COMPONENT COMPOSITION OF ESSENTIAL OIL SHOOTS AND LEAVES OF LAURUS NOBILIS L. UKRAINIAN ORIGIAN

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The aim of our work was to carry out a comparative analysis of the essential oils of shoots and leaves of Laurus nobilis L. Ukrainian flora and to determine the prospects for their use in pharmacy.

Materials and methods. Raw materials for obtaining essential oil (shoots and leaves) of Laurus nobilis L. were harvested in November 2017 in the southern regions of Ukraine.

By the method of chromatography-mass spectrometry using an Agilent Technology 6890N chromatograph, the component composition of 4 samples of essential oil of Laurus nobilis L. raw material was investigated and identified. The composition of the essential oil was identified by comparing the results with the data from the NIST 02 mass spectra library (more than 174,000 substances).

Results. Thus, in the studied series of the essential oil of the shoots, a similarity was observed both in the set of components and in the relative content of a number of individual compounds and compounds of structurally related groups. The content in these samples was dominated by 1,8-cineole (19.63 % of the amount and 12.93 % of the amount, respectively), α-terpinyl acetate (16.22 % of the amount and 16.03 % of the amount, respectively).

In contrast to the series of the essential oil of the shoots, the component composition of the essential oil of the leaves of the two series was significantly different. In both studied series, only 3 compounds were identified that are common—these are aromatic compounds methyleugenol, trans-methylsioveugenol, and the sequiterpenoid carophyllene oxide.

Conclusions. Thus, a comparative analysis of the component composition of biologically active substances in the essential oils of shoots and leaves of the Laurus nobilis L. Ukrainian harvest showed the prospects for further pharmacognostic research of this plant as a source of medicinal raw materials.

Keywords: Laurus nobilis L., shoots, leaves, essential oil, 1,8-cineole, α-terpinyl acetate, spatulenol, methyleugenol

1. Introduction

The search for new medicinal plants and expanding the range of herbal medicines is one of the top priorities of the pharmaceutical industry of any country, Ukraine is no exception. Attention is drawn to those plants that have long been used in folk medicine, are widespread in a particular area, and this is accompanied by an insufficient degree of study. Such a representative of the flora is Laurus nobilis L. of the family Lauraceae [1]. Laurus leaves were used as a seasoning for a huge number of dishes, but its value in a large number of useful properties [2, 3].

Analysis of literature data and previous chemical studies have shown the presence in the raw material of Laurus nobilis L. fatty acids, amino acids, a number of compounds of phenolic nature [4], including tannins and coumarins [5], bitters, essential oils with a wide range of biological activity [6, 7].

Spicy-aromatic plant with healing properties as a valuable crop is grown in many countries. The information of a number of foreign literature sources devoted to the composition of the essential oil of raw materials of this plant of different places of growth is analyzed. Thus, in the essential oil of raw Laurus nobilis L. from Turkey (leaves) [5], Iran (leaves) [8], Bulgaria (leaves, shoots, fruits) [9] and Montenegro (leaves, shoots, fruits) [10] common and main components were 1,8-cineole, sabinen and α-terpinyl acetate, but in the leaves of Laurus nobilis L. from Southern Italy the content of α-terpinyl acetate was negligible [11].

