Advances in Biology & Earth Sciences
Vol.10, No.3, 2025, pp.381-396
https://doi.org/10.62476/abes.103381

CHROMATOGRAPHIC ANALYSIS OF FINGERPRINTS AND METABOLOMIC SCREENING OF COMPOUNDS IN THE RHIZOMES OF *Gynostemma pentaphyllum* (Thunb.) MAKINO

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Abstract. The article is devoted to the chromatographic analysis of fingerprints and the metabolomic screening of compounds in the rhizomes of *Gynostemma pentaphyllum* (Thunb.) Makino. The object of study was the fresh and dried rhizomes of *Gynostemma pentaphyllum* (Thunb.) Makino, grown on the territory of the Republic of Bashkortostan (GPS coordinates of the collection of raw materials: 54.79435 north latitude, 55.85707 east longitude). The fingerprints of flavonoids and saponins were analyzed by thin-layer and high-efficiency thin-layer chromatography (TLC, HETLC). Metabolomic screening of *G. pentaphyllum* rhizomes was performed by gas chromatography with mass spectrometry (GC/MS). According to the TLC and HETLC data, substances of a flavonoid nature were found - rutin (R_f=0.48±0.02), quercetin (R_f=0.89±0.02) and triterpene - β-escin (R_f=0.46±0.02). GC/MS has a component composition consisting of hydrocarbons (cyclic, acyclic, dicarboxylic acids, their derivatives), terpenoids (monoterpenes, sesquiterpenes, diterpenes, their derivatives and from the group of aromatic compounds), flavonoids, derivatives of alkyl carboxylic acids, heterocyclic and steroidal compounds. The component composition of the recognized substances of *G. pentaphyllum* rhizomes may cause cytotoxic, antibacterial, adaptogenic and other effects that require further detailed preclinical and clinical studies.

Keywords: *Gynostemma pentaphyllum* (Thunb.), *Makino rhizomes, high-performance thin-layer chromatography, metabolomic screening, gas chromatography, fingerprints.*

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Received: *26 September 2025;* ***Accepted:*** *20 October 2025;* ***Published:*** *5 December 2025.*

1. Introduction

Gynostemma pentaphyllum was first described in 1406 during the Ming Dynasty by Zhu Xiao in the book "Medicinal Herbs against Hunger" (Blumert & Liu, 1999). The first Western description of the plant was published by the Swedish naturalist Carl Peter Thunberg (1784) under the name *Vitis pentaphylla* (Flora of Japan). Later, the German-Dutch botanist Carl Ludwig Blum (1825) transferred this species to another genus *Gynostemma* (*G. simplicifolium*) (Gan *et al*., 2023). Japanese botanist Makino Tomitaro (1902) retained the specific epithet from Thunberg's first description and thus created the taxon *Gynostemma pentaphyllum* (Thunb.) Makino, which is still in force today.

Systematic classification of the *G. pentaphyllum*:
Kingdom: *Plantae*
Division: *Magnoliophyta*
Class: *Magnoliopsida*
Order: *Cucurbitales*
Family: *Cucurbitaceae*
Genus: *Gynostemma*
Specie: *Gynostemma pentaphyllum* (Gakkai & Gakkai, 1902).

G. pentaphyllum is a perennial herbaceous or sub-woody deciduous liana with a creeping rhizome, dioecious (Figure 1).

Figure 1. Fragment of a wild-growing *Gynostemma pentaphyllum* species

The species is widespread outside of China, from India and Bangladesh to Southeast Asia, Korea, Japan and also in New Guinea (Kharkevich, 1985; Kharkevich & Kachura, 1981; Abid *et al*., 2019). In Russia, this plant is found on the territory of Yuzhno-Kurilsk, located on the island of Kunashir (Kharkevich, 1985).

G. pentaphyllum is called the "second ginseng" because of the similarity of bioactive components and the saponin content is about five times higher than in *Panax ginseng*. In addition, *G. pentaphyllum* is much easier to cultivate and due to its rapid growth, makes it possible to harvest sufficient biomass of plant raw materials (Liang *et al*., 2019).

A comprehensive comprehensive review of electronic databases (Traditional Chinese Medicine Systems Pharmacology, PubChem, CheMDL, online ethnobotanical database) allowed us to analyze over 200 chemical compounds, which are selectively presented in Figure 2 and Table 1 (Belenovskaya *et al*., 2018; Chen & Zhang, 2001; Fang & Zeng, 1989; Kao *et al*., 2008; Li *et al*., 2025; Liang *et al*., 2019; Liu *et al*., 2023; Lu *et al*., 2014; Razmovski-Naumovski *et al*., 2005; Tsai *et al*., 2010; Tsui *et al*., 2014; Xu *et al*., 2013; Zheng *et al*., 2024).

