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LIPIDS FROM FLOWERS AND LEAVES OF *Artemisia annua* AND THEIR BIOLOGICAL ACTIVITY

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Chemical, chromatographic, and spectral methods were used to show that the main components of the lipid extract of flowers and leaves are free and bound aliphatic and cyclic alcohols (sterols and triterpenols) and essential oil. It was shown that the lipid extract of Artemisia annua *has a positive influence on skin metabolism and possesses anti-inflammatory activity.*

Key words: *Artemisia annua*, hydrocarbon extract, polar lipids, fatty acids, skin metabolism, anti-inflammatory activity.

Artemisia annua L. (annual wormwood, Asteraceae) grows in Central Asia, Siberia, Europe, North Africa, and America [1]. The antimalarial preparation artemisinin is isolated from its aerial part [2]. Furthermore, annual wormwood is a source of essential oil, the content of which is highest during flowering, ~0.7% of the dry mass [3].

The plant lipids are practically unstudied. Only the isolation and identification of four lipophilic components have been reported [4]. Data on the pharmacological properties of these lipids are also lacking.

Neutral lipids were isolated from the air-dried flowers and leaves by hydrocarbon (bp 75-80°C) extraction. Then, polar lipids were extracted from the remaining pulp by $CHCl_3$:CH_3OH (2:1 by vol). The $CHCl_3$:CH_3OH extract was purified of nonlipid components by washing with aqueous $CaCl_2$ (0.05%). The yield of neutral lipids was 4.3% of the air-dried mass; of polar, 4.2%.

The hydrocarbon extract was brown and thick and had a characteristic wormwood odor. The carotinoid content was 240 mg%. The acid number of the extract was 1.5 mg KOH.

Essential oil was separated from the hydrocarbon extract by steam distillation. The yield was 13.0% of the extract mass and ~0.55% of the dry mass. We have previously reported the composition of the essential oil [5].

The total substances remaining after removal of essential oil were extracted from the aqueous layer by diethylether. The ether was removed. The extracted substances were separated by column chromatography over silica gel into individual fractions, extracting them with hexane with a gradually increasing concentration of diethylether from 0 to 100%. Substances were identified by TLC on silica gel using solvent systems 1 and 2 and comparison with model samples of lipids and lipophilic components, qualitative reactions, GC, and mass spectrometry. The contents of the substances were determined gravimetrically (Table 1).

It can be seen that paraffins and olefinic hydrocarbons dominate the extract. According to mass spectrometry, they include saturated components of the C_{32}-C_{20} series (*m*/*z* 450-282 $[M]^+$), monoenes C_{38}-C_{20} (*m*/*z* 532-280 $[M]^+$), dienes and trienes C_{38}-C_{28} (*m*/*z* 530-390, 528-388 $[M]^+$), tetraenes C_{38}-C_{27} (*m*/*z* 526-372 $[M]^+$), and pentaenes C_{38}-C_{30} (*m*/*z* 524-412 $[M]^+$).

The fraction of esters of aliphatic and cyclic alcohols consisted according to mass spectrometry of a combination of saturated fatty acids of the series 24:0-10:0 and unsaturated 18:1, 18:2, and 18:3 with aliphatic alcohols of the series C_{30}-C_{16}, stigmasterol, β-sitosterol, and α- and β-amyrins.

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TABLE 1. Composition and Content of Lipids and Lipophilic Components of the Hydrocarbon Extract of *Artemisia annua* Leaves and Flowers

Lipids and lipophilic components	Content, % of extract mass
Hydrocarbons:	
olefins and paraffins	25.5
isoprenoids (including squalene, carotinoids)	0.6
Esters of aliphatic and cyclic alcohols	11.8
Triterpenol acetates	7.0
Triacylglycerides and free fatty acids	3.7
Aliphatic and triterpene alcohols	18.7
Sterols	11.2
Unidentified components	21.5

Total alkaline hydrolysis of the esters isolated the fatty-acid and alcohol fractions. Alcohols were separated into three bands by preparative TLC on silica gel using solvent system 2: aliphatic alcohols, triterpenols, and sterols. The composition of the components in each band was established by GC (SE-30) and comparison with model samples obtained from lipids of *Cousinia severtzovii* seeds [6].

