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Biologically Active Terpenoids *Ligularia Macrophylla* (Ledeb.) DC. and Technology of New Drug Substance

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Abstract

The article presents the results of a chemical study of *Ligularia macrophylla* (Ledeb.) DC. The essential oil was isolated by hydrodistillation using a Clevenger apparatus and microwave extraction using a NEOS installation. Using mass spectrometry, 52 components were identified in the essential oil isolated by hydrodistillation; 63 components were identified in the essential oil isolated by microwave extraction. The main components of the essential oil are Δ^3-carene, β-bisabolene, β-myrcene. It was revealed that the substance obtained from the essential oil of *Ligularia macrophylla* (Ledeb.) DC. has anti-inflammatory and antimicrobial activity. For the first time, the optimal technological parameters for alcohol extraction of *Ligularia macrophylla* (Ledeb.) DC roots were determined using the method of mathematical modeling, providing a quantitative yield of the sesquiterpene lactone furanoeremophilan-14β,6α-olide, which has anti-inflammatory activity.

1. Introduction

The plants of the genus *Ligularia* belong to the large genus of the Asteraceae family, tribe Senecioneae, including about 130 species, which are divided into six sections, according to the classification in botanical taxonomy. On the territory of Kazakhstan, the plants of the genus *Ligularia* are represented by 16 species, 3 of which are endemic [1, 2]. They have antitumor, anti-inflammatory, antibacterial, and antidiabetic activities [3–10].

At the same time, plant species of the genus *Ligularia* Cass. are considered a promising source of new medicinal substances, in particular eremophilane sesquiterpenes and essential oils, among which eremophilane sesquiterpenes are considered the main components of plants of this genus [11–14].

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2. Experimental part

2.1 Materials and methods

Medicinal plant raw materials. Raw materials of the aerial parts (flower baskets, buds, leaves) and underground part (roots) of *Ligularia macrophylla* (Ledeb.) DC. were collected during the flowering phase in the vicinity of the village of Nurken, Karkaraly district, Karaganda region. At the same time, 3.2 kg of aerial parts and 8.2 kg of roots were harvested.

Solvents and reagents: purified water; petroleum ether; ethyl alcohol (70%, 80%, 96%); chloroform; ethyl acetate; desiccants (sodium sulfate anhydrous, calcium chloride, copper sulfate).

Research methods: analytical high-performance liquid chromatography on a Hewlett liquid chromatograph Packard Agilent 1100 Series; chromatography-mass spectrometry method on a gas chromatograph with a mass selective detector Agilent 7890A/5975C; hydrodistillation using a Clevenger apparatus; microwave extraction unit NEOS; IR spectroscopy on the Avatar 360 ESP device; UV spectrophotometry on a Cary 60 UV-Vis device; microanalyzer Eurovector 3000 for elemental analysis; mass spectroscopy; NMR spectroscopy on a spectrometer JNM-ECA 500; study of cytotoxic activity against larvae of sea crustaceans *Artemia salina* (Leach); study of antimicrobial activity; study of anti-inflammatory activity; statistical processing of results.

2.2 Isolation and identification of terpenoids from Ligularia macrophylla (Ledeb.) DC. essential oil. Study of the component composition of essential oil

Essential oils from the aerial parts of *Ligularia macrophylla* (Ledeb.) DC. were isolated by hydrodistillation using a Clevenger apparatus and a NEOS microwave extraction unit (Italy).

Hydrodistillation of the aerial part was carried out on a Clevenger apparatus for 2 h at atmospheric pressure (101.325 kPa).

Microwave extraction of *Ligularia macrophylla* (Ledeb.) DC. was carried out on a NEOS installation at atmospheric pressure (101.325 kPa) for 90 min at a temperature of 100 °C and a radiation power of 550 W.

The component composition of essential oils was studied by chromatography-mass spectrometry on a gas chromatograph with an Agilent 7890B/5977B mass selective detector. Column HP-5MS 5% Phenyl Methyl Silox (30 m × 0.25 mm) with a helium carrier gas flow rate of 1 ml/min. Evaporator temperature 280 °C. The gas chromatography column was kept at a temperature of 50 °C for 2 min; with temperature programming to 280 °C with a temperature change rate of 4 °C/min, and then kept in isothermal mode for 20 minutes. Splitless sample injection mode. Sample volume – 0.2 µl. Conditions for recording mass spectra – 70 eV, mass range – *m/z* 10–350. Essential oil components were identified by comparing their mass spectra and linear retention indices (relative to C7-C40 alkanes) with the data. The quantitative analysis was performed using the method of internal normalization based on the areas of gas chromatographic peaks calculated using the package Agilent ChemStation without using correction factors. The sum of the peak areas of components with linear retention indices in the range of 900–2200 was taken as 100%.

