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COMPOSITION AND BIOLOGICAL ACTIVITY OF LIPIDS FROM LEAVES OF *Limonium gmelinii*

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Lipids of air-dried leaves of the hemi-halophyte plant Limonium gmelinii *(Willd.) Kuntze (Plumbaginaceae) growing on the dry bed of the Aral Sea were studied. The leaves contained neutral lipids (NL, 0.84% of the mass), glycolipids (GL, 1.78), and phospholipids (PhL, 0.74). The compositions of the lipophilic constituents of the NL and hydrocarbons were determined. Fatty acids of NL were dominated by 18:2n6 (53.45%); of GL and PhL, 16:0 (40.14 and 53.98%, respectively). The lipophilic constituents exhibited weak antifungal and high growth-stimulating and stress-protective activity under salt stress conditions.*

Keywords: *Limonium gmelinii*, leaves, lipids, lipophilic constituents, antifungal, growth-stimulating, and stress-protective activities.

Limonium gmelinii (Willd.) Kuntze (Plumbaginaceae) (sea lavender, Siberian statice) is an herbaceous perennial halophyte plant that grows on moist saline soils and saline depressions of European Russia, western Siberia, and Central Asia [1]. It is distributed in Uzbekistan in Karakalpakstan and Bukhara, Navoiy, Samarqand, Jizzakh, and Sirdaryo Regions [2]. This species of *Limonium* Mill. is a medicinal and forage plant and a good dye and ameliorant [3].

The decoction of *L. gmelinii* roots has been used for centuries in traditional medicine of Central Asia to treat inflammatory diseases of the throat and oral cavity, hemorrhages, internal and uterine bleeding, and diseases of the gastrointestinal tract [1, 4, 5]. The plant has been used since antiquity to tan leather and dye wool and as fodder [6]. Roots and rhizomes of *L. gmelinii* were included in the State Pharmacopoeia of the Republic of Kazakhstan as a pharmacopoeial raw material. Biologically active compounds from the subterranean organs are used as a base for anti-inflammatory and antiviral agents, ointments, syrups, and infusions with broad spectra of biological activity [5].

Tanning agents, saponins, polysaccharides, phenolic acids, amino acids, flavonoids, alkaloids, carotenoids, flavones, coumarins, polyphenols, and saponins were observed in the aerial part of *L. gmelinii* [1]. Tanning agents and oxidized flavonoids (mainly myricetin and its glycosides) were dominant [7]. The leaves contained organic acids, flavonoids, alkaloids, tanning agents, carotenoids, organic acids, amino acids, and carbohydrates [1, 4].

Lipids from leaves of sea lavender have been anecdotally reported in the literature. For example, the nonpolar hexane fraction of the aerial part of *L. gmelinii* was reported to contain 14 hydrocarbons (mainly paraffinic) with chain lengths C_{12}–C_{23}, phytol acetate (12.88%), and the higher alcohol 3,7,11,15-tetramethyl-2-hexadecan-1-ol (5.17%) [8]. Lipophilic constituents in lipids from the aerial part of *L. gmelinii* included β-sitosterol [9]. Thirteen fatty acids (FAs) from 12:0 to 22:0 with total unsaturated constituents dominating (80.32%), mainly 18:2n6 (58.1%), were identified in the EtOH (50%) extract of the aerial part [8].

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TABLE 1. Characteristics of Lipids from Leaves of *Limonium gmelinii*

Parameter	Content
Mass fraction moisture and volatiles, mass% of leaves	7.24
Total lipids, % of dry leaves	3.36
In total lipids, %	
neutral lipids	0.84
glycolipids	1.78
phospholipids	0.74
Lipophilic constituents, mass% of NL	7.50
Carotenoids in lipophilic constituents, mg%	35.85
Hydrocarbons, mass% of leaves	0.02

TABLE 2. Composition of Lipophilic Constituents from Leaves of *Limonium gmelinii*

Constituent	Content, mass%
Hydrocarbons	26.91
Carotenoids	0.04
Squalene	0.26
Triterpenols, aliphatic alcohols	28.48
Phytosterols	35.33
Xanthophylls, unidentified compounds	8.98

