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COMPOSITION AND ANTIFUNGAL ACTIVITY OF LIPIDS FROM SEEDS OF *Atriplex tatarica*

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Atriplex tatarica L. (*A. laciniata* L., *A. arenaria* J. Woods; Tatarian orache; Amaranthaceae) is a broadly distributed weedy, medicinal, and food plant that grows in northern Africa, western and Central Asia, and Europe. It is found in Uzbekistan in Tashkent, Namangan, Andijan, and other regions and in Karakalpakstan [1]. The plant is a xero-halophyte species growing in weakly and moderately salty soils [2]. Many halophytes hold economic interest as feed, oil, and medicinal plants and promote phytoremediation and amelioration of degraded grasslands [3]. *A. tatarica* was recommended for introduction to culture for restoring saline sandy clay and sandy soils [4].

Decoctions of *A. tatarica* leaves are used in folk medicine for jaundice and as a diuretic; the seeds, as an emetic and laxative [5]. Extracts of plants of the genus *Atriplex* showed antiparasitic, insecticidal, antimicrobial, cytotoxic, and antioxidant activity [6].

Various organs of representatives of the genus *Atriplex* afforded main constituents such as flavonoids, saponins, alkaloids, and smaller amounts of betaines, amino acids, ascorbic acid, and steroids. Derivatives of flavonoids and saponins from *A. tatarica* exhibited antibacterial activity against the pathogenic bacterium *Pseudomonas aeruginosa* [7]. Lipids from plants of this genus, including *A. tatarica*, are practically unstudied. It is only known that seeds of *A. griffithii* contained 13.5% lipids with 18.6% α-linolenic acid (18:3n3) [8].

The goal of the present work was to determine the compositions of lipids and fatty acids from *A. tatarica* seeds and to test unsaponified substances obtained from neutral and polar lipids for antifungal activity.

Extraction by petroleum ether of air-dried ground seeds isolated neutral lipids (NL). The pulp was dried in air. Polar lipids (PL) were extracted from it. Then, nonpolar impurities were removed as before [9]. Seeds were found to contain moisture and volatile compounds. NL were analyzed for acid number and content of free fatty acids and squalene because this dihydrotriterpene hydrocarbon with high biological activity is characteristic of plants in the family Amaranthaceae [10–12]. NL were first hydrolyzed by alcoholic base because of their light-yellow color. Unsaponified substances (US) were extracted from the hydrolysate. According to photoelectrocalorimetry, they contained carotenoid pigments. PL were fractionated by column chromatography (CC) into glycolipids (GL) and phospholipids (PhL).

Table 1 shows that the content of total lipids (NL and PL) in *A. tatarica* seeds was 6.68%; of squalene and NL, a modest 0.35%; free fatty acids, 0.75%.

The qualitative compositions of NL, GL, PhL, and US constituents were established by analytical TLC over silica gel and Silufol plates using various solvent systems as before [9]. According to the analytical results, NL consisted mainly of triacylglycerides and smaller amounts of hydrocarbons, fatty-acid esters with phytosterols and triterpenols, squalene, free triterpenols, and phytosterols. Phytosterols (main class), hydrocarbons, carotenoids, squalene, fatty aliphatic alcohols, and triterpenols were identified in US.

The compositions of GL and PhL were established by TLC on silica gel as before [9]. GL were dominated by steryl glycosides and minor amounts of monogalactoyl- and digalactoyldiacylglycerides. The principal constituents of PhL were phosphatidylcholines, phosphatidylethanolamines, phosphatidylinositols, and traces of phosphatidic acid.

Acidic methanolysis of NL isolated fatty-acid methyl esters (FAME). Alkaline hydrolysis of GL and PhL and treatment of the isolated FA with diazomethane produced FAME that were analyzed by GC under the published conditions [13].

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TABLE 1. Parameters of Lipids from Seeds of *Atriplex tatarica*

Parameter	Content	Parameter	Content
Moisture and volatiles, % of seed mass	8.00	Unsaponified substances (US), % of NL mass	7.64
NL yield with actual moisture, % of seed mass	5.26	Carotenoids, mg% of US mass	35.93
NL yield per abs. dry substance, % of seed mass	5.71	Polar lipids (PL), % of seed mass, including	0.97
Squalene content, % of NL mass	0.35	glycolipids	0.44
NL acid number, mg KOH/g	1.51	phospholipids	0.53
Free fatty acids, % of NL mass	0.75		

TABLE 2. Fatty-acid Composition of Lipids from Seeds of *Atriplex tatarica*, GC, % of Acid Mass

Acid	RT, min	NL	GL	PhL
10:0	3.030	0.02	0.26	0.14
12:0	5.565	0.05	0.97	0.38
14:0	9.618	0.38	1.34	1.06
15:0	11.897	0.10	0.61	0.68
16:0	14.220	15.89	41.10	50.34
16:1	13.660	0.21	–	0.21
17:0	16.459	0.11	0.63	0.69
18:0	18.684	1.39	8.74	6.27
cis-18:1n9, 18:3n3	18.120	45.73	–	–
cis-18:1n9	18.120	–	13.28	15.44
trans-18:1n9	18.318	0.07	1.82	0.67
18:2n6	17.981	23.08	22.93	15.86
20:0	22.909	0.66	1.34	0.91
20:1n9	22.362	2.24	3.63	2.06
22:0	26.815	1.54	1.64	2.90
22:1n9	26.321	6.54	–	0.90
24:0	30.440	0.59	1.63	1.49
24:1n9	30.007	0.62	–	–
26:0	34.245	0.78	–	–
$\Sigma_{sat.}$		21.51	58.34	64.86
$\Sigma_{unsat.}$		78.49	41.66	35.14

FAME were identified as before [9]. The presence in the FA of three groups of elaidic-acid (*trans*-18:1n9) lipids was confirmed by IR spectra and Ag^+-TLC; of 18:3n3 in FA of NL, by Ag^+-TLC as before [13]. Table 2 presents the analytical results.