The fraction of aliphatic alcohols contained the following components (mass %): C_{16}, 0.7; C_{17}, 1.4; C_{18}, 8.8; C_{19}, 4.0; C_{20}, 23.0; C_{21}, 1.6; C_{22}, 3.6; C_{23}, 1.8; C_{24}, 15.5; C_{25}, 0.4; C_{26}, 22.7; C_{27}, 0.4; C_{28}, 14.0; C_{29}, 0.5; C_{30}, 1.6.

The triterpenol fraction had the following components (mass %): β-amyrin, 33.7; α-amyrin, 58.9; unidentified, 5.6 (with retention time 1.26 for β-sitosterol) and 1.8 (retention time 1.37).

Sterols isolated from esters contained two components: stigmasterol (30.8%) and β-sitosterol (69.2%).

The mass spectrum of triterpenol acetates contained peaks with m/z (I_{rel}, %): 468 (11.1) $[M]^+$, 453 (6.3), 408 (3.2), 393 (4.1), 249 (7.9), 218 (100), 203 (44.4), 189 (25.4), which are consistent with the presence of amyrins acetates [7].

The free-sterol fraction consisted of two components: stigmasterol (m/z 412 $[M]^+$) and β-sitosterol (m/z 414 $[M]^+$), the contents of which according to GC (SE-30) were 74.7 and 25.3%, respectively. Thus, the main component of the sterol fraction is stigmasterol, which is rarely found in plant lipids. The content of sitosterol usually predominates significantly.

Fatty acids of the hydrocarbon extract, polar lipids, ester fraction, free-fatty-acid extract, and essential oil were analyzed by GC as the methyl esters. Table 2 shows that there is a broad set of fatty acids in the initial extracts and its separate fractions. The total mass of saturated components represented by the series 10:0-24:0 dominates in all samples from 55 to 72.0%. The principal saturated acid is 16:0. The unsaturated components made up from 24.0 to 41.0% with 18:2 dominating in the hydrocarbon extract and esters; 18:1, in the free fatty acids and essential oil; and 18:3, in polar lipids. In general, the set and ratio of fatty acids for the *A. annua* extract correspond with those for other plant specimens collected during the same vegetative period [8-11].

Polar lipids were identified by TLC on silica gel using specific reagents. Phospholipids (PL) were determined using two-dimensional TLC and solvent systems 3 and 4; glycolipids (GL), solvent system 5. The PL contained lysophosphatidylcholine, phosphatidylcholine, phosphatidylinosite, and phosphatidic acid. Two PL components were not identified. The main classes of GL were sterolglycosides and their esters in addition to monogalactosyldiglycerides and digalactosyldiglycerides.

The lipid extract containing essential oil was used for the pharmacological investigations. The data from the pharmacological experiments showed that topical (skin) administration of the lipid extract from leaves and flowers of annual wormwood produced a positive shift of carbohydrate metabolism. Administration of a 0.5% solution increased the skin content of glycogen by 15% depending on the concentration used. The dynamics of the skin content of pyruvic (PA) and lactic (LA) acids were rather characteristic. The PA skin content increased by 30% at the administration site whereas that of LA decreased by 33% if the lipid extract (0.5%) was administered for 30 days. The amount of skin glycogen increased by 22% and that of PA by 41% if a 5% concentration was administered for 30 days. The change of LA content also was very distinct and decreased by 36%. Changes in the redox potential (RP) of the LA—PA system upon administration of the different concentrations (0.5 and 5%) of lipid extract were favorable in nature and indicated that aerobic processes of carbohydrate oxidation in skin improved (Table 3). The calculated value of the excess of lactate provided additional conformation of this.

