Chromatographic detection and identification of lipophylic compounds of *Glycine* and *Setaria* genus

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Summary

An investigation of plants reach in lipophylic compounds and searching of sufficient raw material are being conducted due to rising scientists' interest. Among them, we can count the plants of *Fabaceae* and *Poaceae* families. With the use of a gas-liquid chromatography method (GLC), the qualitative composition and the quantitative content of fatty acids and tocopherols in the lipophylic fraction of *Glycine hispida*, as well as sterols and fatty acids in the lipophylic fractions of fruits and grass of *Setaria italica* have been determined. In addition steroid and fatty-acid compositions of fruits of *Setaria italica* have been studied using the methods of gas chromatography/mass-spectrometry (GC/MS).

Key words: *Glycine hispida, Setaria italica, fatty acids, tocopherols, sterols, gas-liquid chromatography, gas chromatography/mass-spectrometry*.

A lot of attention has been paid to the study on lipophylic complexes of medicinal plants as well as the creation of medical and cosmetic preparations, biological active food supplements and foodstuffs of special purposes. This can be explained by the rich chemical composition and pharmacological actions of components of lipophylic fractions, especially fatty acids, tocopherols and sterols. Fatty acids are ones of mandatory components of lipophylic fractions. They take part in biosynthesis of fats and take part in the composition of a plant cell. Fatty acids play a main role in the energetics of living cell, in the sterols metabolism, they are responsible for the pharmacological action of a number of medicines. Tocopherols influence the reproduction and lactation processes. They also have an antioxidant

and antisclerotic action. Furthermore, sterols can play as a bioregulators of living organisms. In addition, sterols have antidiabetic and antineoplastic actions, they are used as a prostate protector and also for treatment of hypercholesterolemia, rheumatoid arthritis [6, 7, 13].

Search for plants with the rich chemical composition of lipophylic compounds and sufficient raw materials base is stipulated by the interest for the study of crops. The plants of *Fabaceae* and *Poaceae* families are among them.

Plants of the *Poaceae* family play an important role in people's life. *Poaceae* is a cosmopolitic family which is present both in tropics and in the areas with temperate and cold climate. Cereals are: rice (*Oryza sativa*), wheat (*Triticum vulgare*), maize (*Zae mays*), barley (*Hordeum vulgare*), rye (*Secale cereale*), sorghum (*Sorghum vulgare*), rape (*Brassica napus*), sugarcane (*Saccharum officinarum*), green foxtail (*Setaria italica*). Crops are very important food, fodder and forage plants. They are the basic component in the natural hayfields and pastures. The best *Poaceaea* species are cultivated.

The *Fabaceae* family is the most important for people after cereals. *Fabaceae* are spread all over the world. A lot of them are widely cultivated both in tropics and in temperate climate. Legume seeds contain protein as a spare compound as well as starch and fatty oil. This fact makes them valuable products for food and fodder. The seeds of soy (*Glycine max*), haricot (*Phaseolus vulgaris*), pea (*Pisum sativum*), peanut (*Arachis hypogaea*) are extensively used as foodstuffs. The best fodder grasses are lucerne (*Medicago sativa*, *falacata*), different clover species (*Trifolium*). A lot of legumes are valuable medicinal plants including licorice (*Glycyrrhiza glabra*), cassia (*Cassia acutifolia*), locoweed (*Astragalus*), sophora (*Sophora japonica*), restharrow (*Ononis arvensis*).

Soybean (*G. hispida* (Moench) Maxim of the *Fabaceae* family) and the green foxtail (*S. italica maxima* Alef. of the *Poaceae* families) were chosen as a subject of research at the Department of Chemistry of Natural Compounds in the National Pharmaceutical University (Kharkiv, Ukraine).

Soybean – *G. hispida* (Moench) Maxim of the *Fabaceae* family is the ancient protein and oil rich plant, which produces actually all necessary compounds during its vegetation period – proteins, fats and also a lot of organic phytogenous compounds. Its seeds contain fatty oil, lecithin and phospholipids.

G. hispida is a very useful plant because it is rich in unsaturated fatty acids and high calorific components (protein, fatty oil). Soybean oil is one of the most popular oils in the world. Soy seeds contain about 30% of this oil. Soybean oil is a nutrient of a big importance and medical properties. It can be consumed as a liquid and in a capsule form. Fatty oil is recommended in cardiovascular, gastrointestinal and endocrine diseases. Soybean oil, lecithin, phospholipids are elements of many drugs and food supplements. The main unsaturated fatty acids in soybean oil are linolenic acid (5–10%), linoleic acid (50–55%) and oleic acid (25–30%). The nonsaponifying compounds of soybean oil are sterols (campesterol, β-sitosterol, stigmasterol) [8, 9].

