygenated monoterpenes, except the oil of oven dried sample at 55°C in which oxygenated sesquiterpene represented as the second main fraction.

Conclusion: The highest essential oil yield was obtained from oven drying at 35°C which conserves the characteristic aroma of the spice, so this method seems to be more advisable for drying of *M. officinalis*.

Keywords: ***Melissa officinalis*, Drying method, Essential oil, GC and GC–MS**

Introduction

Melissa officinalis L. (Family: Lamiaceae), popularly known as lemon balm is one of the oldest and most common aromatic and medicinal herbs [1]. This Plant is native to India, Africa and Southern Asia and nowadays cultivated world-wide for its characteristic lemon-scented leaves [2]. This perennial bushy and upright herb, with local name of "varang-bou" in Iran, puts out numerous herbaceous stems reaching a height of about 1 m, and grows widely in provinces of Alborz, Tehran, Golestan, Azarbayjan, Lorestan and Kermanshah [3, 4]. The leaves of *M. officinalis* have been used traditionally to treat in the folk medicine because of their sedative, aromatic, digestive and antispasmodic properties as well as additive in food, herbal tea, and ingredient in cosmetics, ornamental and medicinal usages [5]. It also has antidepressant [6], neuro-protective [7], anti-inflammatory [8], antioxidant, antifungal, antibacterial [9], antiviral [10] and anti-Alzheimer [11] effects. Nowadays, lemon balm is gaining increasing interest in various branches of industry (such as cosmetics, pharmaceuticals, perfumes and food industries) worldwide [12]. The essential oil of lemon balm is considered the therapeutic principle mainly responsible for most of the biological activities mentioned, but plant phenolic compounds (especially rosmarinic acid and caffeic acid), are also considered to contribute to the therapeutic potential of *M. officinalis* [13]. To date, many investigations have considered the volatile oil composition of *M. officinalis* which contains mainly monoterpenes, sesquiterpenes, alcohols, aldehydes, ketones, esters and miscellaneous compounds [13, 14].

Based on the previous studies lemon balm essential oils showed different chemotypes in various parts of the world due to climatic, geographical and plant genetic background differences. The essential oil composition of *M. officinalis* L. grown in Morocco show citronellal and isogeraniol as the main constituents [15]. In other reports, the major component were cedrane and 2,2,8,8-tetramethy-l-5-nonanone in Iranian lemon balm [16] and β-pinene and sabinene in oil of *M. officinalis* grown in Greece [17]. Citrals (geranial + neral) and citronellal were reported as the main constituents of essential oil in Brazilian [18] and Serbian and Montenegrin [9] lemon balm. Results by Mrlianova *et al.* (2002) cited Citrals and β- Caryophyllene oxide as the major compounds in this plant [19].

In recent decades, the demand for high-quality medicinal herbs is permanently increasing all over the world. Herbs can be marketed as fresh or dried products. Fresh herbs cannot be supplied in a profitable way to all world-wide locations. Drying is the most common and oldest method for post-harvest preservation and a fundamental requirement to achieve a high quality product [20]. The main aim of drying products is to allow longer periods of storage, minimize packaging requirements and reduce shipping weights and improve shelf life in an uncomplicated manner [21, 22]. Volatile constituents are the most sensitive component in the process of food drying. Previous studies showed that the method of drying had a significant effect on

the quality and quantity of the essential oils and the proportion of the various components in medicinal plants [22-24]. The impact of shade, sun and oven drying on the yield and chemical composition of essential oil of *Satureja hortensis* [23] and *Chamaemelum nobile* [25] has been reported in the literatures. Furthermore, it was also reported that essential oil content and chemical profile of *M. officinalis* L. were affected by different drying methods [2, 26-27].

These results demonstrate that the influence of drying methods on lemon balm essential oil depends mainly on plants characteristics and chemotypes as well as drying parameter. However, there is no report on the effect of different drying methods on lemon balm volatile oil in Iran. Thus, the aim of this study was to assess the influence of drying methods (shade and oven drying at 35 °C and 55 °C) in comparison with the fresh aerial part sample on the essential oil content and composition of *M. officinalis* cultivated Karaj, Alborz Province, Iran.

