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Study of the hexane fraction isolated from the substance obtained from the roots *Limonium Gmelinii* by GC-MS

Abstract: Current paper presents the results of chemical study of the non-polar fraction obtained from the substance isolated from the roots of *Limonium gmelinii* (Willd.). *Limonium gmelinii* is a perennial wild-growing plant with a flowering period in June-September. It is found in all regions of Kazakhstan, western and eastern Siberia, European part of Russia, Central Asia, Southeastern Europe, Western China and Mongolia. Roots of *Limonium gmelinii* are introduced into the medicine and State Pharmacopoeia of the Republic of Kazakhstan. In this work, we obtained a substance using ultrasound to intensify the extraction process, which is effective, both from the economic and ecological side. *Limonium gmelinii* plants were harvested in Almaty region in 2018. Extracts were obtained by solvent extraction of a dry substance isolated from the roots of the studied plants with hexane, and studied by chromatography – mass spectrometry on a gas chromatograph with a mass-selective detector. In non-polar extract obtained from such substance, 27 individual compounds were identified, most of them are represented by higher fat-soluble hydrocarbons that are a part of natural waxes and resins; particularly high content of tetradecane, pentadecane, hexadecane, genicosane, octadecane, eicosane and their isomers was noted. In addition, the presence of a quinoline derivative in the form of 1,2-dihydro-2,2,4-trimethyl-quinoline was observed. For comparison, non-polar extract directly from the roots of *Limonium gmelinii* comprised 15 compounds belonging to the classes of hydrocarbons and esters of higher carboxylic acids. Dominating are hexadecanoic acid, ethyl ester, ethyl oleate, linoleic acid ethyl ester. In addition, a number of hydrocarbons, such as heptadecane, octadecane and tetradecane, and a quinoline derivative of 1,2-dihydro-2,2,4-trimethyl quinolone as well as phenol 2,4-bis (1,1-dimethylethyl) have been identified.

Key words: *Limonium gmelinii*, ultrasonic extraction, non-polar fraction, GC-MS, chemical composition.

Introduction

Limonium is a genus of plants that includes more than 300 different species distributed in many regions of the planet, including Central Asia, Southern Europe, North Africa and Middle East. On the territory of CIS, 35 species of the genus were described. Out of 18 *Limonium* species in Kazakhstan, the most industrially significant and productive is *Limonium* (*L.*) *gmelinii* (Willd.), which has wide ecological amplitude and is easily adapted to the environment, normalizing the maintenance of calcium and sodium salts in the soil.

L. gmelinii is a perennial wild-growing plant with a height of 30-80 cm. Roots are thick, nodular, and dark-brown, with thin bark, placed tightly to wood. Stem is truncated, with a little, usually paired branches. Leaves are numerous, green or blue-green, redden at ruptures. Flower stalks are few, apical and axillary. Flowers are in small 1-4 flower clusters, assembled in sufficiently short dense spikes, forming corymbose or pyramidal inflorescences. Spikes are 4.5-6 mm long; the cap is 4-4.5 mm long, at the base and up to the middle densely and reasonably long omitted by veins. Limb is fade white or faded purple, 5-10 notched. Petals are blue-violet, rarely white. Seeds are oblong-ovate, 2 mm in length, 0.6 mm in width, dark-purple-brown. Blooms in June-September, bears fruits in August-September. Area of distribution: all regions of Kazakhstan, western and eastern Siberia, European part of Russia, Central Asia, Southeastern Europe, Western China and Mongolia. Roots of *L. gmelinii* were introduced into the medicine and into the State Pharmacopoeia of the Republic of Kazakhstan, harmonized with the European Pharmacopoeia [8; 9].

On the basis of the plant roots of the *L.gmelinii* species, we obtained and patented a substance in the form of a complex of biologically active compounds, which has antiinflammatory, antioxidant and wound healing effects [10-13].

Considering the possibilities of optimizing the technology for obtaining the substance of our interest, the process of its extraction from the roots of *L.gmelinii* was intensified using ultrasound by varying its power and time of extraction. The amplitude of the ultrasound pulse is 400-1600 watts, pulse duration is 30 minutes. Ultrasonic dispersion was performed using an Elmasonic S450H ultrasonic bath (Elma Schmidbauer GmbH, Germany) at an ultrasound frequency of 37 kHz. The improved technological scheme for substance isolation from plant raw material allowed to increase its yield up to 45-50%. Phytochemical analysis of the substance was conducted as well [14-17].

Materials and methods

The substance was obtained according to the following procedure: 200 g of crushed roots are filled with 1200 ml of 50% aqueous alcohol and extraction is carried out by ultrasonic dispersion with the following electrophysical characteristics: ultrasonic pulse amplitude – 400 V, frequency 37 kHz, pulse duration – 20 min, temperature 35 °C. Ultrasonic dispersion was performed using Elmasonic S 450 (Elma Schmidbauer GmbH, Germany), working volume 45 L. Extraction time 4 hours at room temperature. The extract is filtered and concentrated to dryness on a rotary evaporator. The obtained substance is extracted with hexane in order to isolate from it the non-polar fraction, the composition of which was studied on a gas chromatograph with a mass-selective detector model Agilent 7890N/5973N (CTC Analytics / Leap Technologies, USA). At the same time, the volume of the gas phase to be taken is equal to 1 μl, the sample introduction temperature is 250 °C without dividing the flow. Separation was performed using a DB-35 MS chromatographic capillary column with a length of 30 m, an inner diameter of 0.25 mm and a film thickness of 0.25 μm at a constant carrier gas (helium) rate of 1 ml/min. The temperature of chromatography was programmed from 40 °C to 200 °C with a heating rate of 10 °C/min.

