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**Chromatography-mass-spectrometric determination of the essential oils components of wild lentils.**

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**Introduction**

The genus Lens belongs to the family of legumes (*Fabaceae*) and includes seven species which are annual herbaceous plants. There are six species in the wild form and only one kind - *Lens culinaris M.* is widely cultivated in many countries of the world [1].

Lentil is widespread in Europe, Asia and Africa. Nowadays the main lentil cultivated area is mainly in such countries as Canada, India, Turkey, Australia. Lentil is food culture, the seeds of which are widely used for food purposes. That’s why the chemical composition of lentil has been studied mainly in relation to proteins, amino acids, carbohydrates, vitamins and seed lipids [1]. Protein content of lentil is ahead of such culture as peas and beans. Lentil protein is well absorbed by organism (86%) and contains a sufficient amount of vital amino acids. The plant does not accumulate nitrates, toxic elements and radionuclides and can be considered as ecological clear product [1, 2].

 Lentil has long been considered a medicinal plant. Water infusion made of beans flaps has an anti-inflammatory and antimicrobial action, it is applied externally for ulcers and eczema. Lentil flour has healing effect. Infusion of lentil herbs is also used for obesity, atherosclerosis and urolithiasis [1, 2, 3].

It is known that during the life-cycle the plants produce and accumulate a large amount of a wide variety of substances, many of which are volatile. Information about the volatile components structure of elevated part of the lentil is not found. Thus the aim of this study was extracting essential oils derived of herbs and two kinds of lentils and studying their component structure [4].

**Materials and Methods**

The object of the study was wild herb of lentils: *L. Odemensis Ladiz.* (L. Odem) and *L. tomentosus Ladiz.* (L. Hairy) harvested in Kharkiv region in summer, 2013.

The study of the volatile components was being conducted by using the method of chromatographic mass spectrometry, which is a modern and highly effective way of separating and establishing quantitative content of biologically active compounds, as well as informative when establishing their quality. To study the essential oil we used the method of isolation of essential oil from plant material at limited quantity in raw materials [5, 6]. Weighed sample of material (0.5-5 g) was placed in a vial ‘Agilent’ and internal standard was added. As internal standard, tridecane, based on a sample of 50 g, with followed by calculation of the concentration of the resulting internal standard which was used then for the calculation.

The composition of essential oil was determined by chromatographic mass spectrometry method [6, 7] by Agilent Technology 6890N chromatograph with mass spectrometric detector 5973N. Assay conditions: chromatographic column is silica, capillary HP-5MS. Column length is 30 meters. Internal diameter is 0.25 mm. The rate of gas-carrier (helium): 1 ml / min. Sample volume is 0.1 - 0.5 mkl (for solvents of essential oil). Introduction of the sample flow division is 1/50. The temperature of thermostat is 500 C with 40 C / minute up to 2200 C programming. Detector temperature and evaporator is 2500 C.

**Results and Discussion**

The obtained spectra were treated as based on the general laws of the fragmentation of organic molecules compounds under electron impact, and by comparing the results obtained with those in the mass spectral library Database NIST02 (more substances 174000). Before the search was started we calculated the average of mass spectrum for each chromatographic peak which we deducted from the background spectrum. The identification of the compounds was carried out by comparing the mass spectra of the chromatographic peak with the mass spectra of the reference compounds database. Quantitative content is calculated according to relative peak area sum of squares of components of all peaks in the chromatogram (normalization method). Retention factors of components are calculated by the results of control analyzes of essential oils with a mixture of n-alkanes (C10-C18) [6, 7].

Chromatograms of essential oils of *Odemensis L.* and *L. tomentosus* herbs are shown in Fig. 1 and Fig. 2, accordingly.

The experiment in the essential herbal oil of *L. Odemensis* revealed 32 volatiles and *L. tomentosus* herb - 28. The summarized data of the volatile components of essential oils which were identified in the studied plants is present in the table. The volatiles of the samples are presented by terpenoids and their derivatives, aromatic compounds, aldehydes, ketones, fatty acids, acyclic saturated hydrocarbons.