The significant content of linalool is characteristic of the essential oil of Laurus nobilis L. leaves from Southern Italy, Bulgaria and Montenegro. The highest content of methyleugenol was found in the essential oil of Laurus nobilis L. from Iran and Bulgaria. The essential oil of Laurus nobilis L. from Turkey, Bulgaria, and Montenegro contains a significant amount of α-pinene, and β-pinene was the main component only for the essential oil of raw materials from Turkey and Montenegro. Significant content of 4-terpineol is characteristic of raw materials from Turkey and Bulgaria, and raw materials Laurus nobilis L. Iran contains significant amounts of trans-sabinene hydrate and eugenol. According to literature sources, the composition of the essential oil depends on the place of growth of the plant, the method of production and method of storage.
The raw materials of the plant have long been widely used in folk medicine as a natural antibiotic, anti-septic and anti-inflammatory agent. Extracts from raw materials are relevant for people suffering from respiratory diseases, for the prevention and treatment of tuberculosis, as a protector against colds and viruses. In addition, volatile compounds are able to soothe the baby before bedtime, a decoction of the leaves of Laurus nobilis L. is used for skin manifestations of diaper dermatitis in infants, in adolescence - washing treats acne. Regular washing of hair with this tool gets rid of dandruff. The decoction is widely used in diseases of the oral cavity, it stops bleeding gums, heals small wounds, ulcers. Laurus nobilis L. is recommended for arthritis, rheumatism and gout as an anti-inflammatory, analgesic and anti-edematous. It is used in hypertension to normalize blood pressure, antispasmodic properties of the plant relieve pain in the intestine. Decoction of the leaves of Laurus nobilis L. helps to strengthen the immune system. Foot baths with leaves of Laurus nobilis L. will get rid of sweating feet, rubbing with oil with an extract of Laurus nobilis L. will help with exacerbations of osteochondrosis. In the world scientific literature there is much evidence of a wide range of pharmacological activity of the essential oil Laurus nobilis L. Scientists from different countries have confirmed antifungal and bactericidal activity [12], a beneficial effect on the digestive system [13]. In the literature there is information about the study of essential oil of Laurus nobilis L. leaves as a remedy against nematodes [14], there is information about cytotoxic [15] and antioxidant effects [4, 16, 17], extracts from raw Laurus nobilis L. showed in vitro antidiabetic activity [3]. Raw materials are also widely used in the manufacture of medicines, perfumes and cosmetics as a flavouring and as an insecticide [6, 18, 19].

At the same time, the raw material of Laurus nobilis L. in Ukraine is still not official. The plant is used as an auxiliary in medicines and cosmetics, but, given the chemical composition, a wide range of biological activity, further study of plant raw materials as a source for the creation of medicines is relevant.

**The aim of the study** was to conduct a comparative analysis of essential oils of shoots and leaves of Laurus nobilis L. of Ukrainian flora and to determine the prospects of their use in pharmacy.

### 2. Planning (methodology) of the research

The stages of research of raw materials Laurus nobilis L. are shown in Fig. 1.

![Fig. 1. Stages of the study of the component composition of the essential oil of shoots and leaves of Laurus nobilis L.](https://ssrn.com/abstract=3916275)

### 3. Materials and methods

The object of the study was the essential oil of 4 samples of raw materials Laurus nobilis L. of Ukrainian origin. Shoots (leafless, 1-2 years of vegetation) and leaves of series 1-2 Laurus nobilis L. were harvested in November 2017 in the southern regions of Ukraine.

Confirmation of the systematic species authenticity was carried out by the associate professor of the Department of Botany of NUPh Rudenko V.P.

A portion (60.0 g) of the crushed raw material (leaves or shoots of Laurus nobilis L.) was transferred into a 1 L flask, 300 ml of water was added, and the flask was connected to a vapour tube. Filled with water graduated and drain tubes through the tap with a rubber tube with a funnel at the end. Heated and boiled at an intensity at which the flow rate of the distillate was 60-65 drops per minute. After 5 minutes before the end of the distillation, the tap was opened, gradually lowering the distillate so that the essential oil occupied the graduated part of the receiver tube, measuring the volume of essential oil. The distillation time was 2 hours.

The yield of essential oil (X, %) in terms of absolute dry matter was calculated by the formula:

$$x = \frac{V \cdot 100 \cdot 100}{m \cdot (100 - W)}$$

where V – volume of essential oil, ml; m – mass of raw materials, g; W – weight loss during drying of raw materials, %.

Essential oils were easily mobile liquids with a characteristic odor, well soluble in 95 % ethanol, chloroform.

