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Storage conditions affect the essential oil composition of cultivated Balm Mint Herb (Lamiaceae) in Iran

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ABSTRACT

Lemon balm (*Melissa officinalis* L.) is one of the most important herbs known for curing Alzheimer's disease. In this study, the effect of time and temperature on the quality of essential oils was investigated. The essential oil of air-dried samples was obtained by hydro-distillation and was analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS). Changes in essential oil compositions were detected during storage for four months in a refrigerator (4 °C), a freezer (−20 °C), and at room temperature. The results indicated that, at room temperature, the proportions of compounds with lower boiling temperatures such as citronellal (25.8–12.6%), neral (18.9–4.0%) and geranial (27.0–4.6%) were decreased. Furthermore, the oil compositions showed the least alterations and *M. officinalis* kept its primary quality when stored at low temperatures, particularly at −20 °C.

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1. Introduction

Essential oils are aromatic and volatile liquids extracted from plant material, such as flowers, roots, bark, leaves, seeds, peel, fruits, and wood (Sanchez et al., 2010). Essential oils have been used for centuries in medicine, perfumery, and cosmetics, and have been added to foods as part of spices or herbs. Their initial application was in medicine, but in the nineteenth century, their use as aroma and flavor ingredients increased and became their major employment. Almost 3000 different essential oils are known and 300 are used commercially in the flavor and fragrances market (Burt, 2004). Essential oils are considered to be secondary metabolites and important for plant defense as they often possess antimicrobial properties (Tajkarimi et al., 2010). The antibacterial properties of secondary metabolites were first evaluated using essential oil vapors by De la Croix in 1881 (Burt, 2004). Since then, essential oils or their components have been shown to not only possess broad-range antibacterial properties (Oussalah et al., 2007), but also antiparasitic (George et al., 2009), insecticidal (Kim et al., 2003), antifungal (Silva et al., 2011), and antioxidant (Brenes and Roura, 2010) properties. Furthermore, they also function as growth enhancers for animals (Ahmadifar et al., 2011). Essential oils, also called volatile oils are aromatic oily liquids obtained from different plant parts and are widely used as food flavors. The constituents of the essential oils are mainly monoterpenes and sesquiterpenes, which are hydrocarbons with the general formula $(C_5H_8)_n$. Oxygenated compounds derived from these hydrocarbons, include alcohols, aldehydes, esters, ethers, ketones, phenols and oxides. Volatile oils have been reported to exhibit various antibacterial, antifungal, antiviral and antioxidant properties (Prabuseenivasan et al., 2006). The main class of substances used in aromatherapy is essential oils, which are obtained from various aromatic parts of plants by different methods such as hydro and steam distillations or cold pressing. Essential oils and extracts of various species of edible and medicinal plants, herbs, and spices constitute very potent natural biologically active agents (Nychas et al., 2003). Plants produce a variety of compounds with antimicrobial activity. Some are always present while others are produced in response to microbial invasion or physical injury (Roller, 2003). Identifying the most active antimicrobial compounds of essential oils is cumbersome because essential oils are complex mixtures of up to 45 different constituents (Espina et al., 2011). In addition, the composition of a particular essential oil may vary depending on the season of harvest, and the methods used to extract the oil (Demuner et al., 2011).

Variation in chemical composition of essential oils, in particular, and extracts of medicinal plants may be observed due to the origin, the environmental conditions, and the developmental stage of collected plant materials (Miguel et al., 2004).

Lemon balm, *Melissa officinalis*, is a member of the mint family that is native to Europe. Its use as a medicinal herb dates from the Middle Ages, and it is very well known for its ability to reduce stress

Abbreviations: RI, retention indices; GC/MS, gas chromatography–mass spectrometry; EOs, essential oils.

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and anxiety, promote sleep, improve appetite, and ease pain and discomfort associated with digestion. The medicinal use of essential oils has been known since the early times (Umezu et al., 2002). Popularly, they are used in the control of emotions and mood, for their sedative, anxiolytic (Lehrner et al., 2000), and antidepressant effects (De-Souza et al., 2006) and anticonvulsant (Sousa et al., 2007) among others (Barbosa-Filho et al., 2008). A single, double-blind, placebo-controlled study also assessed the behavioral effects of *M. officinalis* aromatherapy in a group of patients suffering from severe dementia. In comparison to placebo a significant reduction in agitation and social withdrawal, and an increase in constructive activities resulted from the four week treatment with essential oil (Ballard et al., 2002). There is little chemical information about compositional changes of essential oils during storage and/or usage. It is important for consumers and other parties concerned with aromatherapy to ensure the effectiveness and quality of essential oils products, in addition to guaranteeing their safety. The influence of storage on the chemical composition of essential oils from *Leonurus cardiaca* L. grown wild in Vilnius and from commercial herbs showed that, contact with air induced oxidation of β-caryophyllene and α-humulene during storage of essential oils in stoppered glass vessels when keeping samples in refrigerator (Mockute et al., 2005). But, there is no data about the changes on essential oils composition of *M. officinalis* during its storage.