2.3 Chemical study of the roots of Ligularia macrophylla (Ledeb.) DC

2 kg of dried crushed roots of *Ligularia macrophylla* (Ledeb.) DC. were extracted three times with 10 L of ethyl alcohol at room temperature. The extraction was repeated under the same conditions twice. After evaporation of the solvent, the amount of extractive substances (86 g) was treated with a mixture of alcohol:water (2:1), and the filtrate was treated with chloroform three times. After evaporation of the chloroform extract on a rotary evaporator, 65 g of the total extractive substances were obtained, which was chromatographed on a column of large silica gel, large porous at a sum-carrier ratio of 1:5.

Identification of the sesquiterpene lactone was carried out by high-performance liquid chromatography (HPLC) on a Hewlett Packard Agilent 1100 Series device in isocratic mode, using the following composition as a mobile phase: acetonitrile-water (1:1); eluent flow rate 0.5 ml/min. Steel column 150 x 4.6 mm. Sorbent Zorbax SB-C_{18}, particle size 5 µ. The column temperature is room temperature. The volume of the injected sample was 20 µl; the detection of samples was carried out with a UV detector at wavelengths 204 and 215 nm.

Melting points were determined using a Hund Wetzlar. The IR spectrum was recorded in tablets with potassium bromide on an Avatar 360 ESP device. UV spectra – on a Cary 60 UV-Vis in ethanol. Elemental analysis was performed on Eurovector 3000 analyzer.

Individual connections monitored by TLC on Silufol plates. ^{1}H and ^{13}C NMR spectra were recorded on a JNM-ECA 500 spectrometer with an operating frequency of 500.13 MHz for ^{1}H and 125 MHz for ^{13}C. Solvent $CDCl_3$.

3. Results and discussion

3.1 Component composition of essential oils

From the aerial part of *Ligularia macrophylla* (Lebeb.) DC. by hydrodistillation and microwave extraction methods, essential oils were isolated in yields of 0.30% and 0.03%, respectively (based on air-dried raw materials). Essential oils isolated by two methods were mobile light green liquids with characteristic odors.

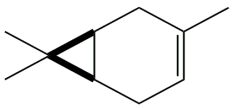
Using chromatography-mass spectrometry in the essential oil from the aerial parts of *Ligularia macrophylla* (Ledeb.) DC., extracted by hydrodistillation, 52 components were found, 43 of which were identified. In the essential oil isolated by microwave extraction, 63 components were found, 55 of which were identified.

Table 1. The main component composition of essential oils isolated by hydrodistillation (HD) and microwave extraction (MWE) from the aerial part of *Ligularia macrophylla* (Ledeb.) DC., collected at flowering stage

RT, min	RI (AMDIS program)	RI (atlas of spectra)	Component	Content, %	
				HD	MWE
7.2155	931.8	932	α-pinene	4.10	1.13
9.0598	990.7	991	β-myrcene	2.90	1.66
9.7152	1010.5	1010	Δ^3-carene	35.16	24.62
10.6862	1038.5	1038	cis-β-ocimene	18.27	15.16
11.0161	1048	1048	trans-β-ocimene	3.61	2.91
11.2844	1055.7	1058	unidentified component, mass spectrum close to γ-terpinene	3.07	2.45
12.5305	1091.6	1092	undec-1-ene	6.60	5.97
25.5322	1482.6	1484	germacrene-D	3.42	7.75

Main components of essential oils from the aerial parts of *Ligularia macrophylla* (Ledeb.) DC. are given in Table 1.

Table 1 shows that the main components of essential oils isolated by hydrodistillation and microwave extraction from the aerial parts of *Ligularia macrophylla* (Lebeb.) DC., collected in the flowering phase, are Δ^3-carene (1), cis-β-ocimene (2) and undec-1-ene (3).