Modern scientific literature contains information the results of the study on *G. hispida's* oil and seeds lipophylic components. Therefore, the aim of our study was to determine the qualitative composition and the quantitative content of some lipophylic compounds of our *G. hispida's* grass lipophylic fraction.

S. italica maxima Alef. is a cultivated, annual plant of the *Poaceae* family. Foxtail Millet, Italian Millet, German Millet, Hay Millet and Green foxtail are cereals and fodder crops. Millets are the oldest of cultivated crops. *S. italica* is cultivated in China, Japan, Korea, Mongolia, India, Morocco and other countries including Ukraine, Russia, Belorussia, Georgia and Kazakhstan. Foxtail Millet is a heat-loving and drought-resistant plant. Its seeds are used for cereals and flour production. They may also be used as forage and fodder source as well as in alcohol production. In medicine it is used to treat dyspepsia, poor digestion and food stagnancy in the abdomen. Grass, green crop and hay of *S. italica* are used as animal husbandry fodder.

According to the latest scientific literature, *S. italica* contains a considerable quantity of biological active compounds: protein, amino acids, mineral substances, vitamins, but the chemical composition of lipophylic compounds has not been studied sufficiently [14, 15]. Therefore the aim of our research was to carry out chemical analyses of S. *italica* biological active compounds of lipophylic nature.

MATERIALS AND METHODS

G. hispida, *Fairy* is a species of a big perspectives grown in the Ukraine, included in the List of species of Ukraine. It has been studied at the stage of flowering. *S. italica* fruits and grass of species Dnyprovsky have been chosen as the subject of our research. This species was discovered after a 48 years of selection work.

Lipophylic fractions of soybean grass (LF-I) as well as green foxtail fruits (LF - II) and grass (LF - III) were obtained by Soxhlet apparatus using chloroform [1]. The solvent was evaporated using a rotary evaporator, then the content of the obtained lipophylic complexes were determined. The yield of LF-I was 2.69% and of LF-II and LF-III were 9.6% and 2.97%, respectively.

By means of a gas-liquid chromatography method (GLC) the qualitative composition and the quantitative content of fatty acids [2, 3, 10, 11] and tocopherols [4, 5] in the LF-I, fatty acids in the LF-II and LF-III and sterols in LF-III have been determined. In addition, steroid and fatty-acid compositions of LF - III have been studied using the methods of a gas chromatography/mass-spectrometry (GC/MS) [1, 12].

The analysis of fatty acids by GLC method was performed under the following conditions: chromatograph Chrom-5 equipped with a flame-ionization detector; column 250 x 0.3 cm coated with Interon-AW with graininess 0.16-0.20 mm as the stationary phase; diethyleneglycol succinate used as the liquid phase in number 10% of carrier mass; separation temperature of 186°C; injector temperature of 230°C; detector temperature 220°C; carrier gas - nitrogen of special purity; the feed rate of carrier gas 30 ml/min. The essence of preparation was in methylation of fatty acids. Reaction mixture has been evaporated to solid residual after methylation finishing. Then the solid residual has been solved in cyclohexane floor amount and studied by GLC method.

The identification of compounds peaks was carried out by comparison of sample retention times with the retention times of prepared compounds standards in

mixes and individual forms. The content of each component was calculated as a ratio of appropriate peak area to the all areas sum of all peaks.

For the studies on tocopherols also the GLC method was used. We used the following test preparations: 100 mg of LF was dissolved in 2 ml of ethanol, then used 3 ml of hexane and centrifugated (3.000 r/min.) this mixture during 10 min. After that the hexane layer has been taken and tocopherols fraction have been collected by eluting with ethyl acetate. After eluate concentration used 5 ml of chloroform, 0.2 ml of hexamethylchlorsilane and 5 drops of trimethylchlorsilane. Reaction mixture has been evaporated to solid residual. Aftrwards, the solid residual has been solved in mixture of hexan-chloroform-methanol (10:10:1). Reaction mixture has been evaporated to solid residual. Then the solid residual has been solved in 1-3 ml of hexane. Analysis conditions were as follows chromatograph Chrom-5 equipped with a flame-ionization detector; column 2.6 m coated with support Interon-super with graininess 0.15 mm, which has been deactivated by hexamethyldisilazane, and with OV-17 in number of 3% of carrier mass as the stationary phase; analysis temperature of 190°C; detector temperature 240°C; carrier gas - nitrogen of special purity; feed rate of carrier gas 40 ml/min.