Lemon balm is beneficial for a wide variety of human disorders such as cancer, HIV-1, Alzheimer's disease, attention deficit hyperactivity disorder, indigestion, gas, insomnia, and hyperthyroidism (De Sousa et al., 2004; Kennedy et al., 2004). Therefore, the aim of the present study was to investigate the influence of storage on the chemical compositions of essential oils in *M. officinalis*. In this regard the composition of *M. officinalis* was measured in different time periods stored at 4 °C, −20 °C, and room temperature.

2. Experimental

2.1. Plant material

The aerial parts of *M. officinalis* were collected from Eram Garden in Fars province. Voucher specimen was deposited at the Herbarium of Fars Research Center for Agriculture, Shiraz, Iran. The plants were shade dried for 14 days at room temperature (20–25 °C). The EOs of all dried samples (100 g) were isolated by hydro-distillation for 3 h, using a Clevenger-type apparatus according to the method recommended by the British Pharmacopoeia (British Pharmacopoeia, 1988). The distillated oils were dried over anhydrous sodium sulfate and put in tightly closed dark vials for further investigations.

2.2. Volatile oils storage conditions

In order to investigate the impacts of different storage conditions on the compositions of distilled oils, the oil samples were subjected to different storage temperatures such as, refrigerator (4 °C), freezer (−20 °C) and at room temperature (25 °C) for four successive months until analysis. The oils analysis of all storage treatments performed monthly. Moreover, to determine the exact effects of storage conditions on EOs compositions during the experiment period, the fresh extracted oil was analyzed immediately after extraction.

2.3. Oil analysis procedure

GC analysis was performed using an Agilent gas chromatograph series 7890-A with a flame ionization detector (FID). The analysis was carried out on fused silica capillary HP-5 column (30 m × 0.32 mm i.d.; film thickness 0.25 μm). The injector and detector temperatures were kept at 250 °C and 280 °C, respectively. Nitrogen was used as carrier gas at a flow rate of 1 ml/min; oven temperature program was 60–210 °C at the rate of 4 °C/min and then programmed to 240 °C at the rate of 20 °C/min and finally held isothermally for 8.5 min; split ratio was 1:50. GC–MS analysis was carried out by use of Agilent gas chromatograph equipped with fused silica capillary HP-5MS column (30 m × 0.25 mm i.d.; film thickness 0.25 μm) coupled with 5975-C mass spectrometer. Helium was used as carrier gas with ionization voltage of 70 eV. Ion source and interface temperatures were 230 °C and 280 °C, respectively. Mass range was from 45 to 550 amu. Oven temperature program was the same given above for the GC.

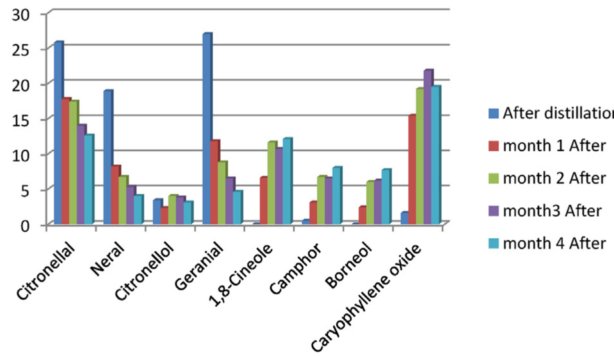

Fig. 1. Changes of *Melissa officinalis* essential oil composition during 4 month storage at room temperature.

2.4. Identification of compounds

The constituents of the EOs were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C8–C25) and the oil on a HP-5 column under the same chromatographic conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literature (Adams, 2001). For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

3. Result and discussion

There are little investigations about plant secondary metabolites storage especially essential oils as these metabolites are volatile and potentially could be subjected to different alterations by storage circumstances. In this study, the compositions of hydro-distilled essential oils of *M. officinalis* were determined at different temperatures and storage times (Tables 1–4). In total, 50 constituents were identified and quantified in the *M. officinalis* essential oils samples. The monoterpenoid fraction constitutes 97.7% of the oil with the main components 1,8-cineole, borneol, citronellal, camphor, citronellol, neral, geranial, (E)-caryophyllene and caryophyllene oxide. Percentage of the identified sesquiterpenes and sesquiterpenoid components was relatively low (2.3%). Monoterpenes are the primary components of plant essential oils and the effects of many medicinal herbs have been attributed to them (Gherlardini et al., 2001; Santos and Rao, 2000). The comparison of essential oils constituents of different temperatures and storage times indicated that the amounts of main compounds were drastically changed during storage at room temperature compared to those of corresponding conditions (Fig. 1 and Table 1). In this regard, the findings of our work showed that the concentration of constituents with a

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