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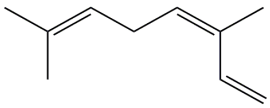

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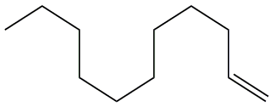


(3)

From the roots of *Ligularia macrophylla* (Lebeb.) DC. using hydrodistillation and microwave extraction methods, light green essential oils were isolated with yields of 0.22% and 0.09%, respectively (based on air-dry raw materials).

Using chromatography-mass spectrometry in the essential oil from the roots of *Ligularia macrophylla* (Ledeb.) DC., isolated by hydrodistillation, 58 components were found, 51 of which were identified; 57 components were found in the essential oil extracted by microwave extraction, 47 of which were identified.

The essential oil substance isolated by microwave extraction from the roots of *Ligularia macrophylla* (Ledeb.) DC.), has a pronounced antimicrobial activity against *E. coli* and moderate activity against *S. aureus* and *B. subtilis*. However, the quantitative yield of essential oil by this method is low (0.09%).

Main components of essential oils from the roots of *Ligularia macrophylla* (Ledeb.) DC. are given in Table 2.

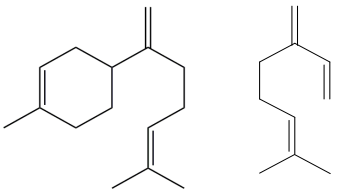
Table 2. Main component composition of essential oils isolated by hydrodistillation and microwave extraction from the roots of *Ligularia macrophylla* (Ledeb.) DC., collected at the flowering stage

RT	RI (AMDIS program)	RI (atlas of spectra)	Component	Content, %	
				HD	MWE
9.0742	991.2	991	β-myrcene	11.94	9.08
9.7140	1010.5	1010	Δ^3-carene	30.68	24.91
26.3807	1510.6	1511	β-bisabolene	16.01	12.37
30.5679	1657.2	-	unidentified component	3.29	4.66
30.8330	1666.8	-	unidentified component	2.95	3.88
34.4233	1800	-	unidentified component	3.37	2.70
35.6564	1849.9	-	unidentified component	4.07	10.24
41.6667	2204.3	-	unidentified component	1.01	4.53

From Table 2 it can be seen that the main components of essential oils from the roots of *Ligularia macrophylla* (Ledeb.) DC. collected in the flowering phase are:

– by hydrodistillation method Δ^3-carene (1) – 30.68%, β-bisabolene (4) – 16.01%, β-myrcene (5) – 11.94%;

– by microwave extraction method Δ^3-carene (1) – 24.91%, β-bisabolene (4) – 12.37%, β-myrcene (5) – 9.08%, unidentified component (RI = 1849.9) – 10.24%.



(4) (5)

When eluting a column with large silica gel, large porous using a mixture of petroleum ether:ethyl acetate (98:2) 3.25 g of white crystalline substance of the composition $C_{15}H_{18}O_3$ was isolated with a purity according to HPLC of 99.53%, ml.p. 135.7–137.1 ºC (petroleum ether:ethyl acetate) with a yield of 0.17% based on air-dried raw materials or 5.4% based on the amount of extractives (Table 3).

The infrared absorption spectrum of the substance furanoeremophilan-14β,6α-olide is recorded on FSM 2201 devices with a Fourier transform in disks with potassium bromide (3 mg of the drug in 300 mg of potassium bromide) in the range from 4000 to 500 cm^{-1}.

The IR spectrum contains absorption bands in the range of 2941–2876 cm^{-1}, which characterize methylene groups -CH_2, carbonyl γ-lactone at 1773 cm^{-1}, and the C=C double carbon bond in the range of 1640 cm^{-1}.

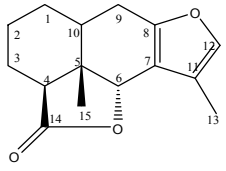
The UV spectrum of 0.001% solution of the substance furanoeremophilan-14β,6α-olide in ethyl alcohol 96% is taken on a Cary 60 device in quartz cuvettes 10 mm thick in the region from 200 nm to 500 nm. The UV spectrum should have an absorption maximum at a wavelength of 215±2 nm (Abs 1.466) with an extinction coefficient ε_{215} = 2.66×10^{-5} l/(mol×cm). The UV spectrum of the studied sample shows an absorption maximum at 216 nm.