The identification of compounds peaks was carried out by comparison of sample retention times with retention times of prepared compounds standards in mixes and individual forms. The content of each component was calculated as a ratio of appropriate peak area to the all areas sum of all peaks.

For LF-II fatty acids study by the GC-MS method double methylation has been carrying out with use of acidulous methalon and diazomethanol. GC-MS was performed under the following conditions: Hewlett Packard HP-6890 with mass-selective detector HP-5972; capillary column HP-5MS (nonpolar), 30 m x 0.25 mm; film thickness 0.25 μm (5% diphenyl); capillary column HP-INNIWAX (polar), 30 m x 0.25 mm; film thickness 0.25 μm (polyethylene glycol); carrier gas helium at flow rate 1 ml/min.; columns were temperature programmed from 60°C to 280°C at 5°C/min. and were maintained at 280°C for 1 min.; injector temperature – 250°C; detector temperature – 280°C; ionization voltage 70eV; mass range was from m/z 40–450.

For LF-II sterols study we used following test preparations: for functional sterol's groups protection double methylation has been carried out by acidulous methalon, diazomethanol and BSTFA (bis[trimethylsilil] – trifluoroacetamide). GC-MS: Hewlett Packard HP-6890 with mass-selective detector HP-5972; capillary column HP-5MS (nonpolar), 30 m x 0.25 mm; film thickness 0.25 μm (5% diphenyl); capillary column HP-INNIWAX (polar), 30 m x 0.25 mm; film thickness 0.25mkm (Polyethylene glycol); carrier gas helium at flow rate 1 ml/min.; columns were temperature programmed from 60°C to 280°C at 5°C/min. and were maintained at 280°C for 1 min.; injector temperature – 250°C; detector temperature – 280°C; ionization voltage 70 eV; mass range was from m/z 40 – 450 amu.

The identification of fatty acids and sterols was carried out by comparison of sample retention times with analogous factors of authentic pure substances using NIST 02 and Wiley 138k library.

RESULTS AND DISCUSSION

During our study 11 fatty acids in LF-I have been identified by GLC, in LF-II and LF-III 4 and 9 fatty acids, respectively. The results of fatty acids quantitative content are presented in table 1 and fig. 1. The highest found amount was that of linolenic acid: 7.5 mg/100 mg, linoleic acid: 3.8 mg/100 mg and palmitic acid: 4.2 mg/100 mg in LF-I. Myristinic acid and linoleic acid in LF-III, valerianic acid and linolenic acid in the LF-II were found in the highest amount. Four tocopherols: Δ-tocopherol, β + γ-tocopherol, α_{1}-tocopherol, α_{2}-tocopherol and their quantitative content in LF-I have been determined by GLC. The results are presented in table 2 and fig. 2.

Table 1.

Fatty acids composition of lipophylic fractions of *G. hispida* grass as well as *S. italica* fruits and grass (GLC)

No.	fatty acids	carbon number	*Glycine hispida* grass [mg/100 mg]	*Setaria italica* grass (% of total fatty acids mass)	*Setaria italica* fruits (% of total fatty acids mass)
			saturated		
1.	valerianic acid	$C_{5:0}$	-	-	37.0
2.	capric acid	$C_{10:0}$	0.023	-	-
3.	lauric acid	$C_{12:0}$	0.07	-	-
4.	myristinic acid	$C_{14:0}$	0.23	24.15	-
5.	palmitic acid	$C_{16:0}$	4.2	-	3.1
6.	stearic acid	$C_{18:0}$	1.6	5.64	0.8
7.	arachidic acid	$C_{20:0}$	t.q.*	-	0.5
			unsaturated		
8.	oleic acid	$C_{18:1}$	0.92	-	9.9
9.	linoleic acid	$C_{18:2}$	3.8	11.3	42.5
10.	linolenic acid	$C_{18:3}$	7.5	58.92	2.1
11.	gondoic acid	$C_{20:1}$	-	-	0.5
12.	arachidonic acid	$C_{20:4}$	1.4	-	-
13.	erucic acid	$C_{22:1}$	-	-	0.3

*t.q. – trace quantity

Table 2.

