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# VARIABILITY IN THE CONTENT AND COMPOSITION OF ESSENTIAL OIL FROM LEMON BALM (*MELISSA OFFICINALIS* L.) CULTIVATED IN POLAND

JOLANTA PATORA[1], TERESA MAJDA[2], JÓZEF GÓRA [2] and BARBARA KLIMEK[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Medical University of Łódź,
[2]Institute of General Food Chemistry, Technical University of Łódź, Łódź, Poland

**Abstract**: Essential oil from a few different population of *M. officinalis* cultivated in Poland has been investigated. The percentage of essential oil ranged from 0.08 to 0.25 ml/100 g in the leaves and from 0.06 to 0.167 ml/100 g in the herb and was higher in the plant material from experimental patch than that from commercial cultivations. Comparative determinations of the essential oil in fresh and dried material showed slightly higher content of the oil in the fresh one. The analysis of the oil composition has been performed by GC and GC/MS. Great differences in the contents of citral, citronellal, linalool, nerol, geraniol β–caryophyllene and β–caryophyllene oxide among the populations has been found. Effect of the harvest time, drying and storage on the composition of lemon balm oil has also been studied.

**Keywords**: *Melissa officinalis*, lemon balm, melissa oil, monoterpene aldehydes, sesquiterpenes, citral, citronellal, GC analysis

Lemon balm – *Melissa officinalis* L. (*Lamiaceae*) is a Mediterranean plant cultivated in many countries for its therapeutic properties. It is used for the production of many phytopharmaceutical preparations and also fragrances and cosmetics.

In therapy, lemon balm has been used for a long time as mild sedative and spasmolytic remedy (1,2). Sedative activity is due to the presence of volatile lipophilic terpenoids (essential oil) (3–5), which show also antibacterial (6) and anti–inflammatory activities (7,8). According to a recent literature report, ethanol extract from lemon balm and also components of *Melissa* essential oil: citral, geraniol and nerol inhibit the formation of proinflammatory eicosanoids : leucotriene $B_4$ ($LTB_4$) and thromboxane $A_2$ ($TXA_2$) [8].

More than fourty compounds were recognized in melissa oil, only some of them, however, occur in the significant quantities. These are monoterpene aldehydes: citral (geranial + neral) and citronellal as well sesquiterpenes: β–caryophyllene and β–caryophyllene oxide; monoterpene alcohols: nerol, geraniol and citronellol are the minor components and additionally 6–methyl–5–hepten–2–one is mentioned as a characteristic constituent of the natural melissa oil. Previous reports (9–16) pointed, that lemon balm plants may differ significantly in the contents of essential oil and also in the oil composition. The differences may be due to external conditions of plant growth ontogenic period (9,14–15) as well as genetic variability (10,17). Possible adulterations of the commercial products by the leaves or herb of *Melissa officinalis* subsp. *altissima* or *Nepeta cataria* var. *citriodora* should also be taken into account (14,18). Because subspecies *altissima* contains only traces of citral and in the oil from *Nepeta cataria* var. *citriodora* monoterpene alcohols are the predominant compounds (19), relative high content of citral and / or citronellal in the oil has recently been proposed as an essential factor helpful to recognize the authentic material derived from *Melissa officinalis* subsp. *officinalis* (14). Amount and composition of the essential oil depend also upon ontogenic stage of the plant (15).

The Polish Pharmacopoeia V (1) recommends to harvest *Melissae folium* from not flowering plants but gives no indications of the time appropriate for harvesting (e.g. before or after blooming). According to PPh V lemon balm leaves should contain not less than 0.05% (v/w) of essential oil containing citral and citronellal (detection by TLC method).

The aim of this study was to determine the contents and composition of essential oil in a raw material from some Polish cultivations, regarding different populations, effect of harvest time, drying and storage.

## EXPERIMENTAL

### Plant material

The investigated plant material was derived mainly from the Garden of Medicinal Plants of the

Medical University of Łódź (samples 1, 3–5 and 10–16). Plants were grown from seeds obtained from Botanical Garden in Essen (Germany). Plant material was collected from two year plants on dry sunny days, dried at a temperature of 20–25°C and stored in closed boxes.

Samples 1, 3, 4, 10, 13 and 14 were collected before and the samples 11, 12, 15 and 16 after blooming time. Commercial raw material was obtained as dried from Herbapol in Lublin (sample 6), Białystok (sample 7) and Łódź (sample 8) and also from one private producer (samples 2 and 9). It was harvested before blooming at the beginning of July in 1998. Samples 2, 5, 8, 9 and 16 were composed of herb and the remaining samples were the leaves of lemon balm.