In addition, the content of other compounds has been established:

- 18 amino acids (including 8 essential ones);
- chlorophylls;
- organic acids (MLI (malonic acid));
- phenolic carboxylic acids (caffeic acid);
- trace elements (Cu, Fe, Zn, Mn, Co, Ni, Se, Mo, Sr);
- coumarins (toddaculin, 1-isochromanone);

● fatty acids (oleic, palmitic, stearic, linoleic and linolenic acids) and their derivatives (ELD (9-octadecenamide), ethylpalmitate);

● nitrogen-containing compounds (allantoin), vitamins and proteins (Deng *et al*., 1994; Huang *et al*., 2008; Kim & Park, 1988; Lu *et al*., 2014; Nookabkaew *et al*., 2016; Razmovski-Naumovski *et al*., 2005; Wang *et al*., 2016; Xu *et al*., 2013; Zou *et al*., 2016).

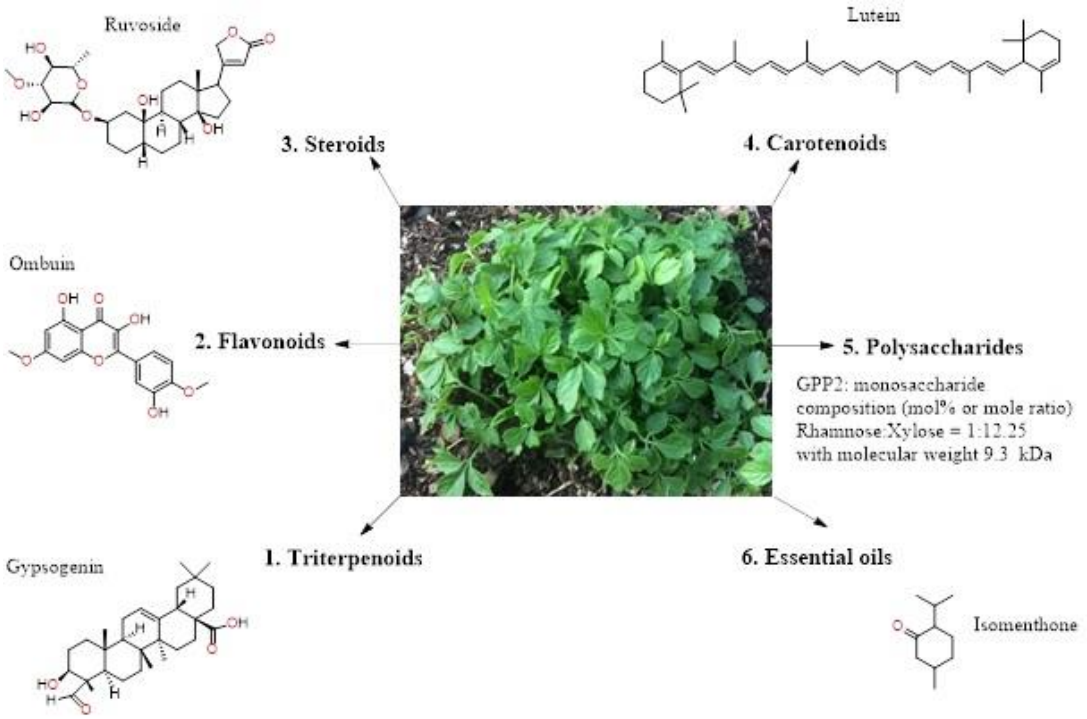


Figure 2. The main bioactive components of *Gynostemma pentaphyllum*

Table 1. Biologically active compounds

No	Name of substances
1.	gypnoside V_qt, ginsenoside-Rb2, gypenoside LXIX, ginsenoside Re, malonylginsenoside Rd, malonylginsenoside Rd_qt, gypenoside XIV_qt, gypenoside I_qt, ginsenoside Rb3, ginsenoside f2, ginsenoside rb1, ginsenoside rd_qt, gypinoside LXVII, gypinoside LXVII_qt, gypinoside VII, gypnoside V, gypsogenin, onjisaponin F and many others
2.	rutin, quercetin, ombuoside, ombuin, isoramnetin-3-o-rutinoside, isoramnetin, quercetin-Di-(ramno)-gexoside, quercetin-ramno-gexoside, camperol-ramno-gexoside, camperol-3-o-rutinoside, benzyl-O-β-d-glycopyranoside, vitexin, rhamnazin, malonylawobanin
3.	ruvoside, sitosterol, spinasterol, campesterol, isofucosterol
4.	α-carotene, β-carotene, cis-neoxanthin, violaxanthin, auxanthin, luteoxanthin, lutein
5.	GPP, GPMPP, CPS-1, GPTP-3, GPWP, GPUP, GPMP, CGPP, GPP1, GP-B1, GP-C1, GP, GPP-TL, GM, PSGP, GP-1, GPP2, GPA1, GPA2. GPA3, GPP1-A, GPP2-B, GPP3-A, ND, GPP-S (carbohydrates are contained in various molar proportions)

Note: qt - sapogenin (a structure that is released from monosaccharides by hydrolysis of glycoside bonds)

Bioactive substances obtained from promising herbal remedies and their synthetic derivatives have a variety of pharmacological properties (anti-inflammatory, antihelicobacterium, antiplatelet, anticoagulant and others) (Abdullina *et al*., 2024; Ksenofontov *et al*., 2022).