Tocopherols composition of *G. hispida* grass (GLC)

No.	tocopherols	content (mg/100 mg)
1.	Δ-tocopherol	0.75
2.	β + γ-tocopherol	0.95
3.	α_{1}-tocopherol	0.75
4.	α_{2}-tocopherol	0.35

The results of the sterols content in the LF - II are presented in table 3. We have found cholesterol and the sum of phytosterols, which consist of campesterol and β-sitosterol by GLC. However, in our opinion, LF-II needs a more detailed study, because its yield was bigger (9.6%). We used GC/MS for our study. We have identified 24 fatty acids (tab. 4) and 12 sterols (tab. 5). Their quantitative content has also been determined.

Table 3.

Sterols content in *S. italica* fruits (GLC)

No.	sterols	mass concentration (% of total sterols mass)
1.	cholesterol	1.0
2.	phytosterols (Σ)	99.0
3.	campesterol	8.1**
4.	β-sitosterol	34.6**

** – % of Σ phytosterols

Table 4.

Fatty acids composition of lipophylic fractions in *S. italica* fruits (GC/MS)

No.	fatty acid	general formula	MW	content [mg/kg]
1.	pentanoic acid (valerianic)	$C_5H_8O_3$	116	5.214
2.	pentadecanoic acid, methyl ether	$C_{16}H_{32}O_2$	256	0.112
3.	pentadecanoic acid, 14-methyl-, methyl ether	$C_{17}H_{34}O_2$	270	3.478
4.	hexadecanoic acid, methyl ether, (palmitic)	$C_{17}H_{34}O_2$	270	4.578
5.	hexadecanoic acid, ethyl ether, (palmitic)	$C_{18}H_{36}O_2$	284	0.089
6.	heptadecanoic acid, methyl ether, (margaric)	$C_{18}H_{36}O_2$	284	0.115
7.	6-octadecenoic acid (Z), (petroselinic)	$C_{18}H_{34}O_2$	282	1.467
8.	9,12-octadecadienoic (Z, Z), (linoleic)	$C_{18}H_{32}O_2$	280	2.610
9.	octadecanoic acid, methyl ether, (stearic)	$C_{19}H_{38}O_2$	298	1.195
10.	9-octadecynoic acid, methyl ether (E), (stearolic)	$C_{19}H_{36}O_2$	296	0.534
11.	9,12-octadecadienoic acid, methyl ether (E, E) (linoleic)	$C_{19}H_{34}O_2$	294	2.623
12.	9,12-octadecadienoic acid, methyl ether, (linoleic)	$C_{19}H_{34}O_2$	294	1.698
13.	9,12,15-octadecatrienoic acid, methyl ether (Z, Z, Z), (linolenic)	$C_{19}H_{32}O_2$	292	0.781
14.	nonadecanoic acid, methyl ether	$C_{20}H_{40}O_2$	312	0.082
15.	linoleic acid, ethyl ether	$C_{20}H_{36}O_2$	308	7.569
16.	eicosanoic acid, methyl ether, (arachidic)	$C_{21}H_{42}O_2$	326	0.402
17.	11-eicosenoic acid, methyl ether, (gondoic)	$C_{21}H_{40}O_2$	324	0.223
18.	heneicosanic acid methyl ether,	$C_{22}H_{44}O_2$	340	0.115
19.	docosanoic acid, methyl ether, (behenic)	$C_{23}H_{46}O_2$	354	0.465
20.	tricosanoic acid, methyl ether	$C_{24}H_{48}O_2$	368	0.233
21.	tetracosanoic acid, methyl ether, (lignoceric)	$C_{25}H_{50}O_2$	382	0.200
22.	hexacosanoic acid, methyl ether	$C_{27}H_{54}O_2$	410	0.223
23.	octacosanoic acid, methyl ether, (montanic)	$C_{29}H_{58}O_2$	438	0.242

Table 5.