Analysis of the component essential oils of shoots, leaves of Laurus nobilis L. was determined
using the chromato-mass spectrometric method using a chromatograph Agilent Technology 6890N, equipped with a mass-selective detector 5973N. Quartz column, capillary HP-5MS with a length of 30 m and an inner diameter of 0.25 mm, filled with 5% phenylmethysiloxane. Previously, the column temperature was programmed: the initial column temperature was 8 min at 50 °C, and then gradually increased to the final – 220 °C. Acceleration rate 4 deg/min. Carrier gas – helium; flow rate 1 ml/min. The duration of acceleration is 1 hour. The sample volume was 0.5 μl at a flow factor of 1:50 and a pressure at the inlet of the column of 40 kPa. The scan was performed in the range of 38-300 a.e.m. Recording time 0.5 s.

The component composition of the essential oil was identified by comparing the results obtained by chromatography of the mass spectra of chemicals included in the test mixture with the data of the library of mass spectra NIsT 02 (more than 174,000 substances) [20].

4. Results
The essential oil of two samples of shoots of different growth places was studied, because it is known from literature sources that shoots, in addition to leaves and fruits, are also a source of essential oil. Thus, the shoots of series 1 contained 0.85±0.05 %, the shoots of series 2 – 0.91±0.04 % of essential oil in terms of dry raw materials.

The study revealed that the component composition of the essential oil of both studied series of shoots is similar in both the set of components and the relative content of a number of individual compounds and compounds of structurally related groups. Gas chromatograms of the components of the essential oil of 2 series of shoots of Laurus nobilis L. are shown in Fig. 2.

![Gas chromatograms of the components of the essential oil of the shoots of Laurus nobilis L.: a – series 1; b – series 2](Electronics available at: https://ssrn.com/abstract=3916275)
It was found that in the shoots of Laurus nobilis L. series 1 were found at least 36 essential oil (Table 1), and in the shoots of series 2 – at least 30 components. 10 compounds (of which 1 is unidentified) are characteristic only of the essential oil of shoots of series 1, and 4 – only in the essential oil of shoots of series 2.

Table 1

| No. | Compound name                  | Shots       |          |          |
|-----|--------------------------------|-------------|----------|----------|
|     |                                | Retention time, min | % of the amount | Retention time, min | % від суми |
| 1   | Sabine | 6.13 | 0.75 | 6.16 | 0.20 |
| 2   | α-Phellandrene | 6.94 | 0.42 | 6.94 | 0.41 |
| 3   | 1,8-Cineole | 7.69 | 19.63 | 7.59 | 12.93 |
| 4   | γ-Terpinene | 8.43 | 0.68 | – | – |
| 5   | trans-Sabinene hydrate | 8.54 | 0.78 | 8.60 | 0.64 |
| 6   | cis-Sabinene hydrate | 9.30 | 0.27 | 9.55 | 2.07 |
| 7   | β-Phenylethyl alcohol | 9.60 | 3.55 | – | – |
| 8   | Pinocarveol | 10.60 | 1.62 | 10.74 | 3.19 |
| 9   | Pinocarvone | 11.06 | 1.14 | – | – |
| 10  | Terpinene-4-ol | 11.98 | 5.73 | 12.12 | 8.58 |
| 11  | Myrtenal | 12.09 | 0.85 | 12.20 | 0.50 |
| 12  | α-Terpineol | 12.36 | 4.84 | 12.56 | 9.00 |
| 13  | Myrtenol | 12.49 | 1.41 | 12.68 | 3.14 |
| 14  | Dodecanone-2 | 14.44 | 0.68 | 14.49 | 0.96 |
| 15  | Bornylacetate | 15.32 | 3.69 | 15.29 | 0.71 |
| 16  | Undecanone-2 | 15.61 | 1.76 | 15.71 | 3.35 |
| 17  | Terpinen-4-ol acetate | 16.27 | 1.36 | 16.34 | 1.67 |
| 18  | α-Terpineyl acetate | 17.54 | 16.22 | 17.58 | 16.03 |
| 19  | 3-Phenyl-1-propyl acetate | 17.70 | 1.68 | – | – |
| 20  | Geranyl acetate | 17.79 | 0.24 | – | – |
| 21  | trans-Methyleugenol | 18.94 | 12.06 | – | – |
| 22  | Methyleugenol | – | – | 19.10 | 15.82 |
| 23  | β-Caryophyllene | 19.71 | 0.49 | 19.73 | 0.76 |
| 24  | Cinnamyl acetate | 20.07 | 12.24 | 19.85 | 1.08 |
| 25  | Tetradecanone-2 | 20.73 | 0.20 | 20.74 | 0.38 |
| 26  | trans-methyleugenol | 21.36 | 0.99 | 21.39 | 0.89 |
| 27  | α-Selinene | – | – | 21.55 | 0.43 |
| 28  | Pentadecanone-2 | 21.76 | 0.20 | 21.80 | 0.43 |
| 29  | α-Amorphene | 22.30 | 0.18 | 22.33 | 0.22 |
| 30  | δ-Cadinene | 22.59 | 0.15 | 22.62 | 0.32 |
| 31  | Elemicin | 23.05 | 0.81 | 23.18 | 0.93 |
| 32  | Spatulenol | 23.96 | 1.55 | 24.16 | 6.13 |
| 33  | Caryophyllene oxide | 24.03 | 1.09 | 24.21 | 1.35 |
| 34  | Unidentified compound | 25.27 | 0.21 | – | – |
| 35  | γ-betaunol | 25.49 | 0.94 | 25.71 | 6.72 |
| 36  | trans-isoelemicin | 25.59 | 0.58 | – | – |
| 37  | Eudesmol | – | – | 25.94 | 0.73 |
| 38  | Nonadecan | 30.88 | 0.62 | – | – |
| 39  | Palmitic acid | – | – | 31.58 | 0.43 |
| 40  | Heneicosane | 33.46 | 0.39 | – | – |

