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COMPARATIVE ANALYSIS OF ESSENTIAL OIL CONTAINING RAW MATERIALS OF HONEYHERB (*LIPPIA DULCIS* TREVIR.) UNDER DIFFERENT GROWING CONDITIONS

Svitlana Marchyshyn, Liudmyla Slobodianiuk, Liliia Budniak, Iryna Dakhym, Larysa Boyko, Mariia Kyryliv, Iryna Bekus

The use of plant raw materials is one of the areas of modern pharmaceutical science in the production of herbal drugs. In this regard, one of the oldest medicinal plants, Lippia dulcis Trevir (Phyla scaberrima (Juss. ex Pers.) Moldenke) from Verbenaceae family, is of great interest. According to traditional Mexican medicine, Lippia dulcis is used in the treatment of colds, coughs, bronchitis, and digestive disorders as an anti-inflammatory, antitussive, antipyretic, expectorant, emollient and diuretic agent.

The aim. *The aim of our study was to identify and determine the quantitative content of essential oils by GC/MS method in Lippia dulcis herb shoots.*

Materials and methods. *The determination of the essential oil composition of Lippia dulcis was conducted using Agilent Technologies' 6890 chromatograph with mass spectrometric detector 5973 (Agilent Technologies, USA).*

Results. *The GC/MS method 19 components of essential oil were identified in L. dulcis shoots grown in open soil conditions, the total content of which was 1274.82 μg/g, in L. dulcis shoots grown in closed soil conditions – 23, the total content of which was 2361.11 μg/g. Thus, the total content of essential oil in shoots of L. dulcis grown in closed soil conditions was 1.8 times higher than in shoots of L. dulcis grown in open soil conditions.*

Conclusions. *The component composition of the essential oil of L. dulcis shoots harvested from plants grown in open soil conditions (LD-1) and closed soil conditions (LD-2) was investigated using the chromato-mass spectrometric method. The following pharmacologically important components – camphor, germacrene D, caryophyllene, α-bisabolene – were found in both samples of the essential oil of L. dulcis shoots in significant quantities, which indicates the prospects for further technological and pharmacological studies of honey herb raw materials*

Keywords: *Lippia dulcis Trevir., shoots, essential oil, GC/MS, camphor, germacrene D, caryophyllene, α-bisabolene, quantitative content*

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1. Introduction

Medicinal plants are unique sources of healing compounds – biologically active substances (BAS), which are used both for the prevention and treatment of various diseases of the human body [1]. In this regard, one of the oldest medicinal plants is of great interest – *Lippia dulcis* Trevir. (*Phyla scaberrima* (Juss. ex Pers.) Moldenke) from *the Verbenaceae* family [2]. The area of distribution of it ranges from southern Mexico to Panama and Colombia. The species grows at an altitude of up to 1,800 m, grows in wet thickets, wooded edges of rivers and ponds, and also invades meadows and fields [3]. *L. dulcis* or honeyherb is also found in Puerto Rico, Cuba, Hispaniola, and other Caribbean islands. Cultivated in Puerto Rico as a medicinal plant [4]. *L. dulcis* include phenolic acids, a wide variety of caffeic acid derivatives, including verbascoside or acteoside [5].

The majority of Lippia species are used as remedies for gastrointestinal and respiratory complaints, lung infections, diarrhea and dysentery, analgesic, antipyretic, anti-inflammatory, stomach ailments, coughs, asthma and colds [6].

L. dulcis is commonly used in traditional medicine to treat inflammatory conditions; an infusion prepared with the aerial part is orally administered [7]. Additionally, this plant is employed as a decoction for the treatment of bronchitis, asthma, and colic [8]. According to traditional Mexican medicine, honeyherb is used in the treatment of colds, coughs, and digestive disorders as an anti-inflammatory, antitussive, antipyretic, expectorant, emollient and diuretic agent [9, 10]. In the 16th century, the Spaniards brought *L. dulcis* to Europe, and it began to be used medicinally in the 19th century. But until today, *L. dulcis* is non-officinal.