Modern mono- and polypharmacological studies of *G. pentaphyllum* have shown anti-inflammatory, cytotoxic, antiallergic, lipid-lowering, immunostimulating effects and others, shown in Figure 3, associated with key components such as saponins, flavonoids and polysaccharides (Belenovskaya *et al*., 2018; Circosta *et al*., 2005; Galiakhmetova *et al*., 2021; Huang *et al*., 2008, 2022; Ibragimova *et al*., 2023; Kodirov *et al*., 2024; Lee *et al*., 2025; Li *et al*., 2025; Liang *et al*., 2019; Liu *et al*., 2023; Razmovski-Naumovski *et al*., 2005; Tan *et al*., 2021, 2023; Tsui *et al*., 2014; Wang *et al*., 2017, 2018; Yang *et al*., 2013; Zheng *et al*., 2024).

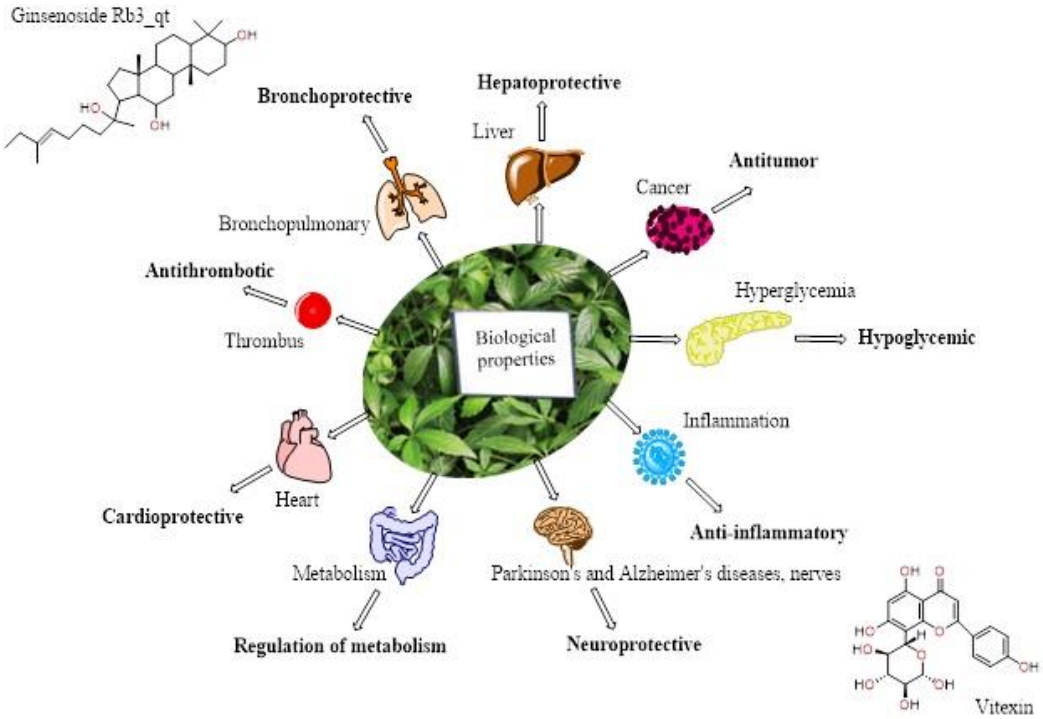


Figure 3. Basic biological properties of *Gynostemma pentaphyllum*

In connection with the above, *G. pentaphyllum* is a good alternative resource and attracts many scientists around the world (Liang *et al*., 2019).

The purpose of the study: chromatographic analysis of fingerprints and metabolomic screening of compounds in the rhizomes of *Gynostemma pentaphyllum* (Thunb.) Makino, grown in the Republic of Bashkortostan.

2. Material and Methods

2.1. Plant materials

The object of the study was *Gynostemma pentaphyllum* (Thunb.) Makino rhizomes grown in the Republic of Bashkortostan (GPS coordinates of raw material collection: 54.79435 north latitude, 55.85707 east longitude). Plant research objects, fresh and dried rhizomes, are harvested during the fruiting period of the producing plant.

Pieces of cord-shaped rhizomes of various lengths from 5 to 10-15 cm, 3.0 mm thick, branching, having a longitudinally wrinkled surface. The color of the rhizomes ranges from light brown to greenish, light yellow at the break (Figure 4).