Materials and Methods

Plant Material

The seeds of *M. officinalis* were provided by Zardband Medicinal Plants Production Co., Tehran, Iran. Planting was done with 30 cm row spacing in plant nursery in mid-February 2012. Transport of transplanting to the main field (Garden of Medicinal Plants Research Campus located in the Botanical Gardens of College of Agriculture and Natural Resources of University of Tehran, Karaj, Iran) was done with 50 cm row spacing and 30 cm plant distance in mid-May 2013. The aerial parts of the plants were harvested at full flowering stage by hand in mid-July 2013. A voucher specimen was deposited at the Herbarium of Horticultural Science Department, University of Tehran, Karaj, Iran. To study the effect of the drying method, different drying treatments including shade-drying and two temperatures of oven-drying at 35 °C and 55 °C in compared with the fresh sample were investigated.

Isolation of the essential oil

The dried aerial parts of every treatment (50 g, three replicates) and the fresh aerial parts (150 g, three replicates) were subjected to hydrodistillation of 3 h using an all-glass Clevenger-type apparatus. The oil was dried over anhydrous sodium sulfate, and then, was kept in a sealed vial at 4 ºC until analysis. The percentage yields of the oils were calculated based on the dried weight of plant material according to volume/weight percent.

Oil analysis procedure

Gas chromatography analysis

GC analyses were performed using a Perkin-Elmer gas chromatograph model 8700, equipped with flame ionization detector (FID) and HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Oven temperature was programmed from 80 °C to 220 °C at the rate of 4 °C/min; initial and final temperatures were held for 3 and 10 minutes, respectively. Detector (FID) temperature was 290 °C and injector temperature was 220°C. Helium was used as carrier gas with a linear velocity of 1.5 ml/ min. The percentages of compounds were calculated by the area normalization method, without considering

any response factors.

Gas chromatography/mass spectrometry (GC-MS) analysis

GC–MS analyses were carried out in an Agilent-Technologies (Little Falls, California, USA) 6890N Network gas chromatographic (GC) system equipped with a HP-5 MS fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm); oven temperature was 80–220 °C at a rate of 4 °C/min, transfer line temperature 290 °C, carrier gas, helium, with a linear velocity of 1.5 ml/ min, split ratio 1:100, ionization energy 70 eV, scan time 1 s, and mass range 50 –550 *m/z*.

Compounds identification

The components of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds, and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature [28]. The mass spectra from the literature were also compared [28]. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes.

Statistical analysis

The experimental design was completely randomized, with three replications. The Analysis of variance (ANOVA) for essential oil content affected by different drying methods and fresh sample was conducted using the SAS 9.1 software. Multiple comparisons of means were carried out by the Duncan's Multiple Range Test. All statistical significance was determined at the 1% significance level.

Results

The analysis of variance for essential oil content of *M. officinalis* is shown in Table 1. Different drying treatments had a significant effect ($P \leq 0.01$) on the content of *M. officinalis* essential oil. Mean comparisons of different drying treatments on oil content (Table 2) showed that dried plant materials in oven-drying at 35 °C (0.3% v/w) and shade-drying (0.3% v/w) yielded more essential oils than the other materials. However, there was no significant difference between these treatments and oil content obtained from fresh sample (0.23% v/w), so they were placed in one group.

The eight important and common constituents which are listed in Table 3 in order of their elution on the HP-5MS column, were the most abundant compounds in different samples under study, representing 58.8-76.6% of the total volatiles concentration. The percentages and relative concentrations of these 8 compounds could be important in determining the quality of the different dried samples of lemon balm. The major components obtained from fresh aerial part were β-caryophyllene (21.8%), germacrene D (15.5%), geranial (12.7%) and γ-cadinene (12%).