In Ukraine, honey herb is cultivated in the Kremenets Botanical Garden on the basis of the collection fund of the Department of Phytosozology, where the initial introduction test of the species was carried out [11, 12]. The analysis of available references showed that the pharmacognostic study of *L. dulcis* is insufficient.

Aim of research was to determine the qualitative and quantitative content of essential oil in *L. dulcis* shoots, which were harvested under conditions of closed and open soil.

2. Planning (methodology) of research

The planning of studies of medicinal plant material *Lippia dulcis* is shown in Fig 1.

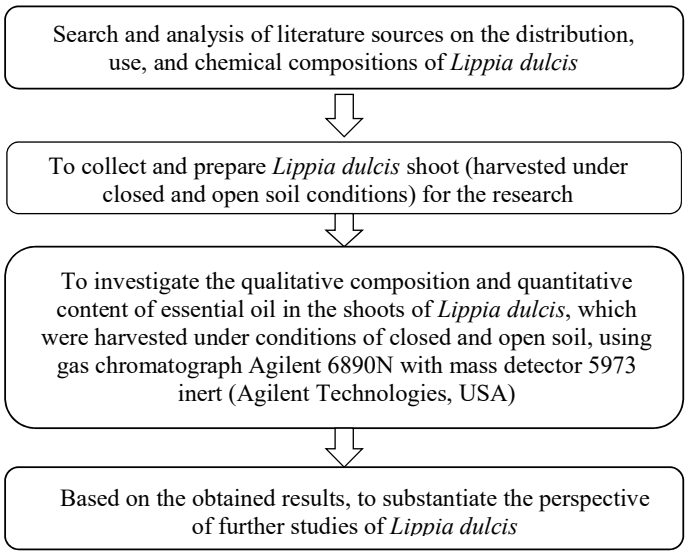

Fig. 1. Design of the experiment

3. Material and method

The material for research were shoots of honeyherb (*Lippia dulcis* Trevir.), which were harvested at the experimental sites in August 2022 (open soil conditions) and shoots of honeyherb, grown indoors in the Kremenets Botanical Garden, in February 2023 (closed soil conditions) [13]. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine) [14, 15]. The voucher specimens of herbal raw materials have been deposited in the departmental herbarium for future records [16].

The quantitative content of the essential oil in the studied raw material of honey herb was determined by the steam distillation method according to the State Pharmacopoeia of Ukraine (SPhU) [17].

The qualitative composition and content (μg/g) of essential oil components were determined by the chromatographic-mass spectrometric method on an Agilent Technologies 6890 gas chromatograph (GC) with a 5973 mass spectrometric detector (MSD).

A sample of shoots of honey herb (1.02 g) was placed in a 20 ml glass vial, with the addition of the internal standard – tridecane – at the rate of 20 μg per weight, followed by the calculation of the obtained concentration of the internal standard for further calculations. 10 ml of purified water was added to the vial, and volatile compounds were distilled with steam for 2 hours. After distillation, the volatile substances adsorbed on the inner surface of the reflux condenser were washed away by slowly adding 3 ml of particularly pure pentane to a 10 ml dry vial. The wash was concentrated by purging (100 mL/min) with particularly pure nitrogen to a final extract volume of 100 μl, which was completely removed by a chromatographic syringe. The sample was injected in a splitless mode. The suitability of the chromatographic system was determined according to the requirements of the SPhU [17].