In the H^1-NMR spectrum of compound (6), singlets are observed in the range of 7.07; 5.06; 2.03 and 1.25 ppm, characteristic of H-12 (proton of the furan nucleus), H-6 (lactone proton), protons of the methyl group at the double bond and angular methyl, respectively (Table 4).

Table 3. Results of chromatographic separation of the amount of extractives from *Ligularia macrophylla* (Ledeb.) DC.

United factions	Weight, g	HPLC, %	IR spectrum, cm^{-1}	UV spectrum, nm	ml.p., ºC
LM 31-35	1.13	99.91	2953; 2938; 2884; 2874; 1773; 1681	216.0	136.3
LM 36-40	0.68	99.92	2954; 2938; 2884: 2874; 1772; 1681	215.0	137.1
LM 41-45	0.41	99.13	2954; 2938; 2884; 2874; 1771; 1682	216.0	137.0
LM 46-60	1.04	98.41	2954; 2939; 2884; 2874; 1772; 1683	216.0	135.7
Total:	3.25				

According to the IR, UV, ^{1}H and ^{13}C NMR, DEPT, COSY, HMQC, HMBS, and physicochemical constants, the compound was identified as the sesquiterpene lactone furanoeremophilan-14β,6α-olide (6).



(6)

When processing medicinal raw materials, one of the main problems is its rational use through complex processing with the most complete extraction of all valuable components.

To prepare pilot industrial regulations for the production of substances based on furanoeremophilan-14β,6α-olide (6) and determine the optimal parameters, a series of extractions of the roots of *Ligularia macrophylla* (Ledeb.) DC., collected in the vicinity of the Nurken village, Karkaraly district, Karaganda region in the rosette phase, using ethanol of

Table 4. NMR spectrum ^{1}H and ^{13}C (500 MHz, $CDCl_3$, ppm, J/Hz) of compound (6)

Atom number	δ^H	δ^C
1	1.59-1.45 (3H, m, H-1, H-2a, H-3a) 1.94-1.85 (2H, m, H-1b, H-3b)	18.86 (t)
2	1.59-1.45 (3H, m, H-1, H-2a, H-3a) 1.81-1.75 (1H, m, H-2b)	20.60(t)
3	1.59-1.45 (3H, m, H-1, H-2a, H-3a) 1.94-1.85 (2H, m, H-lb, H-3b)	25.35 (t)
4	2.32 (1H, dd, J=12.0, 3.0, H-4)	41.43 (d)
5	-	41.56 (s)
6	6.14 (1H, s, H-6)	81.80 (d)
7	-	120.21 (c)
8	-	150.92 (s)
9	2.70-2.60 (2H, m, H-9a, H-9b) 2.70-2.60 (2H, m, H-9b, H-9a)	23.26 (t)
10	2.28-2.22 (1H, m, H-10)	37.07 (d)
11	-	114.78 (s)
12	7.25 (1H, s, H-12)	138.70 (d)
13	1.26 (3H, s, CH_3-13)	8.39 (k)
14	-	176.90 (s)
15	2.02 (3H, s, CH_3-15)	20.16 (k)

various concentrations of 70%, 80% and 96% as an extractant (raw material: extractant ratio = 1:10), at temperatures of 35 °C, 55 °C and 70 °C; a duration of 3 h to study the influence of extraction mode factors on the yield of furanoeremophilan-14β,6α-olide (6) (Table 5).

Based on the obtained data, a regression analysis was carried out and a mathematical model of the dependence of the yield of the amount of extractives has been developed (Fig. 1).

The mathematical model was developed using the GNU Octave software system version 8.4.0.

The equation for the dependence of the yield of the sum of extractive substances on the extractant and the temperature, expressed as a percentage, has the following form:

$$M = -0.024519 * X_1^2 - 0.010308 * X_1 * X_2 + 0.028889 * X_2^2 + 3.761 * X_1 - 1.9214 * X_2 - 58.61$$

M – yield of the sum of extractive substances, X_1 – extractant concentration, X_2 – temperature.

As can be seen from the equation, the main factors influencing the yield of the sum of extractive substances of *Ligularia macrophylla* (Ledeb.) DC. are the concentration of the extractant (X_1) and temperature (X_2). As a result of optimization of the technological regime of extraction of *Ligularia macrophylla* (Ledeb.) DC., it was established that the quantitative yield of the sum of extractive substances is achieved at a temperature of 70 °C and the concentration of ethyl alcohol 70%.