The software Agilent MSD ChemStation (version 1701EA) was used to control the gas chromatography system, record and process the obtained results and data. Data processing included determination of retention times, peak areas, as well as processing of spectral information obtained using a mass spectrometric detector. The Wiley 7th edition and NIST'02 libraries were used to decipher the mass spectra obtained (the total number of spectra in the libraries is more than 550 thousand).

The results of a comparative study of non-polar fractions obtained from the roots of the plant *L.gmelinii* and from the substance isolated from them during the intensification of the extraction process with ultrasound are presented in Tables 1 and 2.

Results and discussion

As a result of studying the hexane fraction of a substance obtained from the roots of plants of the species *L. gmelinii* (50% aqueous alcohol and ultrasound), 27 compounds were identified, most of which were classified as hydrocarbons (Table 1).

Table 1 – Research data of a hexane fraction obtained from a substance isolated from *L. gmelinii* roots by GC-MS

No.	Retention time	Name of compound	Content %
1	6.54	undecane	0.64
2	8.05	dodecane	1.08
3	8.92	2,7,10-trimethyl-dodecane	0.83
4	9.52	tridecane	3.38
5	10.35	2,3-dimethyldodecane	0.48
6	10.42	2,6,10-trimethyl-dodecane	0.50
7	10.91	tetradecane	7.48
8	10.99	1-tetradecene	0.43
9	11.54	2,6,10-trimethyl-tetradecane	1.14

Continuation of table 1

10	11.82	7-hexyl-tridecane	1.00
11	11.99	octyl-cyclohexane	0.55
12	12.23	pentadecane	10.28
13	12.91	1-tetradecanol	2.03
14	12.97	2-methyl-pentadecane	0.79
15	13.10	3-methyl-pentadecane	0.93
16	13.33	n-nonylcyclohexane	0.98
17	13.49	hexadecane	10.77
18	13.88	1,2-dihydro-2,2,4-trimethyl-quinoline,	3.47
19	14.49	2,6,10,14-tetramethyl-pentadecane	4.50
20	14.68	heptadecane	10.67
21	15.47	3-methyl-heptabecane	1.11
22	15.69	2,6,10,14-tetramethyl-hexadecane	3.57
23	15.81	octadecane	10.99
24	18.48	eicosane	7.34
25	19.82	phenanthrene	2.97
26	20.47	heneicosane	6.96
27	23.22	9-hexyl-heptadecane	5.12
Total			100.00

Typical for non-polar plant extracts is the dominance of hydrocarbons in their composition. In particular, a high content of tetradecane, pentadecane, hexadecane, genicosane, octadecane and eicosane was detected. Their total share is 96.52%, with the bulk of them being higher hydrocarbons, such as hexadecane, heptadecane, octadecane. 1,2-dihydro-2,2,4-trimethyl-quinoline, which is a quinoline derivative, is also identified.

Since plants of *L. gmelinii* are distributed mainly in arid zones, long-chain hydrocarbons are typical metabolites for such plants, which are mainly found in the composition of wax-like and resinous compositions on the outer integument, which act as barriers to the drying out and penetration of microorganisms into plants [18-23].

A comparative analysis of the above hexane extract with hexane extract obtained from the roots of the plant *L. gmelinii* was carried out. As a result, 14 compounds were identified, mainly belonging to the class of hydrocarbons, as well as to esters of higher fatty acids (Table 2).

Table 2 – Research data of *L. gmelinii* roots by GC-MS

No.	Retention time	Name of compound	Content %
1	6.53	undecane	0.94
2	8.05	dodecane	2.66
3	9.52	tridecane	4.07
4	10.91	tetradecane	4.15
5	10.99	7-tetradecene, (z)	0.89
6	12.23	9-methylheptadecane	3.22
7	13.49	heptadecane	7.61
8	13.83	phenol 2,4-bis(1,1-dimethylethyl)	1.72
9	13.88	1,2-dihydro-2,2,4-trimethyl quinoline	4.17

Continuation of table 1

10	15.69	tetracosane	3.43
11	15.81	octadecane	5.26
12	19.78	hexadecanoic acid, ethyl ester	24.51
13	25.40	ethyl oleate	11.16
14	25.78	linoleic acid ethyl ester	26.19
Total			100.00

In contrast to the non-polar fraction obtained from the substance isolated from the roots of the plant *Limonium gmelinii*, in the non-polar total extract directly from the roots the hydrocarbon content is 32.23%, which is 3 times less than in the non-polar fraction from the substance. At the same time, heptadecane, octadecane and tetradecane are also the dominant hydrocarbons, which belong to higher fat-soluble hydrocarbons. In addition to hydrocarbons in the roots of *L. gmelinii*, fatty acid esters were identified as hexadecanoic acid, ethyl ester, ethyl oleate, linoleic acid ethyl ester, which together account for 61.86% of the total mass of the identified compounds. Absence of these compounds in the hexane fraction of the substance is explained by the nature of the initially taken extractant in the isolation of the substance from the raw material with 50% aqueous alcohol, which does not dissolve esters of higher fatty acids.

A quinoline derivative in the form of 1,2-dihydro-2,2,4-trimethyl quinoline, different from the quinoline derivative found directly in the roots, was also found in a non-polar extract obtained from a substance isolated from the roots of plants of the species *L. gmelinii*. In addition, phenol 2,4-bis(1,1-dimethylethyl) was identified in the roots in an amount equal to 1.72%.

Conclusion

As a result of the study, the technological scheme for isolating a substance from the roots of plants of the *Limonium gmelinii* species was improved; It has been established that fat-soluble hydrocarbons dominate in the non-polar fraction of the substance obtained from the roots of *Limonium gmelinii* plants, while esters of higher carboxylic acids dominate in the analogous fraction of the roots.

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