Chromatography conditions: Agilent Technologies 6890 chromatograph with mass spectrometric detector 5973; capillary chromatographic column HP-5ms (ext. diam. 0.25 mm and 30 m long); carrier gas velocity (helium) 1.0 ml/min; the temperature of the sample heater – 250 degrees; the temperature of the thermostat is programmed from 50 to 320 degrees with a speed of 4 degrees/min. To identify the components, the spectra obtained were considered on the basis of general laws of fragmentation for molecules of organic compounds under the action of electron impact and using the NIST 08 and WILEY 2007 mass spectrum libraries with a total number of spectra of more than 470,000 in combination with the AMDIS and NIST identification programs. Quantitative determination of the content of substances in the raw material was performed in comparison with a standard sample of tridecane [18, 19].

Statistical analysis.

Statistical processing of the results was performed using the Student's t-test. Statistical properties of random variables with n-dimensional normal distribution are given by their correlation matrices, which can be calculated from the original matrices. The reliability of the compared values was estimated using the Student, Wilcoxon, and Mann-Whitney criteria with a probability level of ≤0.05 on a computer using Statistica 6.0 and Word Excel programs [20, 21]. The results were expressed as mean±standard deviation (SD).

4. Results

While determining the quantitative content of essential oil by the method of steam distillation in the shoots of *L. dulcis*, it was established that its content was higher in raw materials that were harvested under closed soil conditions and was set at 5.51±0.05 %. The content of essential oil in *L. dulcis* shoots harvested in open soil conditions was 3.32±0.03 %.

The results of the determination of essential oil by GC/MS in *L. dulcis* shoots are presented in Fig. 2, 3 and Tables 1, 2.

It was established that the coincidence rate of the detected compounds in the researched raw materials of *L. dulcis* shoots grown in open soil conditions (LD-1) and in closed soil conditions (LD-2) (in percent) with those in the NIST 08 and WILEY 2007 mass spectrum libraries.

As a result of the conducted research, 19 components of essential oil were identified in LD-1, the total content of which was 1274.82 μg/g, and in LD-2 – 23, the total content of which was 2361.11 μg/g. Thus, the total content of essential oil in shoots of *L. dulcis* grown in closed soil conditions was 1.8 times higher than in shoots of *L. dulcis* grown in open soil conditions.

