Influence of vegetation on the composition of essential oil from (*Melissa officinalis* L.)

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Abstract. Lemon balm (*Melissa officinalis* L.) is a perennial plant of the family Lamiaceae, which is used in folk medicine and the food industry, as well as being processed to obtain essential oil. The aim of the special work is to monitor the influence of vegetation on the composition of the essential oil. The chemical composition of essential oils, obtained by steam distillation from lemon balm cultivated in North-eastern Bulgaria, has been determined. The plants were harvested in two periods of vegetation – the first at the end of June and second – at the end of August 2020. The main components in the oil from the first cut are geranial (26.41%), neral (19.55%), (*E*)-*β*-caryophyllene (17.46%), germacrene D (7.86%) and *β*-citronellal (3.24%). In the oil from the second cut, the main components are geranial (37.26%), neral (28.46%), (*E*)-*β*-caryophyllene (6.65%), geraniol (4.72%), and *β*-citronellal (3.04%). The oil from the second harvest has a high content of the main components of geranial, neral and geraniol and a lower content of (E)-*β*-caryophyllene and germacrene D. Variations in the amounts are also present in other compounds.

1 Introduction

Lemon balm (*Melissa officinalis* L) is a perennial herb of the family Lamiaceae, reaching a height of up to 1.25 m. The essential oil is found in glandular trichomes and labiate glands located on the leaves and flower sepals. Its amount reaches 0.01-0.3% and depends on the place of growth, the time of harvesting and the variety. Cultivated and selected lemon balm has more essential oil than wild lemon balm. During distillation, a large part of the oil remains in the distillation waters, which necessitates its extraction from them [1].

Lemon balm is distributed in different countries of Central and Southern Europe, Asia, and America. The wild variety vegetates up to 1200 m above sea level. It is cultivated mainly as a plant for medicinal and honey making purposes and to a lesser extent for essential oil [1].

In our country, it is found growing wild in many areas [2], but it is also cultivated, and the variety “Melpea-2” was created [3].

The essential oil is an easily mobile transparent, pale yellow to yellow-greenish liquid with a specific pleasant lemon smell and a bitter-spicy taste.

Its main indicators are: relative density (d_{20}^{20}) 0.892-0.963, refraction index (n_D^{20}) 1.470-1.506, optical polarization (α_D^{20}) from +2 to -30.08°, acid number 1.2-2.8 mg KOH/g, ester number 12.0-241.4 mg KOH/g, solubility in 90% ethanol 1:0.2-1.5, with a slight opalescence, carbonyl compounds (such as citral) 17-42% reaching up to 69% in some cases [1, 4].

Bulgarian essential oil has the following indicators: relative density (d_{20}^{20}) 0.870-0.906, refraction index (n_D^{20}) 1.460-1.480, optical polarization (α_D^{20}) -32.25°, acid number max 2.5 mg KOH/g, and ester number, 27.0-220 mg KOH/g [1].

More than 130 compounds have been identified in its composition, the amount of which varies depending on the habitat, the stage of development and the variety. The main components determining the odor and biological properties are: citral (geranial 15.0-37.2% and neral 14.0-24.1%), citronellal (0.7-39.0%), caryophyllene oxide (2.5-24%), geraniol and nerol (about 0.1%), and linalool (0.08-0.40%). Also present in the oil are: geranyl acetate (0.5-5.9%), ocimene (0.2-2.2%), 1-octene-3-ol (0.2-2.2%), caryophyllene (9.5%), *α*-cubebene (0.3-1.5%), copaene (0.8-4.0%), *β*-bourbonene (0.06-1.9%), germacrene D (4.2%), *γ*- and *δ*-cadinene (about 1.0%), *etc*. [5-10]. Geranial and neral are different forms of the compound known as citral and all names will be used interchangeably from here on out.

The following main compounds were found in the oil from Bulgaria: citral (16.9-40.4%), citronellal (4.5-25.1%), linalool (0.2-2.9%), geranyl acetate (1.9%), and *β*-caryophyllene (11.1-17.5%) [1].

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It is known that the quantity and chemical composition of essential oils are affected by natural, soil-climatic conditions and technological factors [1]. The influence of some of them on the chemical composition of the essential oil has been studied by a number of foreign authors.

It was found that during the vegetative season, the content of geraniol and caryophyllene oxide in the composition of the essential oil decreases, that of caryophyllene, carvacrol, and methyl citronellate increases, and that of geranyl acetate does not change [11].