Table 2 shows that NL contained 18 esterified FA, where almost 46% were *cis*-18:1n9 and 18:3n3. The total of these FA and 18:2n6 was 68.8%. Constituents 24:1n9 and 26:0 were observed only in NL acids. Acid 22:1n9 amounted to 6.54%. FA of GL (14 acids) and PhL (16 acids) were dominated by 16:0 (41.1 and 50.34%, respectively). Like for reserve lipids of *Amaranthus retroflexus* [9] and *Chenopodium album* [13] (Amaranthaceae), FA of NL, GL, and PhL of *A. tatarica* contained minor amounts of *trans*-oleic acid (*trans*-18:1n9, elaidic).

Biological Activity of *A. tatarica* Lipids. Several FA (12:0–18:0, 18:1, 18:2, and 18:3) are known to exhibit antibacterial and antifungal activity [14]. The antifungal activity of FA depends on the length of their hydrocarbon chain and the presence of unsaturated bonds and oxygenated functional groups. It was found that oxygenated FA 9,10-epoxy-18:1(12*Z*), 9,10-epoxy-18:2(12*Z*,15*Z*), (+)-9-OH-18:2(10*E*,12*Z*), and 9-OH-18:3(10*E*,12*Z*,15*Z*) synthesized in leaves of *Oryza sativa* resistant to infection suppressed the growth and development of the pathogenic fungus *Pyricularia oryzae* and formed phytoimmunity in the plant [15, 16].

Antifungal activity was also observed in lipophilic extracts of several plants. The petroleum ether extract from leaves of plants of the genus *Pistacia* exhibited antifungal activity against three pathogenic fungi of agricultural crops, i.e., *Pythium ultimum*, *Rhizoctonia solani*, and *Fusarium sambucinum* [17]. The hexane and EtOAc extracts of *Chenopodium album*

seeds in *in vitro* experiments suppressed the growth of phytopathogenic fungi *Alternaria alternata* and *Bipolaris sorokiniana* [18]. It was thought that new biofungicides against grain-crop diseases could be designed based on these extracts [18].

Previously, lipids from seeds of *A. retroflexus* and *C. album* and their biological activity were studied by us. It was found the US and FA isolated from NL and their methyl esters possessed cross-protective activity under NaCl stress conditions on cucumber, wheat, and cotton sprouts [9, 13].

The goal of the biological studies of *A. tatarica* lipids was to determine the antifungal activity of seed US and PL against the phytopathogenic fungi *Fusarium oxysporum* Schrf. and *Aspergillus niger*. Antifungal activity was determined by the paper-disk method from the size of the growth inhibition zone of the fungi on the 5th and 7th days [19]. The negative control was MeOH. The standard was the triazole fungicide tebuconazole, which is used in agriculture to protect field and grain crops from pathogenic fungi [20].

The research results demonstrated weak antimicrobial activity of the compounds. The activity of US against *F. oxysporum* was slightly greater than that of PL. The growth inhibition zone on the 5th day was 0.43 cm; on the 7th day, 0.38 cm. The inhibition zone of *A. niger* at the first timepoint was 0.17 cm. Then, the fungal mycelium grew. PL showed significantly low activity. The parameters for *F. oxysporum* were 0.16 cm on the 5th day and 0.05 cm on the 7th day. PL did not exhibit antifungal activity against *A. niger*.

Isolation of lipids, their analysis by TLC, CC of PL, and quantitative HPLC analysis of squalene were performed as before [9, 13].

Carotenoid contents in US were determined on a KFK-2 UKhL4.2 photoelectrocalorimeter. The FA content in NL was calculated based on the acid number [9].

Acid methanolysis of NL used the literature method [21]. The obtained FAME were purified of impurities by TLC on silica gel using hexane–Et_2O (8:2).

Determination of Antifungal Activity. Strains of *F. oxysporum* and *A. niger* were isolated from plant raw material using literature methods [22]. Isolates were identified by cultural and morphological signatures [22, 23]. The surface of potato-dextrose agar in Petri dishes was inoculated with the test cultures. MeOH containing Tween-80 (0.05%) was added to lipids (0.03–0.035 g). The mixture was shaken until an emulsion formed. The lipid emulsion (10 μL, 10 μg of studied material) was placed onto 10-mm paper disks and dried in air. Then, the disks were placed into the Petri dishes on the surface of the growth medium [19]. The dishes were placed into a thermostat and incubated at 25–28°C. Biological and analytical tests were conducted in triplicate. Test results were processed mathematically by dispersion analysis using the Origin Pro computer program [24].

Seeds of *A. tatarica* were collected when fully ripe in Tashkent Region in 2019.

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