Only for the essential oil of shoots of series 1 is characterized by the presence of γ-terpinene, β-phenylethyl alcohol, pinocarvone, 3-phenyl-1-propyl acetate, geranyl acetate, trans-isoelemicin, nonadecane and heneicosane. The presence of methyleugenol, α-selinene, eudesmol and palmitic acid was characteristic only for the essential oil of shoots of series 2.

The content of these samples was dominated by 1,8-cineole (19.63 % of the amount and 12.93 % of the amount, respectively), α-terpinyl acetate (16.22 % of the amount and 16.03 % of the amount, respectively). A high content of methyleugenol (15.82 % of the amount) was determined for the essential oil of Laurus nobilis L. series 2 shoots. Cinnamyl acetate and trans-methyleugenol in significant quantities (12.24 % of the amount and 12.06 % of the amount, respectively) were found in series 1. The essential oil of shoots of series 2 has a significant content of α-terpineol (9.0 % of the amount), terpine-4-ol (8.58 % of the amount) and γ-betaunol (6.72 % of the amount).

The component composition of essential oil compounds of 2 series of Laurus nobilis L. leaves was also...
studied (Fig. 3). The component composition of the compounds of the essential oil of the leaves of Laurus nobilis L. series 1 is shown in Table 2, and series 2 in Table 3.

Fig. 3. Gas chromatogram of compounds of essential oil of leaves of Laurus nobilis L.: a – series 1; b – series 2
Thus, the leaves of series 1 contained 2.15±0.09 %, the leaves of series 2 – 2.75±0.09 % of essential oil in terms of dry raw materials.

In contrast to the series of shoots of Laurus nobilis L., for which there were a total of 28 compounds distilled with water vapour (Table 1), in the series of essential oils of the leaves of this plant there was a different pattern (Tables 2, 3). The composition of the compounds of the essential oil of the leaves of different series differed significantly in both the set of components and the relative content of structurally related groups of compounds.

The leaves in comparison with the shoots are more ontogenetically plastic, vulnerable to changes in a number of environmental factors, part of the plant. And this fact, by the way, can explain a fairly close qualitative composition of the essential oil of shoots from two fairly close to the place of harvesting and such a discrepancy between this figure in the leaf.

In both series, only 3 compounds were found that are common – aromatic compounds methyleugenol (7.14 % of the amount in the leaves of series 1 and 7.57 % of the amount in the leaves of series 2), trans-methylisoeugenol (content was 5.7 % of amounts in the essential oil of leaves of series 1, which is almost 9 times higher than in the essential oil of leaves of series 2 – 0.65 % of the amount) and sequiterpenoid caryophyllene oxide (4 times difference: in series 1 – 13.09 % of the amount against 3.53 % of the amount in series 2).