Treatment of the soil with different elements, such as N, P and K, and their combinations NP, PK and NPK also affects the chemical composition of the essential oil. The maximum amount of essential oil is obtained when treated with P (0.4%). The main components in the oil are: geranial (38.1 - 45.3%), neral (29.2 - 34.1%), pentadecanal (3.7 - 5.4%), geranyl acetate (2.9 - 4.2%), and β-caryophyllene (2.0-3.5%). The highest amount of geranial and neral was obtained when the soil was treated with NP and N [12].

The change in the amount of essential oil and its main components was studied depending on the time of harvesting the plants and the conditions of their drying – in the shade, in the sun and at a temperature of 40ºC, and fresh plants were used as the control. The authors found that the method of drying has a significant effect on both the amount of essential oil and its composition. During drying, oxidation, polymerization and other processes likely occur, which leads to a change in the chemical composition of the oil [13].

The year of cultivation also affects the amount of the main components. It was found that in the second year of the vegetation, the amount of citronellal decreased, geranyl acetate increased, and neral and geranial remained the same [14].

The essential oil has some interesting properties such as antimicrobial activity [5, 9], antioxidant [2], and antiviral effects [5].

It is used in perfumery and cosmetics, as well as for flavouring liqueurs, sweets, jams, *etc*. [1, 4].

In recent years in our country, lemon balm has been cultivated in many regions of the country, because of the interesting smell of the oil, due to its main compound citral. However, research on the influence of some of the above-described factors on the chemical composition of the oil is very scarce.

The aim of this work is to examine the influence of vegetation on the yield and composition of the essential oil, obtained from cultivated lemon balm, raised in Bulgaria. This will allow choosing the most suitable time to harvest from the field, with the maximum amount of essential oil of high quality.

2 Materials and Methods

2.1 Plant material

Lemon balm was cultivated in the North-eastern region of Bulgaria. The plants were harvested in June (first harvest) and in the end of August (second harvest).

The essential oil was obtained in industrial conditions by processing the plants by steam distillation.

After distillation, the essential oils were dried using anhydrous sodium sulphate and stored in tightly closed dark vials at 4ºC until gas chromatographic (GC) analysis.

2.2 Determination of physical and chemical properties

The following properties of the essential oils were determined: appearance, color and odor [15], relative density [16], refractive index [17], optical rotation [18] and acid number [19].

2.3 Gas chromatographic analysis of the essential oil

A GC analysis was performed using an Agilent 7890A gas chromatograph, HP-5 column MS (30 m × 250 mm × 0.25 μm), temperature: 35°C/3 min, 5°C/min to 250°C for 3 min, 49 min in total, helium as a carrier gas, 1mL/min constant speed, 30:1 split ratio. A GC/MS analysis was carried out on an Agilent 5975C mass spectrometer, helium as a carrier gas, column and temperature the same as in the GC analysis. The identification of the chemical compounds was made by comparison to their relative retention time with existing literary data. The identified essential oil constituents were arranged by retention time, and their quantity was calculated in percentages [20].

2.4 Statistical analysis

The experiment was provided with three parallel analysis and presented as ± standard deviations. Statistical analysis was carried out using Excel software.

3 Results and discussion

The essential oil obtained from plants harvested in June has a yield of 0.35%, and from plants harvested in the end of August – 0.01%.

Therefore, physical and chemical parameters were determined only on that of the first harvest, and are presented in Table 1. The data shows that the oil meets the values described in the literature [1, 4].

Table 1. Physical and chemical parameters of essential oil from 1st harvest

Index	Value
Appearance	Mobile liquid
Color	Pale yellow to light brown
Odor	Refreshing, citrusy, sweet
Relative density, d_{20}^{20}	0.890 ± 0.010
Refractive index, (n_D^{20})	1,480 ± 0.01
Optical rotation, (α_D^{20})	-25°
Acid number, mg KOH/g	2.0 ± 0.01

The chemical composition of the essential oils is presented in Table 2. The data shows that for:

Table 2. Chemical composition of balm lemon essential oils (% of TIC[a])