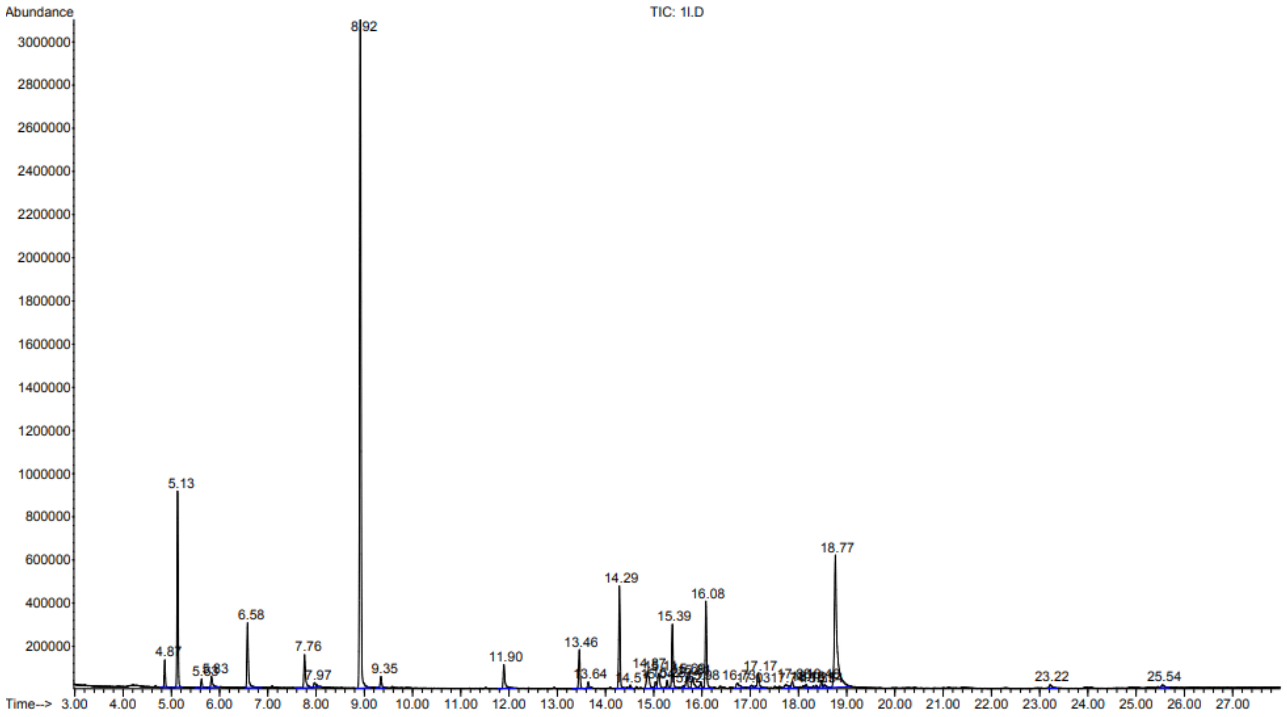

Fig. 2. GC/MS chromatogram of the components of the essential oil of *L. dulcis* shoots grown in open soil conditions (LD-1)

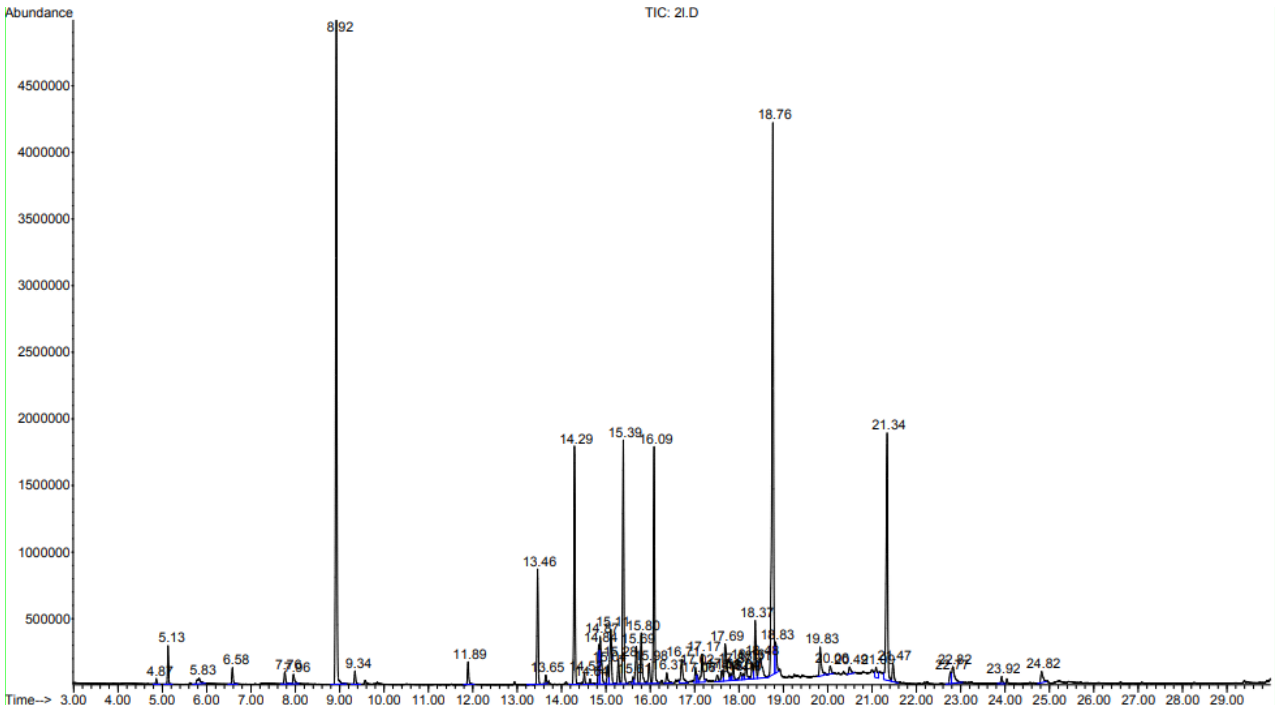

Fig. 3. GC/MS chromatogram of the components of the essential oil of *L. dulcis* shoots grown in closed soil conditions (LD-2)