Figure 4. Plant raw materials: *Gynostemma pentaphyllum* rhizomes

2.2. Chromatographic analysis of fingerprints

Various instrumental analysis methods (chromatographic, spectroscopic, etc.) based on the production of so-called fingerprints are used for authentication and identification of plant raw materials. A chromatographic fingerprint of a medicinal herbal preparation is a chromatographic extraction sample containing components with pharmacologically active and chemical parameters (Giri *et al*., 2010; Nikam *et al*., 2012).

Fingerprints of flavonoids and saponins were analyzed using bottom-up chromatography on plates with fractionated sorbent on an aluminum substrate of the Sorbfil-PTX-AF-A-UV brand and high-performance thin-layer chromatography on plates of the Sorbfil PTX-AF-B-UV brand. The plates were previously activated in a drying laboratory cabinet (LOIP LF series with TS87B control module) at a temperature of 105 °C for 60 minutes.

0.025 ml of the studied extracts and 0.05% alcohol solutions of standard samples were applied to the starting line of chromatographic plates with a microcapillary. The plate was dried at room temperature, then placed in a chamber with a pre-saturated mobile phase and chromatographed in an ascending manner at a temperature of 18-20 °C. After passing approximately 10 cm of the mobile phase, the plate was removed and dried for 20 minutes at room temperature.

Conditions for the analysis of triterpene saponins. The following solvent systems served as mobile phases: petroleum ether-chloroform-acetic acid (10:4:0.4), chloroform - ethyl acetate (9:1), chloroform - methanol - water (26:14:3), benzene - acetone (3:1), chloroform - ethyl alcohol (9:1).

The specific reagent for development is a 20% sulfuric acid solution applied with a spray gun. After development, the plates were heated in a thermostat at 105 °C for 5 minutes.

Conditions for the analysis of flavonoids. The following solvent systems served as mobile phases: ethyl acetate - acetic acid - water (12:1:1), ethyl acetate - formic acid - water (7:1.5:1.7), chloroform - methanol - water (26:14:3), butanol - acetic acid - water (4:1:5), benzene - acetic acid (5:21).

Chromogenic reagent for development is a 5% alcohol solution of aluminum chloride applied with a spray gun.

Chromatograms were detected by viewing in visible and ultraviolet light (*Uf*) at a wavelength of 365 nm. Standard samples were used to identify natural substances: β-escin (CAS No. 11072-93-8, Santa Cruz Biotechnology), rutin (CAS No. 250249-75-3, Biochem), quercetin (CAS No. 849061-97-8, Acros Organics).

Extracts from the rhizomes of *G. pentaphyllum* for chromatography were obtained by extraction with 95% ethyl alcohol and methyl alcohol in a ratio of 1:10 (https://femb.ru/record/pharmacopea15).

Using HETLC, the plates obtained by the TLC method described above were analyzed using a Sorbfil TLC densitometer (Imid, Russia). The device is represented by a Sorbfil KS 4.00.000 lighting camera, a Sony DCR-CX190E video camera and a Sorbfil TLC video sensor program. After the introduction and saving of the chromatogram image in the software, it is possible to select a working area on it; to process the spots and convert them into chromatographic peaks to calculate the values of retention factor (R_f), peak area, peak height and automatically calculate the substance content in the analyzed spots.

2.3. Metabolomic screening

The metabolomic screening of fresh and dried *G. pentaphyllum* rhizomes was performed by gas chromatography with mass spectrometry. Liquid lipophilic fractions were obtained by single maceration. 1.0 g of the crushed raw materials were filled with various solvents: chloroform (sample No 1 and No 4), petroleum ether (sample No 2 and No 5), hexane (sample No 3 and No 6), respectively, in a ratio of 1:10 and infused for a week at a temperature of 20-25 °C in a dark place in a well-sealed container.