The quantitative yield of furanoeremophilan-14β,6α-olide (6), according to HPLC data, is achieved at a temperature of 55 °C and an ethyl alcohol concentration of 96%.

Based on the developed mathematical model, a technological scheme for the isolation of furanoeremophilan-14β,6α-olide (6) from the roots of the *Ligularia macrophylla* (Ledeb.) DC. has been suggested. The main stages of the technological scheme for the production of furanoeremophilan-14β,6α-olide-substance from *Ligularia macrophylla* (Ledeb.) DC. are shown in Fig. 2.

Table 5. Quantitative content of furanoeremophilan-14β,6α-olide (6) in the total extractive substances of *Ligularia macrophylla* (Ledeb.) DC.

Experiment No.	Extragent	Temperature, °C	Yield of the sum of extractive substances, %	Quantitative content (6) according to HPLC data,%
1	96% ethanol	70 °C	11%	12.12
2	96% ethanol	55 °C	10%	19.82
3	96% ethanol	35 °C	7%	15.93
4	80% ethanol	70 °C	36%	7.75
5	80% ethanol	55 °C	18%	6.87
6	80% ethanol	35 °C	27%	5.21
7	70% ethanol	70 °C	43%	5.12
8	70% ethanol	55 °C	24%	3.7
9	70% ethanol	35 °C	28%	4.3

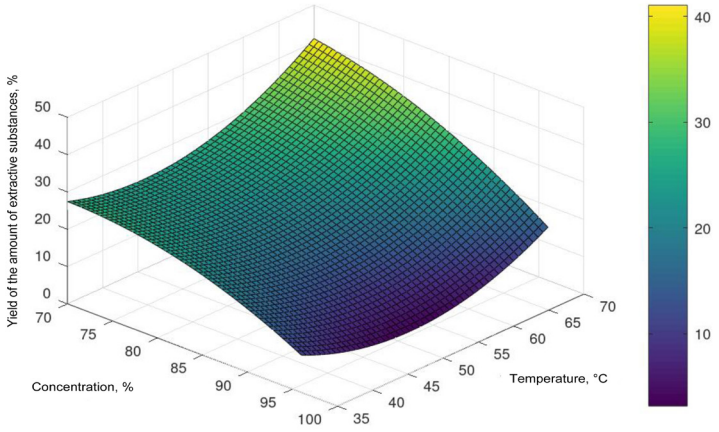


Fig. 1. Dependence of the yield of the amount of extractive substances on temperature and extractant concentration: 3D surface.