Table 1

Component composition of essential oil of *L. dulcis* shoots grown in open soil conditions (LD-1)

No.	Component	Retention time, min	Content, μg/g
1	2	3	4
1	α-Pinene	4.87	16.92
2	Camphene	5.13	115.13
3	β-Pinene	5.63	6.29
4	β-Myrcene	5.83	11.56
5	Limonene	6.54	52.56
6	(+)-4-Carene	7.76	29.77
7	Camphor	8.92	542.14

Continuation of Table 1

1	2	3	4
8	Borneol	9.35	9.59
9	Tridecane	11.89	Internal standard
10	Copaen	13.46	29.92
11	β-bourbonene	13.64	4.36
12	Caryophyllene	14.29	75.76
13	β-Sesquiphelandrene	14.88	25.10
14	Aromadendrene	15.01	5.52
15	Hermacrene D	15.39	50.62
16	γ-Cadinene	15.69	11.70
17	β-Bisabolene	15.80	14.29
18	δ-Cadinene	16.08	67.42
19	Nerolidol	16.73	8.44
20	α-Bisabolol	18.77	191.32

Table 2

Component composition of essential oil of *L. dulcis* shoots grown in close soil conditions (LD-2)

No.	Component	Retention time, min	Content, μg/g
1	α-Pinene	4.87	4.75
2	Camphene	5.13	33.29
3	Limonene	6.54	18.25
4	(+)-4-Carene	7.76	14.88
5	Linalool	7.97	13.65
6	Camphor	8.92	757.74
7	Borneol	9.35	14.67
8	Tridecane	11.89	Internal standard
9	Copaen	13.46	125.89
10	β-Bourbonene	13.64	8.30
11	Caryophyllene	14.29	258.72
12	trans-α-Bergamotene	14.51	12.20
13	β-Farnesene	14.64	5.86
14	trans-β-Farnesene	14.84	41.30
15	β-Sesquiphelandrene	14.88	74.56
16	Aromadendrene	15.01	21.30
17	Hermacrene D	15.39	273.66
18	γ-Cadinene	15.69	52.48
19	β-Bisabolene	15.80	69.33
20	δ-Cadinene	16.08	257.26
21	cis-α-Bisabolene	16.38	17.25
22	Nerolidol	16.73	40.76
23	α-Cadinol	18.30	23.81
24	α-Bisabolol	18.77	191.32

5. Discussion

In LD-1 essential oil, 783.96 μg/g or 61.50 % of monoterpenoids were found, among which the bicyclic monoterpenoid camphene (115.13 μg/g – 9.03 % of the total number of identified components) and the monocyclic monoterpenoid limonene (52.56 μg/g – 4.12 % of the total number of identified components). The major component of the essential oil of *L. dulcis* shoots is the bicyclic monoterpenoid camphor, the content of which was 542.14 μg/g – 42.53 % of the total number of identified components (Table 1). These results are comparable to data from references [9], containing information that the essential oil from the leaves of *L. dulcis*, obtained by steam distillation, is dominated by monoterpenoids and that 33.9-53.0 % of the essential oil of Mexican plants is camphor. Lippia dulcis essential oil from Brazil contains low camphor content (traces) [6].

