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LIPIDS AND FLAVOLIGNANS FROM *Silybum marianum*

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Lipids from the air-dried aerial part (AP) and seeds of Silybum marianum *(L.) Gaerth. (Asteraceae) were studied. The class and fatty acid compositions of neutral lipids (AP, seeds) and glyco- and phospholipids (AP) were determined. Neutral lipids (NL) with a complicated set of lipophilic components, mainly triterpenols, sterols, and their esters predominated in the AP. The fatty acids of the AP were dominated by 16:0, 18:2 (glycolipids), and 18:3 (neutral lipids, phospholipids); of seed NL, by 18:2 and 18:1. The content and composition of flavolignans isolated from defatted seeds and the content of total protein in the meal were found.*

Keywords: *Silybum marianum* (Asteraceae), milk thistle, lipids, aerial part, seeds, fatty acids, flavolignans.

Milk thistle [*Silybum marianum* (L.) Gaerth., Asteraceae] is a biennial (in nature) or annual (in cultivation) herbaceous weed that inhabits many regions of the world including oases in southern Uzbekistan [1, 2]. Seeds of milk thistle contain 28–34% fat [3, 4] and about 0.1% essential oil, biogenic amines, flavonoids, macroscopic and trace elements (predominantly Se), vitamins (A, D, F, E, K, and B complex), and other components [1, 4, 5].

The principal flavonoids of *S. marianum* seeds are flavolignans (up to 4%) with associated minor flavanonols. Medicinal preparations (carsil, siliborum, legalon, etc.), biologically active food additives, and hepatoprotective and antioxidant herbal teas are based on flavonoids of the ripe plant fruit [5]. Milk thistle fatty oil also exhibits hepatoprotective action and simultaneously regenerative and membrane-protective properties [6]. Root and juice of the aerial part (AP) are used in folk medicine to treat various diseases [1].

The content of lipophilic components and the composition of fatty acids of total lipids from the AP [7] in addition to certain physicochemical indicators and the fatty acid composition of seed oil from *S. marianum* [6] growing outside the republic were reported. Lipids of the AP and seed oil of Uzbekistan *S. marianum* are practically uncharacterized.

We investigated lipids of the air-dried AP of *S. marianum* collected at the start of flowering (2009 season) in Kashkadarya Oblast of Uzbekistan and oil (free lipids) from ripe seeds in order to study comprehensively the lipids from this plant.

Total Lipids from the AP. The AP (leaves, stems, heads) had 8.6% moisture. Total lipids were extracted from the AP by $CHCl_3$:MeOH. Non-lipid impurities were removed from the crude extract to afford lipids (3.9% yield of the absolute dry mass). The lipids were dark green and contained chlorophylls (1330 mg%) and carotinoids (37.5 mg%).

Lipids were separated into groups using preparative TLC on silica gel and systems 2 and 7 to produce neutral (NL) (43.6%), glyco- (GL) (37.6%), and phospholipids (PL) (18.8% of the lipid mass). The NL were green; GL and PL, brown.

The component composition of NL was analyzed by TLC on silica gel and Silufol plates using systems 1–6. Model compounds, specific detectors, and literature data were used to identify the spots. The following lipid classes were found: paraffins, olefinic and isoprenoid hydrocarbons, fatty acid (FA) esters with triterpenols and sterols; triterpenols, sterols, triterpene acids, and their acetates; triacylglycerins; free FA, chlorophylls a and b, pheophytins a and b, carotenes, and xanthophylls. The quantitative compositions of the NL classes according to PTLC were:

Lipid class	*Percent of NL*
Parafinic, olefinic, and isoprenoid hydrocarbons, FA esters with triterpenols and sterols	26.6
Acetates of triterpenols, sterols, and triterpene acids	22.4
Triacylglycerins, free FA	9.40
Triterpenols	15.7
Sterols, chlorophylls, pheophytins	13.6
Triterpene acids, chlorophylls, pheophytins	12.3

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TABLE 1. Composition of Lipid Fatty Acids from the Aerial Part and Seeds of *Silybum marianum*, %, GC

Fatty acid	Lipids, aerial part				Lipids, seeds
	total	NL	GL	PL	NL
10:0	1.2	1.7	1.1	2.9	0.3
12:0	2.1	1.9	1.3	3.0	0.1
14:0	5.2	6.7	2.1	8.3	0.2
15:0	1.1	1.2	0.9	1.7	Tr.
16:0	34.3	28.5	40.4	33.9	9.7
16:1	1.8	3.4	1.6	4.7	0.6
18:0	7.2	7.2	8.1	4.3	3.2
18:1	13.0	7.7	17.8	9.9	29.8
18:2	6.2	4.2	8.4	9.7	43.5
18:3	15.2	19.8	11.4	21.6	6.9
20:0	2.1	3.9	6.9	Tr.	2.6
22:0	3.9	5.9	Tr.	Tr.	3.1
24:0	6.7	7.9	Tr.	Tr.	–
$\Sigma_{sat.}$	63.8	64.9	60.8	54.1	19.2
$\Sigma_{unsat.}$	36.2	35.1	39.2	45.9	80.8

Tr.: traces.

The results showed that NL of the AP of *S. marianum* consisted primarily of biologically active components such as sterols, triterpenols, triterpene acids, and their ester including acetates. Several flavolignans as the acetates were also present in seeds of milk thistle [5].