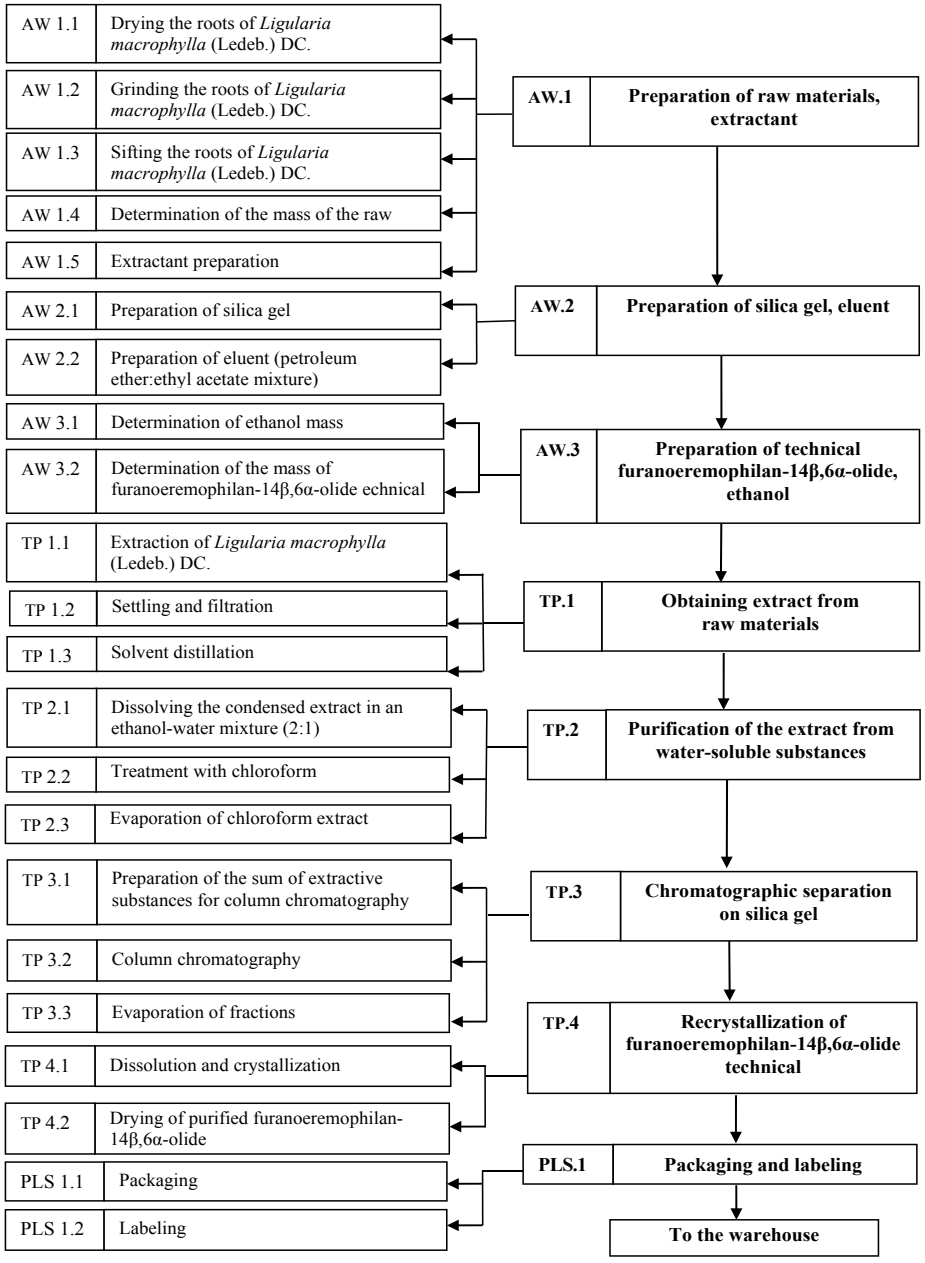


Fig. 2. Technological flow chart for the isolation of furanoeremophilan-14β,6α-olide from the roots of *Ligularia macrophylla (*Ledeb.) DC.

3.2 Biological activity

We performed biological screening of isolated samples of essential oils of *Ligularia macrophylla* (Ledeb.) DC. and furanoeremophilan-14β,6α-olide (6) for anti-inflammatory and antimicrobial activities and cytotoxicity.

Objects of the study:

1. Essential oil of *Ligularia macrophylla* (Ledeb.) DC.-(flower baskets, hydrodistillation)-(LMFHD);
2. Essential oil of *Ligularia macrophylla* (Ledeb.) DC.-(flower baskets, microwave extraction)-(LMFMWE);
3. Essential oil of *Ligularia macrophylla* (Ledeb.) DC. (leaves, hydrodistillation)-(LMLHD);
4. Essential oil of *Ligularia macrophylla* (Ledeb.) DC.-(leaves, microwave extraction)-(LMLMWE);
5. Essential oil of *Ligularia macrophylla* (*Ledeb.*) *DC.* (roots, hydrodistillation)-(LMRHD);
6. Essential oil of *Ligularia macrophylla* (Ledeb.) DC.-*Ligularia macrophylla* (Ledeb.) DC. (roots, microwave extraction)-(LMRMWE);
7. LM 36-40 is furanoeremophilan-14β,6α-olide (6).

3.2.1 Anti-inflammatory activity

Acute exudative reaction (peritonitis) was induced by intraperitoneal injection of 1% acetic acid solution in the volume of 1 ml per 100 g body weight of rats. After 3 h the animals were slaughtered, the abdominal cavity was opened, exudate was collected and its volume was estimated [15]. The study subjects were studied at a dose of 60 mg/kg when administered orally as an oil solution. The comparison drug diclofenac sodium was studied at a dose of 8 mg/kg. Control animals received an equivolume amount of sunflower oil. The studied objects were administered once 1 h before administration of 1% acetic acid solution. The results of the study of the anti-inflammatory activity of essential oil samples are given in Table 6.

The results of the experiment revealed that essential oil samples LMLMWE, LMFMWE, LMRHD, and LMLHD at doses of 60 mg/kg have anti-inflammatory activity on the model of acute exudative reaction.