The petroleum ether fraction of the MeOH extract of the aerial part of *L. gmelinii* contained nine FAs, including high-molecular-mass saturated behenic (22:0) and lignoceric (24:0). Total acids were dominated by 16:0 (39.63%), 18:3n3 (22.98%), and 18:2n6 (19.01%). The extract exhibited fungicidal, antibacterial, and insecticidal activity [9]. Total lipids from chloroplasts of *L. gmelinii* leaves included 12 FAs with 18:3n3 making up 46.6% during the daytime [10].

The present article reports the compositions of lipids and lipophilic constituents from leaves of the hemi-halophyte plant *L. gmelinii* growing on the dry bed of the Aral Sea and the biological activity of the lipophilic constituents.

Total lipids (TL) with residual moisture 7.24% were extracted by the Folch method from air-dried leaves. TL were fractionated by column chromatography into neutral (NL), glycolipids (GL), and phospholipids (PhL). Alkaline hydrolysis showed that NL contained lipophilic constituents. Spectrophotometric methods determined the carotenoid contents in the lipophilic constituents. Furthermore, Table 1 presents the results for a hydrocarbon fraction obtained from a sample of leaves by a brief extraction with hexane.

Table 1 shows that the TL content in *L. gmelinii* leaves was small (3.62%) and consisted mainly of GL. TL included the isoprenoid hydrocarbon squalene, which according to our data is often observed in lipids of hemi-halophyte plant species of the family Amaranthaceae [11–13]. The constituent composition of the lipids was determined by TLC using solvent systems 1–6. The main classes of NL were triacylglycerides in addition to hydrocarbons; carotenoids; esters of aliphatic alcohols, triterpenols, and phytosterols; squalene; free phytosterols (main constituent); and triterpenols. GL consisted of esters of steryl glycosides, free steryl glycosides, and di- and mono-galactosyl diglycerides. Phosphatidylethanolamines, phosphatidylcholines, and phosphatidylinositols were identified in PhL. The main PhL were phosphatidylcholines and phosphatidylinositols.

Lipophilic constituents were separated by preparative TLC using solvent systems 1 and 3 to determine the composition. Table 2 presents the results.

Table 2 shows that triterpenols with aliphatic alcohols and phytosterols made up ~64% of the mass of lipophilic constituents, hydrocarbons, almost 27%. The hydrocarbon fraction was obtained by brief four-fold extraction with hexane of milled leaves. The extracts were combined and concentrated in a rotary evaporator. The composition of the hexane extract was studied by GC-MS (Table 3).

The results showed that the extract contained 10 saturated hydrocarbons with chain lengths C_{25}–C_{35} in addition to palmitic acid. The major constituents in the total were nonacosane (40.1%) and hentriacontane (30.2%). Heptacosane (11.3%) and tritriacontane (9.2%) were present in noticeable amounts. These data differed from those published in the literature.

TABLE 3. Hydrocarbons in Hexane Extract of Leaves of *Limonium gmelinii*, GC-MS

Constituent	RI	Content, %	Constituent	RI	Content, %
Pentacosane	2500	0.3	Triacontane	3000	1.6
Hexacosane	2600	0.3	Hentriacontane	3100	30.2
Heptacosane	2700	11.3	Dotriacontane	3200	1.1
Octacosane	2800	2.2	Tritriacontane	3300	9.2
Palmitic acid	2834	0.8	Pentatriacontane	3500	2.8
Nonacosane	2900	40.1	Total		99.9

TABLE 4. Composition of Fatty Acids in Lipids from Leaves of *Limonium gmelinii*, GC, mass% of Acids