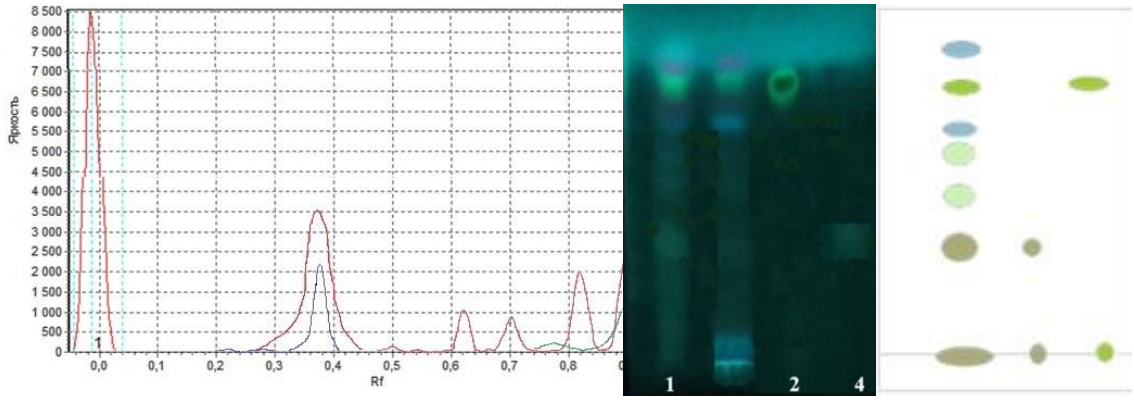
Screening analysis conditions. The device is an Agilent 8890 GC System gas chromatograph with a quadrupole mass-selective detector Agilent Technologies 5977B GC/MSD (USA manufacturer); separation column - capillary HP-5MS 30 m × 0.25 mm × 0.25 mm; sorbent - polydimethylsiloxane (PDMS); carrier gas - helium; carrier gas velocity is 1 ml/min; t° changes in the column: 80 °C - 2 min, then the heating rate was - 5 °/min to 290 °C, the holding time at the initial temperature was 2 min, the holding time at the final temperature was 10 min; t° the injector temperature is 280 °C; the sample volume is 1 µl. The mass-selective detector operated in the electronic shock mode (70 eV), chromatograms were recorded in the ion current scanning mode.

The mass spectra were deciphered in the following programs: Enhanced Data Analysis - EDUCATION.M, Qualitative Analysis Mass Hunter Workstation (Version 10.0) and NIST MS Search 2.2. The coefficient of similarity of the registered mass spectra with the database was at least 80%.

3. Results and Discussions

The best separation of flavonoids was observed in the solvent system ethyl acetate - acetic acid - water (12:1:1) in alcohol extracts with 96% alcohol. The results are shown in Figure 5 and Table 2.

On chromatograms of extracts from rhizomes of *G. pentaphyllum* in visible light and in *Uf* light at 365 nm before and after treatment with the detecting reagent, 5-6 adsorption zones were observed, of which the presence of flavonoids was assumed: quercetin (bright yellow-green fluorescence) and rutin (bright brown-yellow coloration).



A) B) C)

Figure 5. Alcohol extraction densitogram of the rhizome of *G. pentaphyllum*:
A) track 1 red line - rhizome extraction (1), track 2 blue line - standard sample solution of rutin (2), track 3 green line - standard sample solution of quercetin (3); B) chromatogram;
C) diagram

Table 2. Results of densitometric signals alcohol extraction of the rhizome of *G. pentaphyllum*

№	Track 1		Track 2	Track 3	Substance
Peak	R_f±0,02	Peak Area	R_f±0,02	R_f±0,02	
1	0,48	8104	0,49	-	rutin
2	0,63	3016	-	-	-
3	0,71	3003	-	-	-
4	0,84	5227	-	-	-
5	0,89	6964	-	0,88	quercetin

Note: R_f - retention factor

According to the results of the analysis of 5 adsorption zones (Table 2 and Figure 5): peak 1 of track 1 corresponds to peak standard samples of rutin (R_f=0.49, track 2), peak 5 corresponds to peak of standard samples of quercetin (R_f=0.88, track 3). According to the data obtained, the dominant flavonoid substance in the sample is a substance with R_f=0.48, corresponding to the routine.

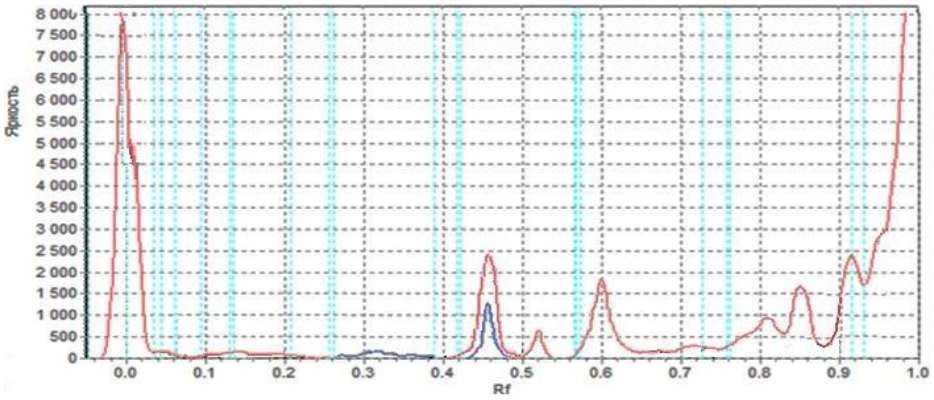
Optimal saponin separation was observed in the chloroform-methanol-water solvent system (26:14:3) in alcohol extracts based on methyl alcohol. The results obtained are shown in Figure 6 and Table 4.