3.2.2 Antimicrobial activity

The antimicrobial activity of the above samples of essential oils was studied in relation to strains of Gram-positive bacteria *S. aureus*, *B. subtilis*, to Gram-negative strain of *Escherichia coli* and to yeast fungus *C. albicans* by the method of diffusion in agar (wells). Comparison drugs were gentamicin for bacteria and nystatin for yeast fungus *C. albicans*.

Table 6. Anti-inflammatory activity of essential oil samples

Groups	Animal weight, g	Amount of exudate, ml
Control, n=6	383.0±19.2	5.1±0.4
Diclofenac sodium, n=6	371.7±16.9	3.5±0.6*
LMFHD, n=6	379.2±9.3	4.5±0.9
LMFMWE, n=6	377.7±5.3	3.3±0.8*
LMLHD, n=6	383.8±6.9	4.2±0.7*
LMLMWE, n=6	382.8±12.4	4.0±1.1*
LMRHD, n=6	382.2±7.6	3.7±1.0*
LMRMWE, n=6	385.0±9.1	5.7±0.5

*Note: * - p<0.05 compared to control*

Cultures were grown on liquid medium pH 7.3±0.2 at 30 to 35 °C for 18–20 h. The cultures were diluted 1:1000 in sterile 0.9% isotonic sodium chloride sterile solution, added 1 ml each into dishes with appropriate elective, nutrient media for the test strains under study and seeded using the 'solid lawn' method. After drying, 6.0 mm wells were formed on the agar surface, into which a solution of the test sample, gentamicin, and nystatin was added. In the control, ethyl alcohol in equivolume amounts was used. Thus, the test sample was tested in the amount of 1 μg and the comparison drug in the amount of 1 mg. The cultures were incubated at 37 °C and counting of growing cultures was done after 24 h. The antimicrobial activity of the samples was evaluated by the diameter of the zones of growth retardation of test strains (mm). The diameter of growth retardation zones less than 10 mm and continuous growth in the cup were evaluated as no antibacterial activity, 10–15 mm – weak activity, 15–20 mm – moderately pronounced activity, and over 20 mm – pronounced activity. Each sample was tested in three parallel experiments. Statistical processing was carried out by parametric statistics methods with the calculation of arithmetic mean and standard error. The results of the study of antimicrobial activity of essential oil samples are shown in Table 7.

It was found that the sample of essential oil from the roots of *Ligularia macrophylla* (Ledeb.) DC. obtained by microwave extraction has pronounced antimicrobial activity against Gram-negative test strain *E. coli*, and moderately pronounced activity against Gram-positive bacteria *S. aureus*, *B. subtilis*.

Table 7. Results of antimicrobial activity study

№	Name of sample	*S. aureus*	*B. subtilis*	*E. coli*	*C. albicans*
1	LMFHD	-	-	12±0.2	-
2	LMFMWE	11±0.2	-	12±0.1*	-
3	LMLHD	-	12±0.2	15±0.1	11±0.2
4	LMLMWE	-	-	14±0.2	-
5	LMRHD	16±0.1	17±0.2	16±0.1*	15±0.1
6	LMRMWE	19±0.1*	18±0.2	21±0.1*	12±0.1
Gentamicin		24±0.1	21±0.1	26±0.1	-
Nystatin		-	-	-	21±0.2

*Note: * - Significance of differences p<0.05 compared to the comparison group*

A sample of essential oil from the roots of *Ligularia macrophylla* (Ledeb.) DC. obtained by hydrodistillation showed moderate antibacterial activity against Gram-positive test strains of *S. aureus*, *B. subtilis* and Gram-negative microorganism *E. coli*.

3.2.3 Cytotoxicity

Determination of cytotoxicity was carried out on marine crayfish *Artemia salina*. The technique is based on the difference between the number of dead larvae of marine crayfish in the analysed sample (experiment) and water that does not contain toxic substances (control). The criterion of acute lethal toxicity of the substance solution is the death of 50% of larvae or more in the experiment compared to the control.

The flask was filled with artificial seawater and *Artemia salina* eggs were added. The flask was incubated for 3 days under mild air supply until the crustaceans hatched from the eggs.