[b]R.T. First harvest	[b]R.T. Second harvest	Components	First harvest	Second harvest
7.075	26.883	6-methyl-5-hepten-2-one	1.69±0.15	1.63±0.15
30.525	30.076	Limonene[c]+*β*-phellandrene[d]	0.11±0.01	0.05±0.0
31.293	30.793	(*Z*)-*β*-Ocymen	0.26±0.02	0.08±0.0
32.157	31.561	(*E*)-*β*-Ocymen	2.30±0.22	0.33±0.03
36.298	35.454	Linalool[c]	0.62±0.05	0.93±0.09
40.580	39.338	*β*-Citronellal	3.24±0.31	3.04±0.30
41.432	40.167	(*Z*)-isocitral[c]	0.49±0.04	1.70±0.16
42.838	41.474	(*E*)-isocitral[c]	0.82±0.08	2.37±0.22
46.413	44.851	Nerol[d]+Citronellol[c]	1.42±0.13	2.80±0.27
47.556	45.838	Neral[c]	19.55±1.80	28.46±2.80
48.390	46.736	Geraniol[c]	2.00±0.19	4.72±0.46
48.735	46.899	Methyl citronelate	0.42±0.03	0.24±0.02
49.800	47.918	Geranial[c]	26.41±2.50	37.26±3.60
53.343	51.166	Methyl geraniate	0.57±0.05	0.60±0.59
57.388	54.771	*α*-Copaene	0.69 ± 0.05	0.05±0.0
57.525	55.035	Geranyl acetate	1.55±0.14	2.74±0.25
60.638	57.672	(*E*)-*β*-Caryophyllene	17.46±1.16	6.65 ±0.65
62.778	59.724	α-Humulene	1.11±0.10	0.32 ±0.30
64.665	61.389	Germacrene D	7.86±0.75	0.40±0.03
65.002	62.699	*α*-Farnesene	0.70±0.06	0.05 ± 0.0
67.192	63.840	*δ*-Cadinene	1.18±0.10	0.04±0.0
71.164	67.481	Caryophyllene oxide	0.71±0.06	0.52±0.04
75.225	71.428	10-epi-*α*-Cadinol	0.30±0.28	0.02±0.0

[a] – total ion current; [b] – retention time, min; [c] – allergens; [d] – could not be separated in the conditions of the gas chromatographic analysis

- The oil obtained from the first harvest, 23 compounds were identified, which represents 91.46% of the total content. Twelve of them have a concentration above 1%, and the remaining 11 are below 1%. The main compounds (concentration above 3%) are: geranial (26.41%), neral (19.55%), (*E*)-*β*-caryophyllene (17.46%), germacrene D (7.86%), and *β*-citronellal (3.24%).

- The oil obtained from the second harvest, 23 compounds were identified, which represent 95.00% of the total content. Ten of them have a concentration above 1% and the remaining 13 are below 1%. The main compounds (concentration above 3%) are: geranial (37.26%), neral (28.46%), (*E*)-*β*-caryophyllene (6.65%), geraniol (4.72%), and *β*-citronellal (3.04%).

The two oils differ in chemical composition, which can be explained by the time of harvesting the raw material related to its vegetation [1].

The chemical composition of the studied oils also differs from that of wild lemon balm in the country [2]. The difference in the composition of its main components caryophyllene oxide (33.99%), *n*-hexadecanoic acid (14.28%), *α*-citral (6.62%), (5E,9E)-farnesyl acetone (5.00%), *β*-caryophyllene (4.82%), (2*E*,6*Z*)-farnesal (4.56%), (5*E*,9*Z*)-farnesyl acetone (4.16%), *β*-citral (3.98%), and *γ*-eudesmol (3.13%) can be explained by the influence of the above-described factors – soil conditions, altitude, harvesting time, processing by water distillation [2]. The oil from the second harvest has a higher content of the main components geranial, neral, and geraniol, and a lower content of (*E*)-*β*-caryophyllene and germacrene D. Variations in the amounts are also found in other compounds, also established by other authors [14].

The distribution of the main groups of compounds in the oils is shown in Fig. 1.

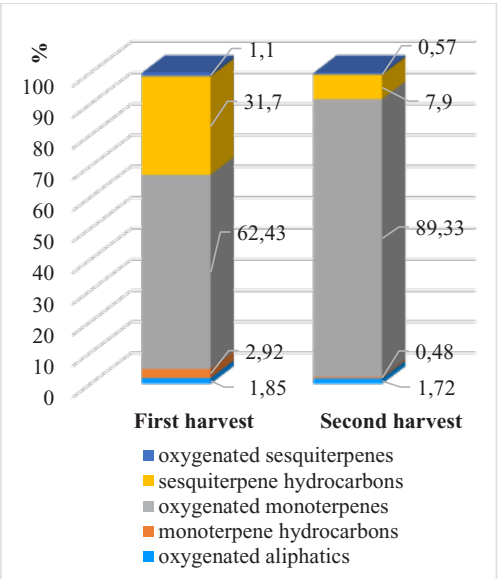

Fig. 1. Groups of components in lemon balm essential oils.

The data shows that:

- Oxygenated monoterpenes predominate in the oil from the first harvest, followed by sesquiterpene hydrocarbons, monoterpene hydrocarbons, oxygenated aliphatics and oxygenated sesquiterpenes.

- The essential oil from the second harvest is mainly comprised of oxygenated monoterpenes, followed by sesquiterpene hydrocarbons, oxygenated aliphatics, oxygenated sesquiterpenes, and monoterpene hydrocarbons.