In series 1 of the essential oil of the leaves of Laurus nobilis L. identified 22 substances, of which dominated by compounds of the caryophyllene type caryophylla-4(12),8(13)-dien-5-ol (26.58 % of the amount) and caryophyllene oxide (13.09 % of the amount), as well as a complex ester of cinnamyl alcohol cinnamyl acetate (16.92 % of the amount). Hydrocarbons are represented by a variety of compounds – groups of normal paraffins (alkanes) from nonadecane to nonacosane (C19-C29), the total content of which was equal to 7.84 % of the amount, as well as unsaturated hydrocarbons of triterpene origin squalene (2.31 % of the amount), for which is characterized by anticancer, antimicrobial, fungicidal and immunostimulatory activity.

Table 3 shows that in the essential oil of the leaves of series 2 Laurus nobilis L. determined the presence of at least 24 substances. Dominated by the content of α-terpinyl acetate (25.02 % of the amount), 1,8-cineole (29.78 % of the amount).

Of the 36 compounds of the essential oil of shoots of the 1st series, only 2 are available in the essential oil of the leaves of the 1st series: cinnamyl acetate (12.24 % of the amount and 16.92 % of the amount, respectively) and caryophyllene oxide (1.09 % of the amount and 13.09 % of the amount, respectively). Of the 30 compounds of the essential oil of shoots 2 series 19 are common to the essential oil of the leaves of the same series with a predominance of 1,8-cineole (12.93 % of the amount and 29.78 % of the amount, respectively), (1.09 % of the amount and 13.09 % of the amount, respectively). These
are cinnamyl acetate (12.24 % of the amount and 16.92 % of the amount, respectively) and caryophyllene oxide (1.09 % of the amount and 13.09 % of the amount, respectively).

5. Discussion of research results

For the first time a comparative analysis of the component composition of essential oil of 2 series of shoots and 2 series of leaves of Laurus nobilis L. of domestic harvest was carried out, general and distinctive features were revealed.

The high content of 1,8-cineole can cause antimicrobial, fungicidal and antiviral action, enhance the drainage function of the ciliated airway epithelium. Quite high content of sesquiterpene alcohol spatulenol type aromadendran can cause fungicidal action, triterpene alcohol of lupan raw betulenol, and its derivatives show different types of biological activity – anti-inflammatory, activate collagen synthesis, inhibit elastase activity.

The advantage of the results of the study is the fact that this is one of the first steps in the study of several types of raw materials widely used in world medicine essential oil plant, whose raw materials are harvested in Ukraine and not yet standardized in pharmacy in our country. Certain disadvantages are the number of analyzed samples and varieties of plant raw materials (bark, flowers, fruits), which could be eliminated by further research.

Some difficulties in comparative analysis of the results with data from researchers from different countries are because sometimes the literature does not indicate the type of raw material at all, or does not indicate what is considered shoots (young leafy or isolated stems).

Thus, the quantitative content of essential oil from shoots of 2 series (shoots of series 1 contained 0.85±0.05 %, shoots of series 2 – 0.91±0.04 % of essential oil) is comparable with the results obtained by researchers from Bulgaria shoots – 0.80 %) [9] and Montenegro (1.4 % in young shoots and 0.80 % in isolated stems) [10]. At that time, the results of the quantitative content of essential oil in the leaves of 2 series (leaves of series 1 contained 2.15±0.09 %, leaves of series 2 – 2.75±0.09 % of essential oil) differ significantly from the results of Bulgarian (3.25 %) [9] and Montenegro (1.5 %) researchers [10].