Dilution was performed at the rate of 1 mg of substance per 1 ml of solvent. Each sample was tested in three parallel experiments. Conducted at temperature 20±2 °C, natural light period. Salinity of control artificial water was 8.0–8.5 (pH). *Artemia larvae* were up to 1 day old at the time of biotesting. The density of larvae planting was 20–40 specimens per one test tube.

Actinomycin D, staurosporine, and Paclitaxel-Teva were used as comparison drugs. The samples were tested with concentrations of 10, 5, and 1 mg/ml. The results of cytotoxic activity studies are shown in Table 8.

As a result of cytotoxic activity study, it was found that LM 36-40 substances at all concentrations exhibited cytotoxicity, larval mortality 50–65%.

Comparison drugs Actinomycin D, Staurosporine and Paclitaxel-Teva in relation to marine crustaceans *Artemia salina* in all concentrations shows cytotoxicity – mortality of larvae is 50–96%.

Thus, the results of the study suggest that furanoeremophilan-14β,6α-olide (6) in all concentrations exhibits cytotoxicity, larval mortality 50–65%.

The anti-inflammatory and antimicrobial activity of the essential oil of *Ligularia macrophylla* (Ledeb.) DC. is manifested due to the relatively high content of Δ^3-carene (1) [16].

4. Conclusion

Thus, for the first time, using the methods of hydrodistillation on a Clevenger apparatus and microwave extraction on a NEOS installation, essential oil substances were isolated and produced from the aerial and the underground parts of *Ligularia macrophylla* (Ledeb.) DC., the main component of which is Δ^3-carene (1) (24.6–35.3%) according to gas chromatography-mass spectrometry. Also essential oil from the aerial part of *Ligularia macrophylla* (Ledeb.) DC, contains cis-β-ocimene (2) (15.2–18.3%), undec-1-ene (3); the substance isolated from the roots contains β-bisabolene (4) (12.4–16.0%), β-myrcene (5) and an unidentified component with RI = 1849.9.

A mathematical model for the extraction of the raw materials of *Ligularia macrophylla* (Ledeb.) DC. has been developed for the first time, as well as the yield of the sum of extractives per air-dry raw material depending on the extractant and the temperature based on a second-order polynomial equation.

Based on the results of experiments, it was established that for the development of the pilot industrial regulations, the optimal technological regime for the production of an anti-inflammatory

Table 8. Results of cytotoxic activity studies

Investigated substances	Concentration, mg/ml	Number of larvae in the control		Number of larvae in the sample		% surviving larvae in control	% surviving larvae in sample	Mortality, %	Presence of neurotoxicity, %
		Survivors	Deceased	Survivors	Deceased				
Actinomycin D	10	25	1	0	29	96	0	96	0
	5	25	1	0	21	96	0	96	0
	1	25	1	6	25	96	19	77	0
Staurosporine	10	25	1	0	24	96	0	96	0
	5	25	1	0	28	96	0	96	0
	1	25	1	16	18	96	47	50	0
Paclitaxel-Teva	10	25	1	0	25	96	0	96	0
	5	25	1	0	22	96	0	96	0
	1	25	1	5	20	96	20	76	0
Furanoeremophilan-14β,6α-olide	10	25	1	11	13	96	46	50	0
	5	25	1	10	12	96	46	50	0
	1	25	1	13	15	96	46	50	0

and antioxidant substance from the roots of *Ligularia macrophylla* (Ledeb.) DC. the extraction should be with 96% ethanol at a raw material:extractant ratio = 1:10, temperature 55 °C, process duration within 3 h, providing a quantitative yield of the amount of extractive substances up to 10% based on air-dry raw materials, as well as a quantitative content according to HPLC data of furanoeremophilan-14β,6α-olide (6) in the extract – 19.82% based on the amount extractives.

Based on the obtained data, a technological scheme for the isolation of furanoeremophilan-14β,6α-olide (6) from the roots of *Ligularia macrophylla* (Ledeb.) DC., including 3 stages of auxiliary work and 4 stages of the technological process, was developed.

Based on the results of the experiments, it was revealed that the substance of the essential oil from the aerial part *Ligularia macrophylla* (Ledeb.) DC., extracted by microwave extraction, has a higher anti-inflammatory activity than the essential oil substance from the roots of the studied plant, isolated by hydrodistillation.

The anti-inflammatory activity of a sample of furanoeremophilan-14β,6α-olide was determined (6).

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