The comparative analysis of the data shows that the content of hydrocarbons in the essential oil from the first harvest is higher, and that of their oxygenated derivatives is lower, which is also confirmed by other authors [12].

The distribution of the compounds in the oils by functional groups, in relation to the identified oxygen derivatives, is presented in Fig. 2.

The data shows that in the oils from the first and second harvest, aldehydes (84.48 and 83.68%) prevail, followed by alcohols (7.26 and 9.74%), esters (4.25 and 4.11%), ketones (2.83 and 1.87%), and oxides (1.18 and 0.60%).

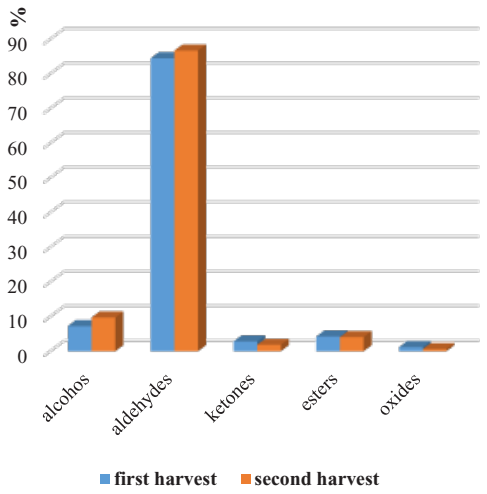

Fig. 2. Groups of oxygenated components in lemon balm essential oils

According to the EU Directive [21], the following eight allergens, arranged in ascending order, which are monoterpene compounds, were found in the both tested lemon balm essential oils, respectively: geranial (26.41 and 37.26%), neral (19.55 and 28.46%), geraniol (2.00 and 4.72%), nerol+citronellol (1.42 and 3.07%), *β*-linalool (0.62 and 0.93%), limonene+(*β*-phellandrene) (0.11 and 0.05%), (*E*)-isocitral (0.82 and 2.37%), and (*Z*)-isocitral (0.49 and 1.70%). According to obtained results their amount is higher in the oil from the second harvest. The low yield of essential oil obtained in the second harvest, as well as the high amount of allergens, makes it pointless to collect the plant in two separate harvests.

The distribution of allergens by functional groups in the studied essential oil is: aldehydes (geranial, neral, (*E*)-isocitral and (*Z*)-isocitral), alcohols (geraniol, citronellol and *β*-linalool) and hydrocarbons (limonene).

Potential allergen in the oil is also the ester geranyl acetate, which in the case of improper storage of the essential oil – presence of water, high temperature or presence of air, can hydrolyze to the corresponding alcohol geraniol, which appear in the EU Directive [21].

It was established that with the use of various cosmetic preparations - emulsion creams, gels, lotions, shampoos, masks, *etc*., containing some of the allergens listed in the Directive, on the skin of the face and hands, as well as on the scalp, may appear various skin changes, the so-called allergic contact dermatitis. Symptoms of allergic reactions are swelling, redness, rashes, severe itching, eczema or increased sensitivity of the skin, expressed by a feeling of pain. These symptoms can appear after hours or days after using the given cosmetic product, which is why specialists often experience difficulties in accurately identifying the allergen. Allergic reactions can also include headache, sneezing, runny nose, watery eyes, *etc*. [22 - 37].

Oxidation of the monoterpene hydrocarbon limonene was found to produce a compound that also causes allergic reactions [38 - 45].

From a medical perspective, citral, which is a major component of lemon balm essential oil, is a type IV contact allergen. This means that symptoms such as skin redness, blistering, itching, breathing disorders or skin inflammation can appear after a maximum of 72 h. People with sensitive skin or known chronic respiratory diseases should refrain from using products containing citral. For this reason, it is included in Regulation (EC) No 1223/2009 as one of the 25 aromatic allergens [21].

4 Conclusiion

The influence of vegetation on the chemical composition of essential oils obtained from lemon balm cultivated in North-eastern Bulgaria was monitored. The plants were processed by steam distillation after their harvest in 2020 at the end of June (first harvest) and at the end of August (second harvest). The essential oil from the second harvest has a higher content of the main compounds, oxygenated monoterpenes: geranial, neral and geraniol; which are, however, allergenic, and lower in sesquiterpene hydrocarbons: (*E*)-*β*-caryophyllene and germacrene D. Therefore, in our opinion, the second harvest is not recommended. The essential oil from the first harvest meets the data from the literature in terms of physical and chemical parameters, which is a reason for searching for possibilities of its application in rinse-off cosmetic preparations.

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