Sterols content in *S. italica* fruits (GC/MS)

No.	sterols	general formula	MW	content [mg/kg]
1.	ergostanol	$C_{28}H_{50}O$	402	0.164
2.	γ-sitosterol	$C_{29}H_{50}O$	414	2.781
3.	stigmasterol	$C_{29}H_{48}O$	412	0.398
4.	cholest-5-en-3-ol,24-propylidene-, (3β)-	$C_{30}H_{50}O$	426	0.390

No.	sterols	general formula	MW	content [mg/kg]
5.	lanosterol	$C_{30}H_{50}O$	426	0.185
6.	stigmasta-5,24(28)-dien-3-ol,(3β, 24 Z)-	$C_{29}H_{48}O$	412	0.200
7.	5-cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400	0.913
8.	stigmastanol	$C_{29}H_{52}O$	416	1.006
9.	stigmastan-3,5-diene	$C_{29}H_{48}$	396	0.376
10	campesterol	$C_{28}H_{48}O$	400	0.867
11.	stigmast-4-en-3-one	$C_{29}H_{48}O$	412	0.215
12.	9,19-cyclolanost-24-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O$	468	0.229

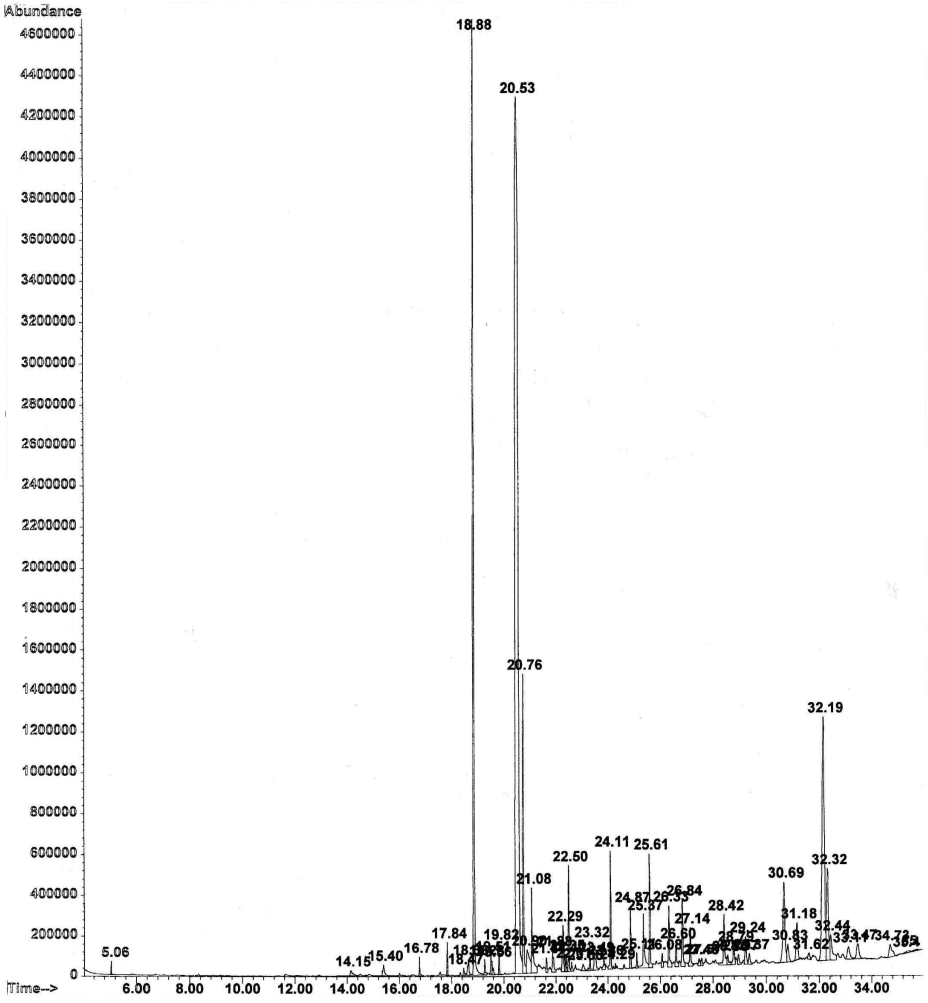

Figure 1. Chromatographic scheme of fatty acids of *S. italica* fruits lipophylic fraction (column HP-5MS)

