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Variation of essential oil composition of *Melissa officinalis* L. leaves during different stages of plant growth

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ABSTRACT

Objective: To determine the best time of harvest for *Melissa officinalis* (*M. officinalis*) L. to gain highest amounts essential oil.**Methods:** *M. officinalis* leaves were harvested in three different stages (before flowering stage, flowering stage and after of flowering stage) and were dried. The essential oils were isolated by hydro- distillation and analyzed by GC/MS.**Results:** It showed that most essential oils of plants were in before flowering stage. In before flowering stage 37 compounds were identified in leaves oil of *M.officinalis*. The major components before flowering stage were decadienal (29.38%), geraniol (25.3%), caryophyllene oxide (8.75%), geranyl acetate (5.41%). In the flowering stage 36 compounds were identified as the major components of plant essential oils: decadienal (28.04%), geraniol (24.97%), caryophyllene oxide (7.55%), caryophyllene E (4.65%) and 16 components in the after flowering stage of plant were identified as the major components carvacrol (37.62%), methyl citronellate (32.34%), geranyl acetate (5.82%), caryophyllene (5.50%).**Conclusions:** The essential oils yields vary considerably from month-to-month and is also influenced by the micro-environment (sun or shade) in which the plant is growing. We found that the essential oil content of *M. officinalis* L. of leaves is significantly affected by harvesting stages.

1. Introduction

Lemon balm, member of the family Lamiaceae (formerly Labiatae) is a perennial bushy plant and is upright, reaching a height of about 1 m. The soft, hairy leaves are 2 to 8 cm long and either heart-shaped[1]. Lemon balm can rapidly grow at temperature range 15 to 35℃ and requires 500 to 600 mm precipitation well distributed throughout the growing season, otherwise it should be irrigated. The leaf surface is coarse and deeply veined, and the leaf edge is scalloped or toothed. Flowers white or pale pink consisting of small clusters of 4 to 12 blossom in the summer. *Melissa officinalis*(*M. officinalis*) is used in herbal medicine and is native to the eastern Mediterranean region and western Asia[2].

In the Lamiaceae family, essential oils are mainly produced in secretary structures known as glandular trachoma, of which there are two main kinds, peltate and capitates. In this respect, *M. officinalis* is no exception[3]. The amount of essential oils produced is directly connected with the number and physiology of these structures. In *M. officinalis*, the peltate glands are very sparsely distributed on the leaf and, furthermore, once the leaves have grown to 4 mm in size. The glandular pattern is fully developed and the number of peltate trichomes does not increase[4]. This may be a physiological reason for low yield in *M. officinalis*. Lemon balm essential oil, obtained from fresh or dried flower, leaf, and branches of this plant by water steam distillation or chemical extraction, is characteristic with fresh lemon odor, and light yellow colored. Essential oil of lemon balm and extracts (*M. officinalis* subsp. officinalis and of *M. officinalis* subsp. inodora) can be used as antioxidant[5] have described antimicrobial and free radical scavenging capacitytogether with the effects on lipid preoxidation of lemon balm essential oil in their study. Essential oils of lemon balm are used as an anti-tumeral agent as a potential for cancer remedy or prevention[6]. The volatile oils of lemon balm may also be used as an anti-virus agent and contains as anti- herpes simplex virus type 2 substances. The essential oils find wide and varied application in many industries such as cosmetics and perfumes, beverages and ice creams, confectionary and backed food products, etc for the scenting and flavouring of consumer's finished products. Essential oils are very complicated mixtures of natural compounds at quite different concentrations[7,8]. Essential oils are made up of three elements almost exclusively carbon, hydrogen,

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and oxygen. By far the most common component class is the terpenes. Terpenes are made from combinations of several 5-carbon- base units called isoprene. Terpenes can form building blocks by joining together in a "head-to-tail" configuration to form monoterpene, sesquiterpenes, diterpene and larger sequences. Factors that determine the composition and yield of the essential oil obtained are numerous. In some instances it is difficult to segregate these factors from each other, since many are interdependent and influence one each other[9]. These variables may include seasonal and maturity variation, geographical origin, genetic variation, growth stages, part of plant utilized and postharvest drying and storage[10].