**Isolation and determination of the content of essential oil**

Determinations of the volatile oil contents were done by steam distillation in a Deryng's apparatus according to PPh V. 40 g of the dried material or 130 g of the fresh material were placed in a round–bottom flask, 1000 ml of distilled water was added and kept at boiling in the Deryng's apparatus for 3.5 h. The volume of the distilled oil was measured in accordance with PPh V. The oil was separated, diluted to 1:1 with petroleum ether and kept with a drying agent in a refrigerator.

The oils obtained by the above described manner were used to the chromatographic analysis (GC and GC–MS).

**GC analysis**

A gas chromatograph Carlo Erba Instruments type HRGC 5300 Mega Series (with the computerized system Chrom–Card) was used. An injector (SSL) and a detector (FID) were used. The temperature was 320°C. A capillary column DB–17 (30 m : 0.32 mm, film thickness 0.25 μm).

Table 1. Average retention times of main constituens of mellisa oil

| Compound | Retention time (min) |
|---|---|
| 6–methyl–5–hepten–2–on | 9.21 |
| linalool | 12.08 |
| citronellal | 14.76 |
| citronellol | 17.39 |
| nerol | 17.81 |
| methylcitronellate | 18.17 |
| geraniol | 19.07 |
| neral (citral b) | 19.37 |
| geranial (citral a) | 20.63 |
| geranic acid | 22.23 |
| β–caryophyllene | 23.12 |
| geranylacetate | 23.37 |
| α–humulene | 24.68 |
| γ–cadinene | 25.05 |
| germacrane D | 25.55 |
| cadina–3,9–diene | 27.56 |
| β–caryophyllene oxide | 30.97 |
| humulene oxide | 32.07 |

Table 2. The content of essential oil in different samples of lemon balm

| Sample No. | Material | Time of harvesting | Time of determination | ml/100 g in dried material | ml/100 g in fresh material (based on dry mass) |
|---|---|---|---|---|---|
| 1. | leaves | VII 95 | III 98 | 0.085 | – |
| 2. | herb | VII 97 | III 98 | 0.060 | – |
| 3. | leaves | V/VI 98 | VI 98 | 0.220 | 0.250 |
| 4. | leaves | VI 98 | VI 98 | 0.233 | – |
| 5. | herb | V/VI 98 | VI 98 | 0.167 | – |
| 6. | leaves | VII 98 | V 99 | 0.100 | – |
| 7. | leaves | VII 98 | V 99 | 0.080 | – |
| 8. | herb | VII 98 | V 99 | 0.080 | – |
| 9. | herb | VII 98 | V 99 | 0.100 | – |
| 10. | leaves | VII 98 | V 99 | 0.220 | – |
| 11. | leaves | IX 98 | V 99 | 0.220 | – |
| 12. | leaves | X 98 | V 99 | 0.230 | – |
| 13. | leaves | VI 99 | VI 99 | 0.160 | 0.180 |
| 14. | leaves | VII 99 | I 00 | 0.180 | 0.200 |
| 15. | leaves | VIII 99 | IX 99 | 0.220 | 0.240 |
| 16. | herb | IX/X 99 | XI 99 | 0.140 | 0.140 |

Table 3. The contents of the main constituents in essential oil obtained from different populations of *Melissa officinalis* (% of the total oil)