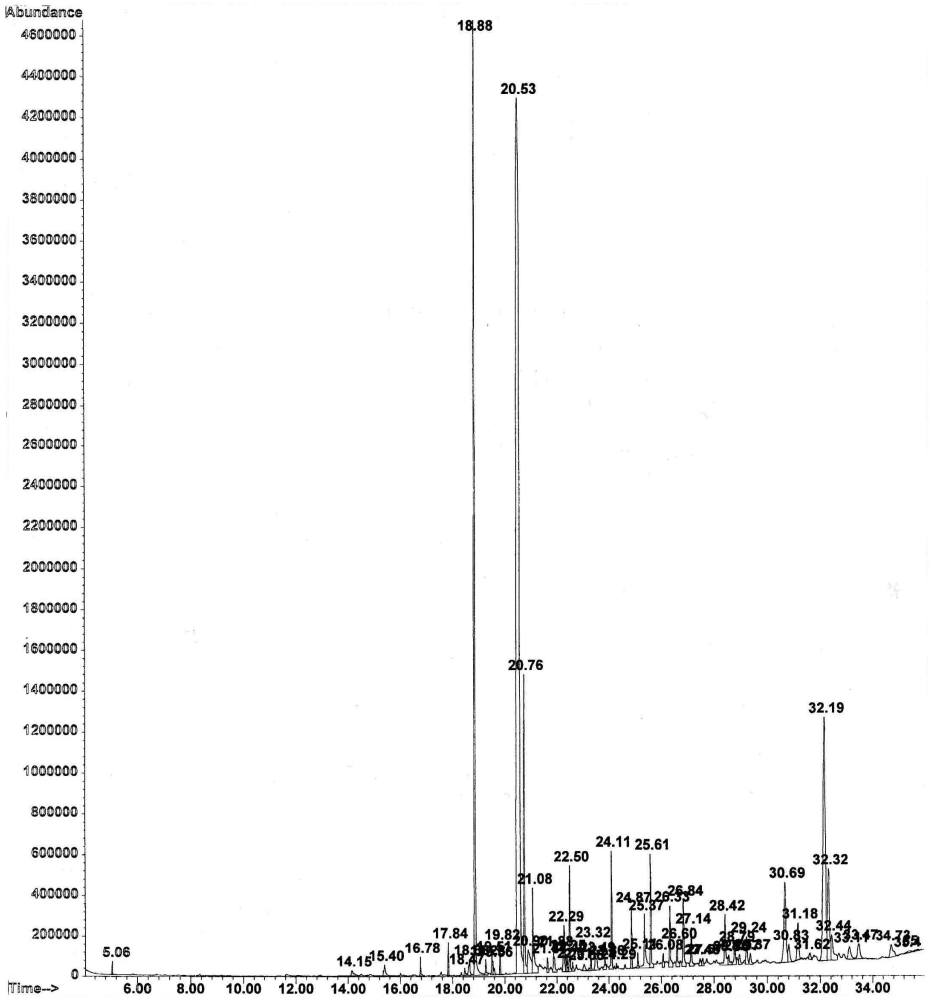

Figure 1. Chromatographic scheme of fatty acids of *S. italica* fruits lipophylic fraction (column HP-5MS)

CONCLUSIONS

Lipophylic fractions of soybean grass and foxtail millet fruits and grass were obtained and their yields were determined. The qualitative composition and the quantitative content of fatty acids and tocopherols in the LF-I as well as sterols and fatty acids in the LF-II and LF-III have been determined by a method of the GLC. Sterols and fatty acid compositions of LF - II have been studied using GC/MS methods.

All analyzed samples have unsaturated acids as a dominate component. This can help us to prognosticate F-vitamin action of our LF. The LF of *S. italica* fruits also contains valerianic acid, which can be used as a "marker" for raw material standardization.

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POSZUKIWANIE I OZNACZANIE SUBSTANCJI LIPOFILNYCH W ROŚLINACH Z GATUNKÓW *GLYCINE* I *SETARIA*

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Streszczenie

W kręgu zainteresowań naukowców leży poszukiwanie roślin bogatych w składniki lipofilne, jak również znajdowanie wystarczających źródeł surowca. Do takich roślin można zaliczyć gatunki z rodzin *Fabicae* i *Poaceae*. W przedstawionej pracy stosując chromatografię gazowo-cieczowej (GLC) określono skład jakościowy i zawartość kwasów tłuszczowych

w owocach i zielu *Setoria italica*. Dodatkowo za pomocą chromatografii gazowej sprzężonej ze spektrometrią masową (GC/MS) zbadano zawartość steroidów i kwasów tłuszczowych w owocach *Setoria italica*.

Słowa kluczowe: *Glycine hispida, Setaria italica, kwasy tłuszczowe, tokoferole, sterole, chromatografia cieczowo-gazowa, chromatografia gazowa/spektrometria masowa*