Fatty acid	NL	GL	PhL	Fatty acid	NL	GL	PhL
10:0	0.72	–	0.10	18:2n6	53.45	5.94	8.05
12:0	1.18	5.85	2.09	20:0	0.46	0.74	0.56
14:0	2.25	7.76	3.57	20:1n9	1.20	2.62	0.95
15:0	0.20	0.67	0.25	22:0	3.54	1.19	0.96
16:0	7.85	40.14	53.98	22:1n9	1.94	–	–
16:1	1.66	0.41	1.73	24:0	2.17	0.65	0.83
17:0	Tr.	Tr.	0.69	24:1n9	0.20	1.36	–
18:0	2.64	7.78	6.79	26:0	0.25	–	0.36
cis-18:1n9, 18:3n3	18.96	23.07	17.38	$\Sigma_{sat.}$	21.26	64.78	69.82
trans-18:1n9	1.33	1.82	1.71	$\Sigma_{unsat.}$	78.74	35.22	30.18

TABLE 5. Effect of Pretreatment of Wheat and Cucumber Seeds with Lipophilic Constituents from Leaves of *Limonium gmelinii*

Expt. Version	Concentration, %	Wheat (length, cm)		Cucumber (length, cm)	
		roots	stems	roots	stems
Control	–	2.88 ± 0.584	1.70 ± 0.249	3.07 ± 0.409	1.41 ± 0.348
Floroxan	0.00001	3.24 ± 0.372	1.96 ± 0.782	3.79 ± 0.754	1.87 ± 0.501
Lipophilic constituents	0.001	3.19 ± 0.465	1.93 ± 0.380	3.75 ± 0.431	2.05 ± 0.463
	0.0001	2.30 ± 0.247	1.59 ± 0.395	4.01 ± 0.649	1.69 ± 0.329

For example, the nonpolar fraction obtained from the aerial part of *L. gmelinii* of the Kazakhstan population via extraction by hexane for 48 h with a 1:5 raw-material–extract ratio consisted of saturated hydrocarbons C_{12}–C_{23} with tricosane dominating (47.4%) [8].

Next, fatty acids (FAs) were isolated from the lipids by producing their methyl esters (FAMEs). The composition of the FAMEs was analyzed by GC. Table 4 presents the results.

Peaks for the FAMEs *cis*-18:1n9 and α-18:3 were not separated under the used GC analysis conditions. Therefore, total FAMEs of NL were also analyzed by TLC on silica gel with 30% $AgNO_3$ in benzene as described before [11]. The results showed that the FAMEs included acids *trans*-18:1n9 (R_f 0.65), *cis*-18:1n9 (R_f 0.55), 18:2n6 (R_f 0.46), and 18:3n3 (R_f 0.40). Acid *trans*-18:1n9 was identified in all lipid groups.

Table 4 shows that *L. gmelinii* leaf lipids contained 19 (NL), 15 (GL), or 16 (PhL) FAs. The major saturated FA in all lipid groups was 16:0. Its content in NL was 7.85%; in PhL, almost 54%. The unsaturated acids were dominated in NL by 18:2n6 (53.45%); in GL and PhL, by total *cis*-18:1n9 and 18:3n3 (23.07 and 17.38%, respectively). The greatest total unsaturated FAs was found in NL (78.74%) whereas it was less than half that value in other lipid groups. FAs 20:2, 21:0, 17:1, 18:1n11, and 22:2, which were found in *L. gmelinii* leaves of Kazakhstan [7] and Russian origin [10], were not detected.

Biological Activity. The goal of the research was to find the antifungal activity of *L. gmelinii* lipophilic constituents against the phytopathogenic fungi *Fusarium oxysporum* Schrf. and *Aspergillus niger* and their growth-stimulating and stress-protective activities.

Antifungal Activity. Weak antifungal activity was found according to research results for lipophilic constituents from *L. gmelinii* leaves at a concentration of 0.5%. The growth inhibition zone against *F. oxysporum* was 0.27 cm; against *A. niger*, 0.16 cm. The growth inhibition zones of tebuconazole against the fungi were 17.5 cm for *F. oxysporum* and 15.5 cm for *A. niger*.

Growth-Stimulating and Stress-Protective Activities. The research showed that the lipophilic constituents from *L. gmelinii* at a concentration of 0.01% inhibited the growth of wheat and cucumber sprouts (Table 5).