| Compound | Sample number | | | | |
|---|---|---|---|---|---|
| | 6 Commercial cultivation VII 1998 (oil from leaves) | 7 Commercial cultivation VII 1998 (oil from leaves) | 8 Commercial cultivation VII 1998 (oil from herb) | 9 Commercial cultivation VII 1998 (oil from herb) | 10 Garden of Medicinal Plants, VII 1998 (oil from leaves) |
| 6-methyl-5-hepten-2-one | 0.04 | 0.24 | 0.20 | 0.71 | 0.45 |
| linalool | 0.99 | 1.32 | 1.15 | 1.12 | 0.45 |
| citronellal | 6.45 | 3.68 | 1.33 | 7.01 | 15.18 |
| citronellol | 0.08 | 0.93 | 0.07 | 7.69 | 0.03 |
| nerol | – | – | – | 7.89 | – |
| methylcitronellate | 1.72 | 1.37 | 0.56 | 0.57 | 1.49 |
| geraniol | 0.06 | 1.20 | 0.65 | 11.83 | 0.12 |
| neral (citral b) | 6.85 | 4.59 | 4.84 | 7.41 | 17.37 |
| geranial (citral a) | 9.79 | 6.27 | 6.62 | 10.08 | 32.92 |
| geranylacetate | 1.45 | 2.05 | 3.07 | 1.51 | 0.01 |
| geranic acid | – | – | – | – | – |
| β-caryophyllene | 12.20 | 9.39 | 5.75 | 4.53 | 0.01 |
| α-humulene | 0.49 | 0.06 | 0.28 | 0.58 | 0.04 |
| γ-cadinene | 1.41 | 1.23 | 0.99 | 0.08 | 0.27 |
| germacrene D | 1.52 | 0.99 | 0.86 | 0.34 | 0.06 |
| cadina-3,9-diene | 0.65 | 0.59 | 0.31 | 0.19 | 0.16 |
| β-caryophyllene oxide | 18.62 | 25.31 | 31.73 | 10.89 | 5.88 |
| humulene oxide | 1.26 | 1.39 | 0.79 | 0.71 | 0.13 |
| sum of monoterpene aldehydes | 23.09 | 14.54 | 12.79 | 24.50 | 65.47 |
| sum of monoterpene alcohols | 1.13 | 3.45 | 1.87 | 28.53 | 0.60 |
| sum of sesquiterpenes and sesquiterpene oxides | 36.15 | 38.96 | 40.71 | 17.32 | 6.55 |

Carrier gas: $N_2$ with a flow rate of 1.5 ml/min. Temperature program: 60°C for 10 min isothermal, then 4°C/min to 300°C.

### GC/MS analysis

A gas chromatograph type GC 8000 coupled with a mass spectrometer MD 800 (Fisons Instruments) operated at 70eV. The capillary column DB-17 (30 m : 0.32 mm, film thickness 0.25 μm) with an injector on-column was used. Carried gas: He with a flow rate of 1ml/min. Temperature was programmed as 60–250°C at 4°C/min.

Components of the oils were indentified by both retention times and MS spectra using database of Technical University of Łódź and quantifying was calculated from area percent; FID response factor = 1. The retention times of identified compounds are given in Table 1.

## RESULTS AND DISCUSSION

The content of essential oil in the raw material from five populations cultivated in Poland determined by steam distillation ranged from 0.08 to 0.25 ml/100 g of dried leaves and from 0.06 to 0.167 ml/100 g of dried herb (see Table 2) and it was in accordance with the Polish Pharmacopoeia prescription. In the material from commercial cultivations (samples 2 and 6–9) it did not exceed 0.1%, while in the material from the Garden of Medicinal Plants, situated in an enclosed area in the city, it was nearly twice as high (Table 1).

Comparative determinations of the essential oil contents in the freshly collected material (determinations performed on the day of harvesting) and in the dried material revealed that the oil content in the freshly collected material was higher by

Table 4. The percentages of the main constituents in melissa oil distilled from dried and fresh plant material in dependance upon harvesting time (the plant material obtained from the Garden of Medicinal Plants in Łódź