Chromatograms of extracts of *G. pentaphyllum* (Thunb.) Makino rhizomes in *Uf* light at 365 nm showed about 10-11 adsorption zones after staining with a detecting reagent, of which at least 7-8 zones had a staining from light pink to dark purple, indicating the presence of substances of a triterpene nature.

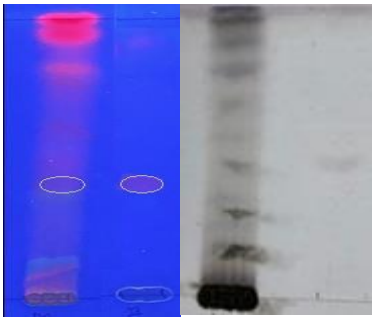
According to the results of the analysis of alcohol extraction (Table 3 and Figure 7) peak 1 of track 1 corresponds to the peak of standard sample β-escin (R_f=0.45, track 2). The dominant substances of the sample have not been identified.

Method GC/MS from fresh and dried rhizomes of *G. pentaphyllum* (Thunb.) Makino were studied using several solvents (Tables 4-5, Figures 7-9 and 10-12).

A qualitative analysis of sample No 1 revealed 9 compounds (Figures 7, Table 4), of which the structure has been established in 3 substances related to steroid compounds (*14-.beta.-H-pregna*), flavonoids (*4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy*) and heterocyclic compounds (*2,2,7-trimethyl-2,3-dihydro-1-benzofuran*); the dominant substances have not been identified.



A)



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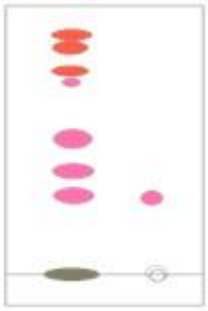

C)

Figure 6. Alcohol extraction densitogram of the rhizome of *G. pentaphyllum*:
A) red line - extraction, blue line - standard sample solution of β-escin; B) chromatograms;
C) diagram

Table 3. Results of densitometric signals alcohol extraction of the rhizome of *G. pentaphyllum*

No	**Track 1**		**Track 2**	**Substance**
Peak	R_f±0,02	Peak Area	R_f±0,02	
1	0,46	4935	0,45	β-escin
2	0,53	937	-	-
3	0,62	4074	-	-
4	0,81	1005	-	-
5	0,85	2310	-	-
6	0,90	6151	-	-
7	0,94	11262	-	-

Note: R_f - retention factor

A qualitative analysis of sample No 2 revealed 8 compounds (Figures 8, Table 4), of which the structure is established by 2 substances: monoterpene (*p-Cymene*) and phenylpropanoid (*Eugenol*). The dominant compounds in the sample were not identified. It should also be noted that the eugenol content in the sample is 6.421%.

A qualitative analysis of sample No 3 revealed 15 compounds (Figures 9, Table 4), of which the structure has been established in 7 substances belonging mainly to the group of terpenes (monoterpene derivatives from the group of aromatic compounds) and the flavonoid (*4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy*); the dominant compounds identified in the sample are *D-Limonene* (4659403 and 8.161%) and *Eugenol* (3393663 and 5.944%).

Table 4. Results of metabolomic screening of *G. pentaphyllum* rhizomes (dry raw materials, n=5)

No	Name of the compound	Ret Time, min	Extractions					
			Peak Area	Content in the sample (% of total)	Peak Area	Content in the sample (% of total)	Peak Area	Content in the sample (% of total)
			chloroform (sample No 1)		petroleum ether (sample No 2)		hexane (sample No 3)	
1	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(1-methylethyl)- *Dehydrosabinene*	3,241	-	-	-	-	355075	0,622
2	D-Limonene	3,780	-	-	-	-	4659403	8,161
3	Eucalyptol	3,827	-	-	-	-	1047848	1,835
4	p-Cymene	4,073	-	-	1840659	1,875	-	-
5	L-Menthone	5,226	-	-	-	-	676432	2,266
6	.alpha.-Terpinyl acetate	7,666	-	-	-	-	1350405	2,365
7	Eugenol	7,770	-	-	1005432	6,421	3393663	5,944
8	2,2,7-trimethyl-2,3-dihydro-1-benzofuran	8,678	614289	0,613				
9	3,5-ditert-butylphenyl)methanol	9,689	-	-	-	-	835701	1,464
10	14-.beta.-H-pregna	17,528	1806165	1,802	-	-	-	-
11	4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy *Luteolin 6,8-di-C-glucoside*	19,230	3460972	3,453	-	-	1866305	3,269