2. Materials and methods

Preparation of plant samples lemon balm was collected in three different times of growth plant (before flowering, flowering and after flowering stage) respectively, in 10 June, 19 august and 23 September 2011.

The leaves of *M. officinalis* were shade dried (12 days) at room temperature, minced and immediately hydrodistilled (100 g) for 2 h, using a modified clevenger type apparatus. Then the plant oils used was injected into the Gas chromatography-mass spectrometry (GC-MS) system to determine the type of their constituents.

An Agilent model 6890 GC interfaced to a 5973 mass selective detector was used for mass spectral identification of the components of the oils. HP-5MS capillary columns (30 m × 0.25 mm × 0.25 μm film thick-ness) were used for GC. The oven temperature was maintained at 60℃ for 6 min then programmed to 240℃ at 5 ℃/min. The carrier gas was helium, at a flow rate of 0.9 mL/min, and the injection volume was 1 μL. In mass spectrometry electron-impact ionization was performed at electron energy of 70 eV.

Components of the oils were identified by comparison of their mass spectra and retention indices with those published in the literature[11].

3. Results

In the leaf essential oil of M.officinalis in the before flowering stage, 37 compounds were identified. The major components were decadienal (29.38%) and geraniol (25.3%), other components present in appreciable contents were: caryophyllene oxide (8.75%), geranyl acetate (5.41%), geranyl (3.15%), caryophyllene E (2.17%), carvacrol (2.04) and linalool (0.1%).

In the leaf essential oil of *M. officinalis* in the flowering stage, 36 compounds were identified. The major components were decadienal (28.04%) and geraniol (24.97%), other components were caryophyllene oxide (7.55%), caryophyllene E (4.65%), geranyl (4.11%), carvacrol (2.75%), geranyl acetate (2.53%), neryl acetate (2.31%) and linalool (0.2%).

In the leaf essential oil of *M. officinalis* in after flowering stage, 16 components were identified. The major components were carvacrol (37.62%) and methyl citronellate (32.34%), other components were geranyl acetate (5.82%), caryophyllene (5.50%), geraniol (4.58%), hepten (3.35%), trans pulegol (3.21%) and linalool (0.72%). The highest essential oil content was obtained at before flowering stage and the lowest was obtained at after flowering stage of *M. officinalis*. The essential oil of *M. officinalis* leaves was decrease at after flowering stage.

Table 1.
The rate of change in the components of essential of *M. officinalis* leaves in various stages.

Compounent	Before flowering	Flowering	After flowering
Caryophyllene	2.17	4.65	5.50
Caryophyllene oxide	8.75	7.55	0.95
Carvacrol	2.04	2.75	37.62
Geraniol	25.03	24.97	4.58
Geranyl acetate	5.41	2.53	5.82
Hepten-2-one<6- methyle-5>	4.82	5.11	3.35
Hexenal (E- 2)	0.22	0.61	0.10
Iso menthol	1.15	1.41	2.35
Linalool	0.83	0.18	0.72
Methyl citronellate	0.98	0.33	32.34
Methyl docanoate	0.60	0.85	0.64

The compounds in essential oil from *M. officinalis* leaves were divided to two main groups monoterpene and sesquiterpene. Monoterpene hydrocarbons were found to be the major group of compounds. Monoterpene accounted for 80% and sesquiterpene for 20% of oil volume form *M. officinalis* leaf were included. The essential oils of *M. officinalis* leaves in the before flowering and flowering stage were monoterpene rich (Figure 1). In monoterpene, oxygenate monoterpenes components were highest at before flowering stage in compared with the after flowering stage.

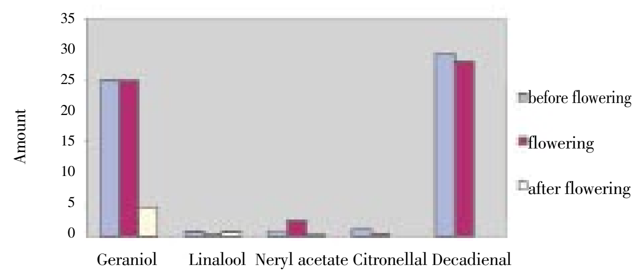

Figure 1. Monoterpene composition of essential oil of *M. officinalis* L. leaves in various stages of plant growth.