| Compound (% of the total oil) | 1998 | | | 1999 | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | VII[a] (10)[b] | IX (11) | X (12) | VI (13) | | VII (14) | | VIII (15) | | IX/X (16) | |
| | dried | dried | dried | fresh | dried | fresh | dried | fresh | dried | fresh | dried |
| 6–methyl–5–hepten–2–one | 0.45 | 0.21 | 0.20 | 0.49 | 1.43 | 0.83 | 0.40 | 0.42 | 0.54 | tr | tr |
| linalool | 0.45 | 0.22 | 0.09 | 0.35 | 0.57 | 0.61 | 0.43 | 0.47 | 0.22 | 0.22 | 0.16 |
| citronellal | 15.18 | 19.94 | 60.78 | 16.59 | 20.76 | 5.42 | 9.76 | 13.62 | 11.89 | 39.82 | 46.77 |
| citronellol | 0.03 | 0.18 | 0.84 | 10.52 | 0.31 | 3.74 | 0.45 | 6.03 | 0.85 | 12.79 | 2.65 |
| nerol | – | – | – | 2.92 | 0.11 | 2.76 | 0.79 | 1.97 | 0.51 | 1.42 | 0.18 |
| methylcitronellate | 1.49 | 1.95 | 5.71 | 2.91 | 2.83 | 2.31 | 1.07 | 2.59 | 0.90 | 4.84 | 4.57 |
| geraniol | 0.12 | 0.12 | 0.15 | 8.01 | 0.26 | 6.22 | 1.42 | 3.24 | 0.05 | 2.98 | 0.51 |
| neral (citral b) | 17.37 | 25.20 | 6.55 | 10.08 | 19.05 | 19.37 | 24.38 | 13.95 | 27.39 | 6.77 | 8.46 |
| geranial (citral a) | 32.92 | 36.54 | 9.53 | 14.29 | 26.62 | 29.06 | 33.75 | 22.44 | 37.17 | 9.89 | 14.05 |
| geranylacetate | 0.01 | 0.86 | 0.39 | 0.36 | 0.21 | 1.22 | 0.07 | 1.41 | 0.53 | 0.50 | 0.21 |
| geranic acid | – | – | – | – | – | – | – | – | – | – | – |
| β–caryophyllene | 0.01 | 2.72 | 4.06 | 7.69 | 5.75 | 7.14 | 1.94 | 2.74 | 3.74 | 4.45 | 5.10 |
| α–humulene | 0.04 | tr[c] | tr | tr | tr | 0.54 | 0.20 | 0.38 | 0.29 | 0.55 | 0.54 |
| γ–cadinene | 0.27 | 0.04 | 0.05 | 0.61 | 0.24 | – | – | 0.02 | – | – | – |
| germacrene D | 0.06 | 0.06 | 0.38 | 6.81 | 1.81 | 0.19 | 0.14 | 0.12 | 0.45 | 1.86 | 1.13 |
| cadina–3,9–diene | 0.16 | 0.07 | 0.09 | 1.27 | 0.70 | 0.54 | 0.38 | – | 0.25 | 0.49 | 0.37 |
| β–caryophyllene oxide | 5.88 | 2.71 | 1.63 | 0.46 | 0.90 | 4.07 | 2.84 | 4.80 | 2.66 | 1.01 | 2.41 |
| humulene oxide | 0.13 | – | – | – | – | 0.13 | 0.24 | 0.36 | 0.21 | 0.11 | 0.17 |
| sum of monoterpene aldehydes | 65.47 | 81.68 | 76.86 | 40.96 | 66.43 | 53.85 | 67.89 | 50.01 | 76.45 | 56.48 | 69.28 |
| sum of monoterpene alcohols | 0.60 | 0.52 | 1.08 | 21.8 | 1.25 | 13.33 | 3.09 | 11.71 | 1.63 | 17.41 | 3.50 |
| sum of sesquiterpenes and sesquiterpene oxides | 6.38 | 5.60 | 6.21 | 8.15 | 6.65 | 11.21 | 4.78 | 7.54 | 6.40 | 5.46 | 7.51 |

[a] – month of the harvest [b] – sample number [c] – trace

0.02–0.03 ml/100 g when calculated on dry mass than in dried material.

The analysis of the oil components performed by gas chromatography (GC) and GC/MS showed that each of the samples contained components typical for the oil from *Melissa officinalis* subsp. *officinalis* such as: citral (neral and geranial), citronellal, β–caryophyllene, 6–methyl–5–hepten–2–one, but the percentages of particular components of the oils obtained from various populations was different (see Table 3).

In three out of five studied samples collected in 1998 from five different populations of lemon balm, the essential oil contained a large quantity of sesquiterpenes (36.15–40.71%) namely β–caryophyllene (5.75–12.2%) and β–caryophyllene oxide (18.62–31.73%). One sample contained significant amount of monoterpene alcohols – geraniol, nerol, citronellol and linalool (28.53% of the total oil), and only in one of the five samples derived from the Garden of Medicinal Plants aldehydes neral, geranial and citronellal predominated in the essential oil (65.47% of the total) at 6.55% content of sesquiterpenes and 0.6% content of monoterpene alcohols.