Comparing the results of determining the component composition of the essential oil series of Laurus nobilis L. shoots of Ukrainian origin, for example, with the essential oil of this raw material from Bulgaria, we can say that the percentage of α-terpinyl acetate in two series of essential oil shoots of Ukrainian origin was higher (16.22 % of the amount and 16.03 % of the amount, respectively) than in the essential oil of Bulgaria (13.1 % of the amount) [9]. It should be noted that the percentage of 1,8-cineole is much higher in the essential oil of raw materials of Bulgarian origin (48.5 % of the amount), which is almost 2.5 times higher than the percentage of this compound in the essential oil from Ukraine (19.63 % of amounts in 1 series).

For the two batches of the essential oil of the leaves, the common components were methyleugenol (7.14 % of the amount and 7.57 % of the amount, respectively), trans-methylsioxugeneol (5.7 % of the amount and 0.65 % of the amount) and sequiterpenoid caryophyllene oxide (13.09 % of the amount and 3.53 % of the amount). It should be noted that the content of methyleugenol and caryophyllene oxide in the essential oil of leaves of Laurus nobilis L. from Bulgaria is much lower (6.03 % of the amount and 0.34 % of the amount, respectively) [9] than in the essential oil of this raw material of Ukrainian origin. In the essential oil of the leaves of the Egyptian harvest (17) the percentage of methyleugenol (7.67 % of the amount) is comparable to this indicator of the essential oil of the leaves of the Ukrainian harvest in the absence of trans-methylsioxugeneol, and in the essential oil of raw materials harvested in Turkey, the percentage of methyleugenol 8 % of the amount in the absence of trans-methylsioxugeneol and caryophyllene oxide [18].

Thus, the component composition of essential oils of shoots and leaves of Laurus nobilis L. of Ukrainian harvest is comparable to this indicator of raw materials of other countries of harvest.

A limitation of the study could be considered the volume of the library of mass spectra of compounds, which leads to the presence of unidentified compounds. In addition, the metrological evaluation of the measurement results was not performed because these studies are the first step towards the standardization of raw materials and are likely to have an indicative value in terms of the component composition of the raw material series. However, due to the high reproducibility and accuracy of the GC/MS method, these results can be taken into account in the further study of essential oils of shoots and leaves of Laurus nobilis L., in particular, artificial cultivation.

Promising areas for further research are similar studies of raw materials cultivated in artificial conditions Laurus nobilis L., which will improve the availability of raw materials and, of course, expand its boundaries. Also promising is further study of the chemical composition of shoots and leaves (especially phenolic compounds, in particular tannins) and phytotechnological research with the development of a dosage form in the form of a galenic drug or solid dosage form (capsules, tablets).

6. Conclusion

The component composition of essential oil of 4 samples of raw material Laurus nobilis L. (2 series of shoots and 2 series of leaves) was investigated and established.

The composition of compounds that are distilled with water vapour, a series of shoots is similar both in the set of components and in the relative content of a number of individual compounds and structurally related groups of compounds. Thus, in the shoots of Laurus nobilis L. series 1 were identified 36 essential oil, and in series 2 – 30 components. 10 compounds (of which 1 is unidentified) are characteristic of the essential oil of shoots of series 1, and 4 – is contained only in series 2.

The component composition of the essential oil of the leaves of the two studied series differed significantly in the set of components and their relative content. This may indicate that the accumulation of a number of substances in the same type of raw material depends on the location and conditions of plant growth. In both series, only 3 com-
pounds were invented, which are common – the aromatic compounds methyleugenol, trans-methyleloseugenol and the sequarendoid carpyrhylene oxide.

Comparative analysis of the component composition of biologically active substances in the essential oil of shoots and leaves of Laurus nobilis L. showed the prospects for further pharmacognosy study of this plant as a source of medicinal raw materials.

Conflict of interests.
The authors declare that they have no conflicts of interest

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Financing
The study was performed as part of a comprehensive research work of the National University of Pharmacy “Pharmacognostic study of medicinal plant raw materials and development of phytotherapeutic agents based on it” (state registration number 0114U000946).

Acknowledgements
We express our deep gratitude to the associate professor of the Department of Botany of NUPh Rudenko V. P. for the botanical determination of the plant, the raw material of which we studied.
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Received date 06.07.2021
Accepted date 24.08.2021
Published date 31.08.2021

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Electronic copy available at: https://ssrn.com/abstract=3916275