The drying method caused some variation on the relative proportions of the components. The all major compounds showed no sharp difference among the drying methods except neral and geranial that did not exist in oil obtained from oven dried at 55 °C.

Table 1- Analysis of variance for essential oil content of *Melissa officinalis*

Source of variation	DF	MS
Treatment	3	0.034**
Error	8	0.002
Total	11	-

CV=22; **significant at P ≤ 0.01

Table 2- Mean comparisons of different drying treatments and fresh sample on essential oil content of *Melissa officinalis*

Treatment	Means of oil content (% v/w)
shade-drying	0.3 [a]
oven-drying at 35 °C	0.3 [a]
oven-drying at 55 °C	0.08 [b]
Fresh sample	0.23 [a]

Similar letters in means column are not statistically different at 1% level of probability using Duncan's multiple range tests.

Table 3- Chemical composition of essential oils (%) in *Melissa officinalis* using different drying treatments

No.	Compound	RI	Shade-drying (%)	Oven-drying at 35 °C (%)	Oven-drying at 55 °C (%)	Fresh sample (%)
1	Neral	1245	8.1	5.3	*tr*	5.4
2	Geranial	1271	13.0	18.9	*tr*	12.7
3	Neryl acetate	1365	1.6	1.9	*tr*	1.6
4	β-Caryophyllene	1421	14.9	17.9	12.6	21.8
5	α-Caryophyllene	1455	1.5	1.8	2.2	2.5
6	Germacrene D	1489	11.0	10.5	11.6	15.5
7	γ -Cadinene	1514	8.0	11.3	20.6	12.0
8	Caryophyllene oxide	1583	2.5	6.5	11.5	5.2
Oxygenated monoterpenes			21.1	24.2	0.0	18.1
Sesquiterpen hydrocarbon			37.0	43.4	47	53.4
Oxygenated Sesquiterpenes			2.5	6.5	11.5	5.2

RI, retention indices in elution order from HP-5MS column. *tr*, less than 0.05%

Discussion

In almost all plant species of the Lamiaceae, the essential oil is synthesized in specialized secretory organs such as glandular trichomes [29]. Considerable levels of essential oil yield (>2%) were recorded for genera *Mentha*, *Lavandula, Origanum, Salvia* and *Satureja* as essential to the oil-rich plant [30, 31]. The total oil content in *M. officinalis* is relatively low [27]. The essential oil content of *M. officinalis* in our study (range, 0.08 –0.3 % v/w; overall average of 0.22% v/w) was relatively higher than when compared with literature reports in India [32] Turkey [33] and Germany [34]. Also, in another study that was conducted among the three methods of drying (shadow drying, sun drying and fresh sample) the highest oil content (0.084% and 0.122% w/w, from two locations) was obtained from shadow drying,

and the lowest (0.099% and 0.06% w/w, from two locations) was observed from fresh plant [35]. The Essential oil yield obtained from fresh material for this species in present study is comparable to results reported by Abdellatif *et al* (2015) [14] and Adinee *et al*. (2009) [36] for the oil yield extracted from the fresh lemon balm, but still lower than those recorded by Blank *et al.* (2005) [37] and Pino *et al.* (1999) [38] for fresh and air-dried sample, respectively. Although, the samples dried in oven at 35 °C and shade had considerable oil yield, there were no significant differences between essential oil content of these treatments with oil content of fresh sample. Sefidkon *et al.* (2006) revealed that the oven drying at 45 °C and shade drying had no significant effect on oil yield of *Satureja hortensis* [23]. These findings contradict those obtained by Sellami *et al.* (2011) in *Laurus nobilis* [39] Ghasemi Pirbalouti *et al.* (2013) in *Satureja bachtiarica* [20], and Rahimmalek and Goli (2013) in *Thymys daenensis* [22] which reported the essential oil yields of fresh sample and those dried by oven temperature (at 35- 45 °C) and shade were not similar and they had significant differences.