The content of citral was within a wide range from 10.86% (sample 7) to 64.56% (sample 15)

Table 5. The effect of storage time on the composition of lemon balm oil

| Compound (% of the total oil) | Time of storage | | | |
|---|---|---|---|---|
| | Six days | | Six months | |
| | sample 14 | sample 15 | sample 14 | sample 15 |
| 6–methyl–5–hepten–2–one | 0.40 | 0.54 | 0.49 | 0.60 |
| linalool | 0.43 | 0.22 | 0.51 | 0.19 |
| citronellal | 9.76 | 11.89 | 4.47 | 2.94 |
| citronellol | 0.45 | 0.85 | 0.25 | 0.07 |
| nerol | 0.79 | 0.51 | 0.04 | 0.07 |
| methylcitronellate | 1.07 | 0.90 | 3.16 | 0.68 |
| geraniol | 1.42 | 0.05 | 0.15 | 0.15 |
| neral | 24.38 | 27.39 | 5.74 | 3.56 |
| geranial | 33.75 | 37.17 | 7.67 | 4.99 |
| geranylacetate | 0.07 | 0.53 | 3.20 | 2.91 |
| geranic acid | – | – | 3.56 | 5.40 |
| β–caryophyllene | 1.94 | 3.74 | 0.03 | 1.41 |
| α–humulene | 0.20 | 0.29 | 0.20 | – |
| γ–cadinene | 0.04 | 0.06 | 0.18 | 0.20 |
| germacrene D | 0.14 | 0.45 | 0.39 | 0.41 |
| cadina–3.9–diene | 0.38 | 0.25 | 0.67 | 0.78 |
| β–caryophyllene oxide | 2.28 | 2.66 | 9.76 | 7.79 |
| humulene oxide | 0.24 | 0.21 | 0.56 | 1.24 |

and the amount of monoterpene alcohols in the majority of oils from dried plant material did not exceed 3.5%. However, in one sample as much as 28.5% of monoterpene alcohols in the essential oil was found, which may suggest that an adulteration with lemon catnip (*Nepeta cataria* var. *citriodora*) could take place (19).

The content of β–caryophyllene in oils from commercial samples was comparable to that found in *Melissa officinalis* subsp. *officinalis* cultivated in Germany (14). High amount of sesquiterpenes has recently been determined in the essential oil from *M. officinalis* subsp. *altissima* (14). In any of the samples, however, we did not observe larger amounts of germacrene D, a component characteristic of subsp. *altissima* (14). Cubebene, which has recently been detected in larger quantities in subsp. *inodora* (13) was not found in the investigated plants. β–caryophyllene and β–caryophyllene oxide were the predominant constituents in the sesquiterpene fraction of the investigated *Melissa* oils.

Comparative GC determinations of the oils obtained from the fresh and dried plant material showed that the content of monoterpene alcohols significantly decreased and the content of aldehydes increased during drying (Table 4).

The composition of the essential oil changed much during vegetation time from June to October (Table 4). The highest content of citral was in the *Melissa* oil from leaves collected in July, and the lowest in the *Melissa* herb collected in October. In autumn harvest, the main component of the oil was citronellal (up to 46.8% of the total oil from herb in 1999 and up to 60.8% of the total oil from leaves in 1998). This result supports the hypothesis that citral may be converted to citronellal with ageing of lemon balm plant (15).

Quantitative and qualitative changes in the *Melissa* oil components during its storage (Table 5) were also found. After six months of cold storage (at about 10°C) in non–hermetic bottles, the content of monoterpene aldehydes decreased while a significant amount of geranic acid occurred (3.56–5.40%) in addition to an increase of geranyl acetate and β–caryophyllene oxide percentages. This statement may indicate that oxidation process occurs not only during drying but also during storage of the oil. Increasing content of β–caryophyllene oxide during storage of lemon balm oil has already been described by German authors (20).

The obtained results of the essential oil analysis showed that the investigated *Melissa* populations can be classed among the plants of the taxon *Melissa officinalis* L. subsp. *officinalis*, even though the percentages of components in the oil varied. Great differences in the content of citral between the samples might be caused, among others, by different conditions of plant growing,

plant age and vegetation period of the plant. At the time of the rapid growth of plant (the time before blooming) large amounts of young leaves occur, which are very rich of citral (15). Therefore, the harvesting time just before flowering may be proper to obtain *Melissae* folium with high content of essential oil and high percentage of citral in the oil. In the plant material from autumn collection, monoterpene aldehydes were also the predominant fraction in the oil, but the percentages of citronellal were higher than those of citral.

Besides *Melissae* folium also the herb of *Melissa officinalis* is offered on the pharmaceutical market (21). Comparing the content and composition of the essential oils in significant differences between leaves and herb of lemon balm have been found.

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