The other composition of essential oil constituents in leaves of *M. officinalis* in different stages of growth were shown in Table 1. In total 11 compositions in essential oils in three stages of plant growth (before flowering, flowering and after flowering stage) were observed. The most of these components were oxygenated monoterpenes. The major component found in the leaf oil of *M. officinalis* in three stages was geraniol. The content of geraniol in the essential oil in three different stages was 25.03 (before flowering), 24.97 (flowering) and 4.58 (after flowering). Sari and Ceylan (2002) reported that the essential oil ranged from 0.03% to 0.067% besides, the main component in essential oil was geraniol (33.13% to 53.68 %), and the others were neral, citronellal, and β- pinen in the study, carried out with 11 Melissa populations in Menemen and Bozdağ.

4 Discussion

The amount and composition of essential oil is strongly dependent on the developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factors influencing mint oil quality.

Harvesting a crop early or late resulted in a low yield of leaves as well as the essential oil content because at an earlier or later stage of harvesting, the crop was immature or over mature resulting in a poor yield of herb and oil content. These two factors are interlinked with each other, because the specific ontogenic growth stage will differ as the season progresses. There are many reports in literature regarding the variation in the chemical profile of essential oils from various plants collected during different seasons[3,12,13]. Essential oil rate in drug herb changes between 0.02% to 0.30%, which is quite low compared to other member of the family labiatae. That is why the production cost and price of essential oil is very high in the market. Meftahizade et al(2010), reported that the main constituent of the essential oil *M. officinalis* are citral (Geranial and Neral), citronellal, geraniol, beta-pinene, alpha- pinene, beta-caryophyllene, comprising 96% of the oil ingredients. Farahani et al(2009) in a research about effects of irrigation levels on essential oil of balm, reported that, highest shoot yield and height plant were relate to non stress treatment, highest essential oil yield was achieved under 40% FC and essential oil percent related to 20% FC. Highest stem diameter was related to 20% FC. It could be concluded that moderate drought stress is beneficial for balm essential oil. The essential oils yields varied considerably from month-to-month and was also influenced by the micro-environment (sun or shade) in which the plant was growing. Filiz Ayanoglu (2005) reported essential oil content was affected by harvesting stages, harvesting hours and drying methods and the highest oil content was obtained in the before flowering stage lemon balm[14]. Pala-Paul et al (2001) reported month-to-month variations in the essential oil composition and yield of Santolina rosmarinifolia, which could be attributed to precipitation and temperature. Results obtained by Badi et al(2004) for Thymus vulgaris (thyme) also indicated that the timing of harvest is critical to both yield and oil composition. McGimpsey *et al*(2006) reported seasonal variation in the essential oil composition and yield from naturalized Thymus vulgaris collected from Newzeland. The higher essential oil yield from Thymus vulgaris was reported in spring and over the nine month harvesting period, thymol was found to vary from 31.5%-52.4%. With respect to the harvesting time, the thyme essential oils were richer in oxygenated compounds in the spring, followed by summer, autumn and winter[15]. The maximum yields and phenol content of the oil from naturalized thyme were exhibited from crop harvested after flowering in December[16].

Emadi et al(2007) reported the rate of constituents in leaves of Rosmarinus officinalis plant being collected in three periods (before, after and during blooming) as α-pinene (20.08%, 27.65% and 17.82%), 1.8-cineole (7.32%, 7.55% and 9.99%) and camphor (9.11%, 8.84 and 15.68%). Furthermore, studies on the chemistry of Iran[4].

This study has been concerned with determining the chemical composition characteristics of essential oils extracted from *M. officinalis*.

In the current study, we found that the essential oil content of *M. officinalis* L. of leaves was significantly affected by harvesting stages. Harvesting at before flower and at flowering stages were found to be the best stages to harvest the plant to obtain the highest essential oil yield.

Conflict of interest statement

We declare that we have no conflict of interest.

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