Total lipids were hydrolyzed by methanolic KOH (10%). Unsaponified substances were isolated in 27.6% yield (of the lipid mass). The aforementioned hydrocarbon classes, isoprenoid alcohols, and triterpene acids were identified in them. The content of carotinoids in the unsaponified substances was 136.0 mg%.

GL were separated by TLC on silica gel using system 8. The following components characteristic of higher plant photosynthetic tissues were identified: sulfolipids, digalactosyldiglycerides, cerebrosides, sterylglycosides, and monogalactosyldiglycerides. The principal GL classes were digalactosyldiglycerides and sterylglycosides. The PL of the AP consisted of phosphatidylcholines, phosphatidylethanolamines, and phosphatidic acids according to two-dimensional TLC on silica gel using systems 9 and 10.

Alkaline hydrolysis of the lipids isolated FA, which were converted to methyl esters by treatment with diazomethane. The composition was analyzed by GC. Table 1 presents the FA composition of lipids from the AP.

Seed Lipids. Ripe seeds of milk thistle were extracted with benzine in order to compare lipids from this plant. NL (oils) were isolated in 23.7% yield (of absolute dry mass). Seed NL were light yellow and consisted (TLC, systems 1–6) of mainly triacylglycerins. Hydrocarbons, free FA, sterols, triterpenols, and triterpene acids were also identified. There were no acetates of cyclic alcohols and acids. A spot of compounds with R_f 0.52 (system 6) was not identified.

The acid number of NL was 1.9 mg KOH. The content of unsaponified substances in seed NL was 0.72%. The unsaponified substances contained hydrocarbons, sterols, triterpenols, and triterpene acids. Table 1 presents the composition of seed NL FA.

The results showed that NL of the AP of milk thistle differed from those of seeds by the presence of a high content (22.4%) of acetates of sterols, triterpenols, and triterpene acids. The component compositions of NL FA from the AP and seeds were similar and characteristically had 13 components (Table 1). However, significant differences were observed in the amounts of individual FA. The NL from the AP had 1.8 times more saturated medium- (12:0 and 14:0) and high-molecular-weight (16:0 and 24:0) FA than those from seeds. The unsaturated components of these same lipids contained almost three times more 18:3 acid.

The NL of seeds consisted primarily of 18:1 and 18:2 acids (73.3%). The level of 16:0 acid was less than 10%. The contents of 18:0, 20:0, and 22:0 acids were about the same (2.6–3.2%). Seed oil of milk thistle growing outside of Uzbekistan in more northern regions contained just as much 18:0 acid (3.5–4.0%) and slightly less 18:1 (21.0–22.0%). However, as expected [8], it had 1.4 times more 18:2 acid (61.0–62.0%) [6].

Biologically active flavolignans that are recovered by extraction with aqueous EtOH are known to remain in the meal after isolation of oil by pressing *S. marianum* fruit [9]. After isolating free lipids from seeds, we extracted the meal with EtOH

(80%). Flavolignans were isolated in 1.8% yield (of seed mass) from the resulting extract. The UV spectrum of the EtOH solution of flavolignans showed an absorption maximum λ_{max} at 288.15 nm that is characteristic of this group [5]. Separation of the flavolignans by TLC on Silufol plates using system 11 detected two principal spots that corresponded to silybin (R_f 0.75) and silychristin (R_f 0.50) [5].

The meal remaining after isolation of seed oil was analyzed for nitrogen content. The result was 6.8% N, which gave 42.5% when calculated for total protein. This highly proteinaceous waste is promising as a food additive.

Thus, the AP of *S. marianum* inhabiting Uzbekistan contained about 4% total lipids with a slight predominance of NL of complicated composition and a high level of acetates of sterols, triterpenols, and triterpene acids. Seeds of the plant from Uzbekistan had slightly less fatty oil (about 24%) and flavolignans (2%) [3, 4] but more protein [9] than seeds of milk thistle from European regions.

EXPERIMENTAL

GC of FA methyl esters was performed on a Chrom-5 instrument under the published conditions [10]. The carotinoid content was determined on a KFK-2-UKL 4.2 photocolorimeter as before [11]; chlorophylls, on an SF-46 instrument by spectrophotometry [12].

Lipids were analyzed by TLC on silica gel and Silufol UV-254 plates (Czech. Rep.) using solvent systems benzine:Et_2O (3:7, 6:4, 1:1, 95:5; 1–4); benzine:acetone (4:1, 5); heptane:MEK:HOAc (43:7:0.5, 6, double development); acetone:toluene:HOAc:H_2O (60:60:2:1, 7); $CHCl_3$:acetone:MeOH:HOAc:H_2O (65:20:10:10:3, 8 [13]); $CHCl_3$:MeOH:NH_4OH (7 N) (65:35:5, 9); $CHCl_3$:MeOH:HOAc:H_2O (14:5:1:1, 10); and CCl_4:MeCN (6:4, 11 [5]).

Spots of compounds were detected using iodine vapor and H_2SO_4 (50%) with subsequent heating. Known components of plant lipids [14] and qualitative reactions with specific reagents [15] were used to identify compounds.

Qualitative analysis of chlorophylls and carotinoids was performed using TLC on silica gel and hexane:acetone:benzene:isopropanol (69.5:25:4:1.5 [16]).

Total lipids from the air-dried raw material were extracted by $CHCl_3$:MeOH (2:1, v/v) by the published method [15]. Non-lipid impurities were removed from the extracts by washing with aqueous $CaCl_2$ solution (0.04%).

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