In the overall evaluation of identified substances we should note that quantitatively (relative to all identified components) in studied raw materials hydrophobic compounds such as fatty acids were dominated (more than 60% of the total content of volatiles). The saturated fatty acids among all fatty acids were dominated in a qualitative sense (89.6% in *L. tomentosus* and 84,5% *L. Odemensis*). The *L. Odemensis* herb contained tridecanoic and heptadecanoic acids which were not found out in *L. tomentosus* herb. As concerning saturated fatty acids palmitic, lauric, myristic predominated (almost in equal proportions). Only the content of lauric acid in *L. tomentosus* herb was almost two times higher than in *L. Odemensis* herb.

Lauric acid has a broad antimicrobial and antibacterial spectrum of action. It fights with such pathogens microorganisms as viruses, bacteria, fungi. Palmitic and myristic acids contribute to the restoration of protective properties of skin. That’s why they are widely used in cosmetics. Linoleic and linolenic acids are part of the complex of vitamin F, which has a beneficial effect on the musculoskeletal system: nourishes the tissues of the joints, prevents the development of rheumatic diseases, osteoarthritis [8, 9].

Terpenoid compounds are of special scientific interest the content of which in essential oil of *L. Odemensis* and *L. tomentosus* herbs is 7.4% and 11.7% accordingly. Among terpenoids there were contained a large amount of acyclic triterpenoid squalene (143.4 mg / kg in *L. Odemensis* and 289.2 mg / kg in *L. Tomentosus*). The biological activity of squalene is quite diverse. This substance in human body shows anticarcinogenic, antimicrobial, fungicide, radioprotection properties, also increases immunity. Squalene is derivative of vitamin A, and during the cholesterol synthesis it promotes cholesterol conversion to the 7-dehydrocholesterol analog which in turn is converted into vitamin D in sun. As a precursor of steroids it affects steroid metabolism, which can be used in diet therapy of cardiovascular disease [10]. Also the bi-cyclic sesquiterpenoids geksagidrofarnezilatseton dominated among volatile terpenoids, and β-phenylethyl alcohol dominated among aromatics. We should note that contain of these compounds were two times more in *L. Tomentosus* herb than in *L. Odemensis* one. Other components were found out in minor quantities.

Analyzing the composition of the volatile compounds of studied samples we should note that in essential oil of *L. Odemensis* herb there were identified such substances as bisabolen, caryophyllene, phenylacetaldehyde, which in *L. tomentosus* herb were not found. Conversely among volatile components of *L. tomentosus* there had been identified terpenoid alcohols β-evdesmol and apiol.

**Conclusions**

The content of volatile compounds of two herbs of wild lentils (*L. Odemensis* and *L. tomentosus*) by chromatography-mass spectrometry method was studied. Quantitatively in studied raw material saturated (palmitic, lauric, myristic acid) and unsaturated (linoleic, linolenic) fatty acids (more than 60% of the total content of volatile substances) were dominated. There were identified dominant terpenoid compounds of *L. Odemensis* and *L. tomentosus*: squalene hexahydrofarnesylacetone, β-phenylethyl alcohol. Such components of essential oil as β-bisabolen, β-caryophyllene, phenylacetaldehyde were revealed only in *L. Odemensis* herb and terpenoid alcohols β-evdesmol and apiol were revealed only in *L. tomentosus* herb.

Thus, the results of studies suggest that plants of the lentils are perspective drug raw materials for more detailed study of the chemical composition and creation new drug substances on their basis.

**Summary**

Applying the chromatography-mass spectrometry method there were made the qualitative and quantitative determination of volatiles of essential oils among wild lentil: *L. Odemensis Ladiz.* (L. Odem) and *L. tomentosus Ladiz.* (L. Hairy). They were harvested in Kharkiv region in summer, 2013. There were identified 32 components in the essential oil of *L. Odemensis* and 28 components in the oil of *L. tomentosus* which are belonged to different groups of chemical compounds, among which terpenoids and their derivatives, aromatic compounds, aldehydes, ketones, fatty acids, and saturated alicyclic hydrocarbons.