TABLE 2. Composition of Fatty Acids of the Hydrocarbon Extract (1), Fatty Acid Esters (2), Free Fatty Acids (3), Polar Lipids (4), and Essential-Oil Acids (5) from *Artemisia annua* Flowers and Leaves, GC, mass %

Acid	1	2	3	4	5
10:0	5.0	5.0	0.6	4.8	3.6
12:0	4.7	5.0	0.6	6.7	8.7
13:0	1.5	Tr.	0.2	0.8	0.5
14:0	2.4	7.5	1.4	1.4	12.4
15:0	1.1	5.1	0.9	0.9	1.6
16:0	16.5	24.2	32.0	30.2	20.9
16:1	4.7	Tr.	3.3	5.4	2.9
17:0	3.3	1.8	6.3	0.6	0.7
18:0	3.2	5.4	13.1	2.6	20.5
18:1	8.0	10.0	12.7	3.7	17.3
18:2	18.3	14.3	11.0	13.7	3.9
18:3	8.0	6.3	1.9	17.8	-
$\Sigma_{20:0,\ 22:0,\ 24:0}$	17.2	15.4	14.7	9.2	3.4
$\Sigma_{unident}$	6.1	-	1.3	2.2	3.6
$\Sigma_{sat.}$	54.9	69.4	69.8	57.2	72.3
$\Sigma_{unsat.}$	39.0	30.6	28.9	40.6	24.1

TABLE 3. Effect of the Lipid Extract of *Artemisia annua* Flowers and Leaves on Certain Biochemical Properties of Rat Skin (M ± m, n = 6-8)

Experimental conditions	Preparation conc., %	Ratio coefficient		RP of LA/PA system, mV	Excess of lactate	CS/PL
		K/Na	LA/PA			
Intact animals	-	0.56±0.025	12.6±0.72	-232.9	-	0.32±0.014
Control (cotton oil)	-	0.58±0.028	13.9±0.87	-242.4	-	0.33±0.01
Lipid concentrate	0.5	0.48±0.03	7.2±0.44*	-230.6	-15.2	0.26±0.013*
Lipid concentrate	5	0.45±0.04*	8.1±0.52*	-233.4	-18.6	0.28±0.015*

Reliability of differences and excess-lactate index were calculated relative to the appropriate control.
*Differences are reliable ($P < 0.05$).

An important indicator that characterizes the functional state of skin is the level of the principal lipid-exchange parameters. The experimental results showed that administering the studied preparation (0.5%) to skin decreased the cholesterol content rather clearly by 36%. Application of the 5% solution decreased cholesterol by 22%. The cholesterol decrease was statistically reliable for use of both concentrations. The PL content at the administration site was increased after 30 days for both preparation concentrations.

Table 3 shows that the lipid extract from annual wormwood leaves and flowers has a significant effect on skin electrolyte exchange. Thus, topical administration of the preparation (0.5 and 5%) over a month caused a slight decrease of K ions at the administration site although the Na content did not change noticeably. The changes in the contents of K and Na ions caused a significant reduction in the K/Na coefficient, which was statistically reliable at 17-22%. All data are indirectly consistent with a positive influence of the studied extract on skin hydration processes. It was also found that the lipid extract of annual wormwood leaves and flowers exhibits distinct anti-inflammatory activity under formalin and ovalbumin adema conditions. The exudate from the inflammation was suppressed under formalin adema conditions by 18-28%; ovalbumin, 8-31% depending on the dose used (Table 4).

TABLE 4. Effect of *Artemisia annua* Lipid Extract on Formalin and Ovalbumin Adema in Rat Paws (M ± m, n = 6)

Experimental conditions	Starting paw volume	Paw volume after treatment	Growth of paw volume	Effect, %
		Formalin adema		
Control	1.1±0.036	1.53±0.033*	0.43±0.021	
Lipid extract, mg/kg				
0.1	1.13±0.033	1.48±0.030*	0.35±0.034*	18.6
0.5	1.08±0.054	1.40±0.04*	0.31±0.027	27.9
5	1.1±0.04	1.45±0.05*	0.35±0.022*	18.6
		Ovalbumin inflammation		
Control	1.06±0.04	1.7±0.08*	0.63±0.05	-
Lipid extract, mg/kg				
0.1	1.13±0.021	1.71±0.01*	0.58±0.04	8
0.5	1.10±0.035	1.53±0.033*	0.43±0.04*	31.7
5	1.13±0.02	1.56±0.033*	0.43±0.021*	31.7

*Reliable relative to control ($P < 0.05$)

Thus, the lipid extract from annual wormwood leaves and flowers is definitely interesting as a biocomponnet for preparing various cosmetics and perfumes intended for skin prophylaxis and inflammation treatment.