Note: Ret Time - retention time

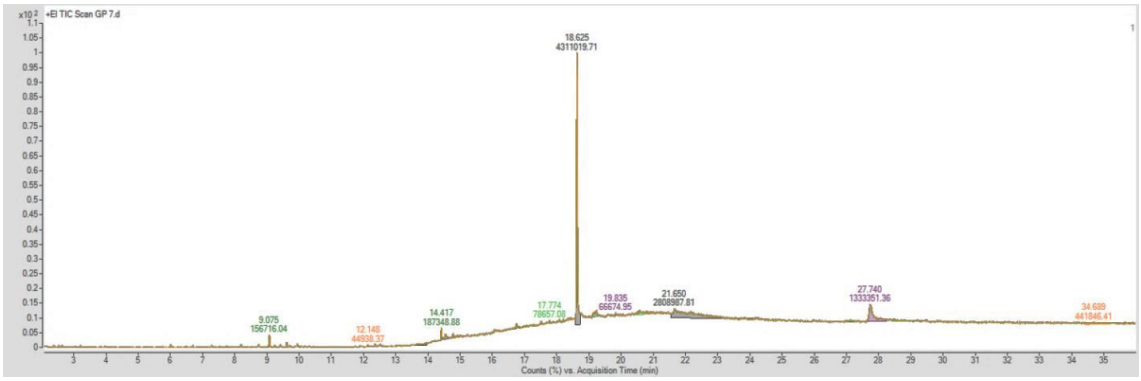

Figure 7. GC/MS chromatogram of chloroform extraction (sample No 1) from *G. pentaphyllum* rhizomes (dry raw materials)

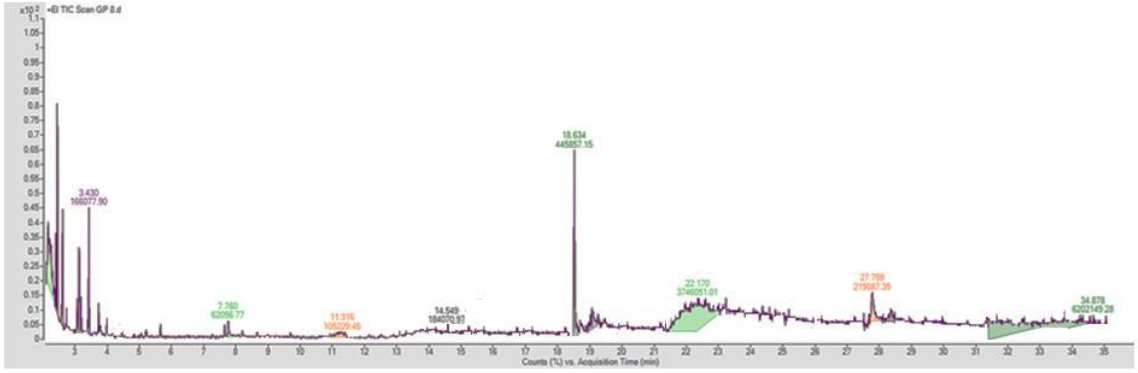

Figure 8. GC/MS chromatogram of extraction from petroleum ether (sample No 2) from *G. pentaphyllum* rhizomes (dry raw materials)

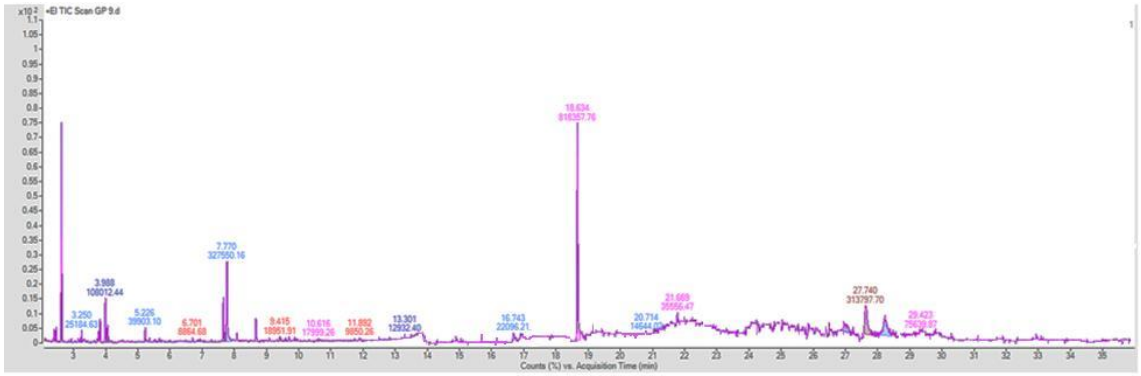

Figure 9. GC/MS chromatogram of hexane extraction (sample No 3) from *G. pentaphyllum* rhizomes (dry raw materials)

A comparative qualitative analysis of the phytochemical profile of samples from extracts of *G. pentaphyllum* rhizomes (dry raw materials) using all solvents (chloroform, petroleum ether and hexane) showed that approximately equal amounts of substances are released in the samples. At the same time, it turned out that of the identified 12 compounds isolated by all solvents, there are no substances that would be found in all three samples. There were also no matches in samples No 1 and No 2 and in samples No 1 and No 3 and in samples No 2 and No 3 for 1 compound: *4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy* (19,230 min) and *Eugenol* (7,770 min) and 9 substances were detected only singly in different samples.