**Keywords:** plants of lentils, essential oil, chromatography-mass spectrometry.

**Хромато-масс-спектрометрическое определение компонентов эфирных масел диких видов чечевицы**

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**Резюме.** Методом хромато-масс-спектрометрии проведено качественное и количественное определение летучих веществ эфирных масел травы диких видов чечевицы: *L. Оdemensis Ladiz.* (ч. Одемена) и *L. tomentosus Ladiz.* (ч. опушенная), заготовленных в Харьковской области летом 2013 г. Идентифицировано 32 компонента в эфирном масле *L. Оdemensis* и 28 компонентов в масле *L. tomentosus*, относящихся к разным группам химических соединений, среди которых терпеноиды и их производные, ароматические соединения, альдегиды, кетоны, жирные кислоты, а также ациклические насыщенные углеводороды.

**Ключевые слова:** растения рода чечевица, эфирное масло, хромато-масс-спектрометрия.

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Fig. 1. The chromatogram of essential oil of *L. Оdemensis* herb



Fig. 2. The chromatogram of essential oil of *L. tomentosus* herb

*Table*

The content of volatile compounds in *L. Odemensis* and *L. tomentosus* herb (mg/kg)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| №п/п | Keeping time, min. | The identified compounds  | *L. Оdemensis*  | *L. tomentosus* |
| 1 | 6.63 | capron acid | 126.5 | 142,8 |
| 2 | 7.16 | рhenylacetaldehyde | 6.8 | - |
| 3 | 7.62 | benzylic alcohol | 24.4 | 86,2 |
| 4 | 8.8 | heptanoic acid | 33.7 | 43,1 |
| 5 | 10.15 | β-phenylethyl alcohol | 100.8 | 170,5 |
| 6 | 12.51 | caprylic acid | 186.0 | 241,2 |
| 7 | 15.13 | nonanoic acid | 113.4 | 102,7 |
| 8 | 16.1 | 2-nonenoic acid | 12.1 | - |
| 9 | 16.57 | 5-pentyl-2(5Н)- pentyl-furanone | 36.7 | 54,5 |
| 10 | 17.15 | dihydro-5-pentyl-2(3Н)-furanone  | 8.9 | 13,2 |
| 11 | 18.29 | capric acid | 149.2 | 174,6 |
| 12 | 18.71 | β-caryophyllene | 8.8 | - |
| 13 | 20.71 | undecanoic acid или tridecylic acid | 18.4 | 15,9 |
| 14 | 20.87 | β-ionone-epoxide | 12.8 | 21,0 |
| 15 | 21.5 | β-bisabolen | 6.2 | - |
| 16 | 24.07 | lauric acid | 342.1 | 634,5 |
| 17 | 24.8 | benzophenone | 10.8 | 16,0 |
| 18 | 25.47 | β-evdesmol  | - | 13.8 |
| 19 | 26.12 | tridecanoic acid | 18.4 | - |
| 20 | 26.34 | apiol | - | 92.9 |
| 21 | 28.47 | myristic acid  | 287.2 | 653,8 |
| 22 | 29.41 | hexahydrofarnesylacetone | 82.7 | 205,4 |
| 23 | 29.91 | pentadecanoic acid | 45.3 | 124,6 |
| 24 | 31 | palmitoleic acid | 59.6 | 149,0 |
| 25 | 31.75 | palmitic acid | 1519.4 | 1774,1 |
| 26 | 32.61 | heptadecanoic acid | 13.0 | - |
| 27 | 33.61 | linoleic acid | 234.1 | 119,7 |
| 28 | 33.69 | linolenic acid  | 200.4 | 175,9 |
| 29 | 33.86 | stearic acid | 37.4 | 77,1 |
| 30 | 35,17 | tricosane | - | 21,6 |
| 31 | 37.22 | pentacosane | 28.9 | 88,3 |
| 32 | 39.1 | heptacosane | 67.7 | 70,9 |
| 33 | 40.27 | squalene | 143.4 | 298,2 |
| 34 | 40.85 | nonacosane | 92.4 | 86,9 |

Note: ‘-‘ compound wasn’t revealed