EXPERIMENTAL

Mass spectra were recorded in an MX-1310 instrument at electron ionization energy 40/50 eV and ionization chamber temperature 190/180°C.

GC was performed in a Chrom-41 instrument with a flame-ionization detector. The analysis conditions have been described [12].

TLC of lipids was carried out on silica gel L 5/40 with 10% gypsum. The solvent systems were: hexane:diethylether (1, 4:1; 2, 1:1), $CHCl_3$:CH_3OH:NH_4OH (25%) (3, 13:5:1), $CHCl_3$:CH_3OH:CH_3CO_2H:H_2O (4, 14:5:1:1), $CHCl_3$:$(CH_3)_2CO$:CH_3OH:CH_3CO_2H:H_2O (5, 65:20:10:10:2). Bands were developed by spraying plates with aqueous H_2SO_4 (50%) (lipids and free cyclic alcohols and their esters), α-naphthol and the same H_2SO_4 solution (GL), Vaskovsky reagent, Dragendorff's solution, and ninhydrin solution (PL) [13].

The carotinoid content was found by spectrophotometry [14]; acid number, by the literature method [15].

The hydrocarbon extract and ester fraction were hydrolyzed by methanolic KOH (30%) on a boiling-water bath for 3 h; polar lipids, by KOH solution (10%) with boiling for 30 min. Then, the reaction products were diluted with water. Unsaponified substances and residual essential-oil components were extracted with diethylether. The remaining fatty acids salts were decomposed by aqueous H_2SO_4 (10%). The released fatty acids were also extracted by diethylether and methylated by diazomethane. Free fatty acids in the triglyceride and essential-oil fractions (Table 1) were isolated by preparative TLC using solvent system 1 after they were methylated by diazomethane. Diazomethane was prepared as before [16].

Mass spectrum, molecular ions with *m*/*z* 804, 790, 776, 774, 762, 748, 746, 734, 732, 720, 718, 706, 704, 702, 700, 692, 690, 688, 686, 678, 676, 674, 672, 664, 662, 660, 658, 652, 648, 646, 638, 636, 624, 622, 620, 618, 594, 592, 590, 578, 562, 560, 558, 536, 534, 532, 522, 518, 506, 466, 452, 438, 424; groups of peaks for $[RCO_2H_2]^+$ fragments with *m*/*z* 369-173 and 283, 281, 279; $[RCOO]^+$ with *m*/*z* 367-171 and 281, 279, 277; $[RCO]^+$ with *m*/*z* 351-155 and 265, 263, 261; for fatty acids and peaks for [R′ - 18] fragments with *m*/*z* 420-224, 396, 394, and 408 for aliphatic and cyclic alcohols.

Pharmacological experiments were carried out using male rats of mass 160-190 g. Lipid extract from annual wormwood leaves and flowers was administered to animals on shaved skin of the spine (3 × 3 cm) once daily as 0.5 and 5% concentrations in cotton oil. Controls received only cotton oil. Animals were observed for 30 days, after which they were

decapitated. The treated skin sections were isolated, fully depilated, purified of subcutaneous fat cells [17], and used for the biochemical investigations. The skin electrolyte contents were determined by flame photometry. The effect of the lipid concentrate on carbohydrate—lipid exchange in skin was evaluated from the change in content of glycogen [18], pyruvic acid [19], lactic acid [20], cholesterol [21], and phospholipids [22]. The RP of LA/PA was calculated based on the LA and PA content by the literature method [23] and the excess of lactate [24].

The antiphlogistic activity was estimated from the ability of the lipid extract (0.1:0.5 and 5 mg/kg *per os*) to inhibit paw adema caused by administering formalin and ovalbumin. The results were processed by variational statistics using the Student *t*-criteria.

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