857.23 μg/g or 36.31 % of monoterpenoids were found in the essential oil of honey herb shoots grown in closed soil conditions (LD-2), among which camphor was also quantitatively predominant – 757.74 μg/g or 32.01 % from the total number of identified components and 88.39 % from identified monoterpenoids. The quantitative content of other monoterpenoids was insignificant (Table 2).

490.86 μg/g of sesquiterpenoids (38.50 % of the total amount of all identified components) were found in the essential oil of honey herb shoots grown in open soil conditions (LD-1), among which 33.54 μg/g (2.63 %) of the total number of identified components) are acyclic sesquiterpenoids, 262.64 μg/g (20.60 %) are monocyclic

sesquiterpenoids, 154.88 μg/g (12.15 %) are bicyclic and 39.80 μg/g (3.12 %) – tricyclic sesquiterpenoids. The predominant sesquiterpenoid is α-bisabolol, a powerful anti-inflammatory, wound-healing, antibacterial and antifungal agent, the content of which was 191.32 μg/g – 38.98 % of the total number of identified sesquiterpenoids.

1503.88 μg/g of sesquiterpenoids (63.69 % of the total number of all identified components) were found in the essential oil of *L. dulcis* shoots grown in closed soil conditions (LD-2). Acyclic sesquiterpenoids were found to be 162.48 μg/g, which was 6.88 % of the total number of all identified components, monocyclic – 581.44 μg/g (24.63 %), bicyclic – 580.66 μg/g (24.59 %), tricyclic – 155.49 μg/g (6.59 % of the total amount of all identified components).

Dominated among sesquiterpenoids from monocyclic type were germacrene D – 273.66 μg/g (18.20 % of the total amount of all identified sesquiterpenoids) and α-bisabolol – 191.32 μg/g (12.72 %); from bicyclics - caryophyllene (258.72 μg/g or 17.20 %) and δ-cadinene (257.26 μg/g or 17.11 %).

The studied essential oils contained basically the same components, which differed in their quantitative content. However, the essential oil of honey herb, grown under closed soil conditions, contained small amounts of acyclic sesquiterpenoids β-farnesene (5.86 μg/g) and trans-β-farnesene (41.30 μg/g) and monocyclic sesquiterpenoids cis-α -bisabolene (17.25 μg/g) and α-cadinol (23.81 μg/g). *L. dulcis* essential oil grown in open soil contained 6.99 μg/g of β-pinene.

It should also be noted that the quantitative content of camphor, which dominated in both samples of essential oil of *L. dulcis*, was higher in the raw materials obtained from plants that grew in closed soil conditions.

Study limitations. Through the study of the essential oils by GC/MS, several compounds were not identified due to the absence of their characteristics in NIST 08 mass spectra libraries, as well as in AMDIS and NIST programs.

Prospects for further research. The obtained results might be used in the standardization and quality assurance of new remedies containing *Lippia dulcis*. Further phytochemical and pharmacological studies of the *Lippia dulcis* shoots and their essential oil will show the prospect of creating new pharmaceuticals.

6. Conclusion

Using the method of steam distillation, the essential oil of *L. dulcis* was obtained, and the quantitative content of it was established. It was set that a higher content of essential oil is observed in raw materials harvested under conditions of closed soil, which amounted to 5.51 %.

The component composition of the essential oil of *L. dulcis* shoots harvested from plants grown in open soil conditions (LD-1) and closed soil conditions (LD-2) was investigated using the chromato-mass spectrometric method.

As a result of the conducted research, 19 components of essential oil were identified in LD-1, the total content of which was 1274.82 μg/g, and in LD-2 – 23 components, the total content of which was 2361.11 μg/g.

The following pharmacologically important components – camphor, germacrene D, caryophyllene, α-bisabolene - were found in both samples of the essential oil of *L. dulcis* shoots in significant quantities, which indicates the prospects for further technological and pharmacological studies of honey herb raw materials.

Conflict of interests

The authors declare that they have no conflict of interest in relation to this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this article.

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Data availability

Data will be made available on reasonable request.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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