Two studied concentrations, 0.001% and 0.0001%, exhibited stimulatory activity on both cultures. However, the maximum activity was found for the former, at which the lengths of wheat roots exceeded those of control plants by 10.8% (3.19 cm); stems, by 13.5% (1.93 cm). These parameters for growth of cucumbers were 3.75 and 2.05 cm, respectively, and exceeded the controls by 22.1 and 45.4%.

Thus, the lipophilic constituents from *L. gmelinii* leaves at a dose of 0.001% possessed high growth-stimulating and stress-protective activities under saline conditions.

EXPERIMENTAL

GC-MS Study of Hexane Extract. The analysis used an Agilent 5975C inert MSD/7890A GC with an Agilent HP-INNOWax capillary column (30 m × 250 μm × 0.25 μm) in temperature regime 60°C (2 min), 4°C/min to 220°C (10 min), and 1°C/min to 240°C (20 min). The injected sample volume was 1.0 μL. The mobile phase (H_2) flow rate was 1.1 mL/min. The vaporizer temperature was 220°C; ion-source, 230°C. EI-MS spectra were measured in the range *m*/*z* 45–550 amu. Constituents were identified by comparison of mass spectral characteristics with data in electronic libraries W9N11.L (Wiley Registry of Mass Spectral Data, 9th Ed., NIST Mass Spectral Library, 2011) and retention indices (RI) of the compounds determined relative to retention times of a mixture of *n*-alkanes (C_9–C_{36}). The quantitative content of constituents was calculated from chromatographic peak areas. GC of FAMEs was performed as before [12]. Spectrophotometric determination of carotenoids used a Cary 60 spectrophotometer (Germany). Column chromatography of total lipids used Chemapol silica gel of particle size 100/160 μm; TLC, the same silica gel of particle size 5/40 μm [12].

The solvent systems were hexane–Et_2O (4:1, 1; 7:3, 2; 3:2, 3); heptane–C_6H_6 (9:1, 4); $CHCl_3$–Me_2CO–MeOH–AcOH–H_2O (65:20:10:10:3, 5), and $CHCl_3$–MeOH–NH_4OH (65:35:5, 6). Lipids were identified based on model compounds and qualitative reactions with specific reagents. Spots of NL were detected in I_2 vapor and by spraying plates with aqueous H_2SO_4 solution (50%) followed by heating; GL, by α-naphthol; PhL, by Vaskovsky and Dragendorff's reagents [14].

Lipophilic compounds were isolated by the literature method [15] and were identified using the Liebermann–Burchard qualitative reaction for triterpenols and sterols and model samples of alcohols obtained by us earlier from natural sources.

The isolation of FAs, preparation of their methyl esters, and analysis of FAMEs by GC have been described [11]. FAs were identified by comparing retention times of peaks with those of peaks of a standard sample mixture of 37 FAMEs (Supelco® 37 Component FAME Mix, Sigma-Aldrich, USA).

The hydrocarbon fraction was obtained via extraction (4 ×) by hexane at room temperature with periodic shaking for 4 h for each extraction with a 1:4 milled-leaves–hexane ratio. The extracts were combined and concentrated in a rotary evaporator.

The antifungal activity of the lipophilic constituents of *L. gmelinii* was determined by the disk diffusion method [16]. The standard was the triazole fungicide tebuconazole, which is used in agriculture to protect field and grain crops from pathogenic fungi [17]. The phytopathogenic fungi were *F. oxysporum* and *A. niger* [12].

The growth-stimulating and stress-protective activities were studied under laboratory conditions by the Rakitin and Rudnik method [18]. Control wheat and cucumber seeds were wetted in water; test seeds, in solutions of the tested compounds at various concentrations for 18 h. Wetted seeds were spread on filter paper in Petri dishes, treated with tap water or NaCl solution (1%), and cultivated at 25–27°C. The lengths of sprout roots and heights of stems were measured on the fifth day. The standard was the synthetic growth regulator floroxan. Test results were mathematically processed using the Origin Pro computer program [19].

The plant was collected during flowering on the dry bed of the Aral Sea in 2021.

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