Increase in the essential oil content of dried plant materials compared with the oil yielded from the fresh sample can be attributed to the structural changes of specific cells containing the essential oil with special structures and lignified cell walls. Such cells are located in the leaves of parenchymatous tissue [40]. Opposite results may be due to the differences of plant species, the secretory structures and their position in plant body, and the chemical composition of essential oil [41]. In this study, the lowest amount of essential oil yield was resulted through oven drying at 55 °C which indicates that increasing the drying temperature would significantly decrease the essential oil content. At high temperatures, the biological structure of the oil glands of aromatic plants can be affected significantly, and the epithelial cells in the dried samples of some sensible plants can collapse resulting in more volatile oil diffusion during the drying, which could explain the loss of essential oil in high temperatures [42, 43]. In the same way, Khangholil and Rezaeinodehi (2008) [41], Braga *et al.* (2005) [44], and Argyropoulos and Müllera (2011) [2] held that increasing the drying temperature would result in a significant decrease in the essential oil content.

Concerning the essential oil extracted from the fresh sample, β-Caryophyllene and Germacrene D were the two major components. According to previous reports regarding this species, this combination of major compounds was rare for lemon balm in other regions. To the best of our knowledge, there is only one published case report [35] which cited β-Caryophyllene and Germacrene D as the main constituents of essential oil of fresh lemon balm. Our results confirm previous reports which cite different chemotypes for the species *M. officinalis*. The chemical composition of medicinal and aromatic plants can be altered in response to different environmental conditions and developmental stages or in different plant parts [45]. Many researchers have reported that the main components of fresh lemon balm are neral and geranial [13, 14, 33, 37, 46, 47]. In other reports, the major compounds of fresh

sample of *M.officinalis* essential oils were geraniol and citronellol in Iran [36], Nerol and Citral in Morocco [8], and Citronellal and Citronellol in China [26].

Drying of lemon balm caused not only the quality deterioration of the essential oil, but also resulted in some quantitative variations of relative proportion of the components. The chemical compounds fluctuation in different drying treatments (such as shade or oven drying) was reported previously in lemon balm [26, 27], *Satureja bachtiarica* [20], *Laurus nobilis* [39] and sage [42].

In the present study, some of the components were missing in the essential oil of oven dried sample at 55°C. The components of the essential oils that are lost in the dried samples are those stored on or near the leaf and stem surfaces [48]. Also Sellami *et al.* (2011) reported that relatively long period of drying may cause oxidation process and chemical rearrangements which lead to the disappearance of some oil constituents [39]. Besides, in accordance with our results, Rahimmalek and Goli (2013) reported that the oven drying resulted in the loss of some components in thyme compared with the shade dried and the fresh sample. On the other hand, some compounds seem to have more affinity to the water fraction contained in thyme leaves and thereby, they were lost with water during the drying process.

In general, although the behavior of the various compounds during different drying methods is not fully understood, it can be hypothesized that compounds with lower boiling points (retention time) will show a lower share in the total oil at higher drying temperatures [27].

Sesquiterpene hydrocarbons constituted the principal fraction of all samples, followed by oxygenated monoterpenes, except oil of oven dried sample at 55°C in which the oxygenated sesquiterpene represented the second main fraction. Lemon balm belongs to the Lamiaceae family of plants, which are known to store their essential oils on or near the leaf surfaces [48]. Besides, increasing the temperature to 55°C, would increase the chance to collapse the cuticle layer and damage the extensive cell of the epidermis which Causes monoterpenes (the low molecular components) to leave the plant organ more rapidly compared to sesquiterpenes [27].

Overall, the development of the essential oil sector has a direct relation to the improvement of postharvest process especially the drying technology in industrial plants. Results obtained from experimental data could be recommended to their uses in functional food and pharmaceutical applications. In this study, oven drying at 35°C was faster than other treatments and resulted in appreciable essential oil yield, and helped to conserve the characteristic aroma of the spice, so would seem to be more advisable for drying the lemon balm.

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