A qualitative analysis of sample No 4 revealed 11 compounds (Figure 10, Table 5), of which the structure of 5 organic compounds from various groups has been established: hydrocarbons, including an alkyl carboxylic acid derivative (*pentanoic acid, 5-hydroxy-, 2,4-di-tert-butylphenyl esters*), a heterocyclic (*2,2,7-trimethyl-2,3-dihydro-1-benzofuran*) and a steroid compound (*14-.beta.-H-pregna*), which is dominant in the sample), as well as the flavonoid (*4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy*).

Table 5. Results of metabolomic screening of *G. pentaphyllum* rhizomes (fresh raw materials, n=*5*)

No	Name of the compound	Ret Time, min	Extractions					
			Peak Area	Content in the sample (% of total)	Peak Area	Content in the sample (% of total)	Peak Area	Content in the sample (% of total)
			chloroform (sample No 4)		petroleum ether (sample No 5)		hexane (sample No 6)	
1	2-Hexylcyclobutanone	2,305	-	-	-	-	636709	0,394
2	2-Oxepanone, 5-methyl-	2,314	-	-	918610	0,547	-	-
3	meso-2,5-Dimethyl-3,4-hexanediol	2,740	-	-	-	-	6338399	3,918
4	Heptane, 2,2,3,5-tetramethyl-	3,071	-	-	-	-	2633143	1,627
5	Heptane, 2,2,4,6,6-pentamethyl-	3,647	-	-	-	-	2623934	1,622
6	Nonane, 2,2,4,4,6,8,8-heptamethyl-	4,073	-	-	-	-	5254752	3,248
7	Nonane, 3-methyl-5-propyl-	4,234	-	-	-	-	4663109	2,882
8	Carbonic acid, eicosyl vinyl ester	4,357	-	-	-	-	3866900	2,390
9	Cyclohexane, 1,2-dimethyl-3-pentyl-4-propyl-	4,829	-	-	-	-	2676748	1,654

10	Oxalic acid,1-methyl pentadecyl ester	5,085	-	-	-	-	1703051	1,053
11	(E)-2-nonadecene	5,141	-	-	-	-	1143599	0,707
12	4,6-Dimethyldecan-1-ol	5,453	-	-	-	-	1023571	0,515
13	Naphthalene,decahydro-1,6-dimethyl	6,172	-	-	-	-	2291292	1,416
14	Naphthalene, decahydro-1,5-dimethyl-	6,238	-	-	-	-	2057744	1,272
15	.alpha-Terpinyl acetate	7,666	-	-	638386	0,380	-	-
16	2,2,7-trimethyl-2,3-dihydro-1-benzofuran	8,715	458192	0,272	-	-	-	-
17	Octadecane, 1-iodo-	9,415	802982	0,477	-	-	-	-
18	Pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters	9,614	646304	0,384	-	-	-	-
19	14-.beta.-H-pregna	16,753	3770164	2,239	9060143	5,394	-	-
20	4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy *Luteolin 6,8-di-C-glucoside*	19,139 19,147	2299528	1,365	9430824	5,615	-	-

Note: Ret Time - retention time

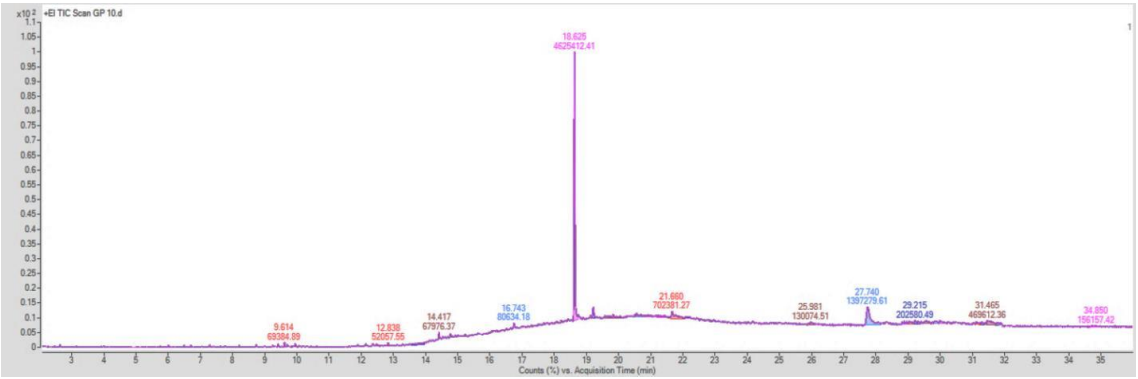

Figure 10. GC/MS chromatogram of chloroform extraction (sample No 4) from *G. pentaphyllum* rhizomes (fresh raw materials)

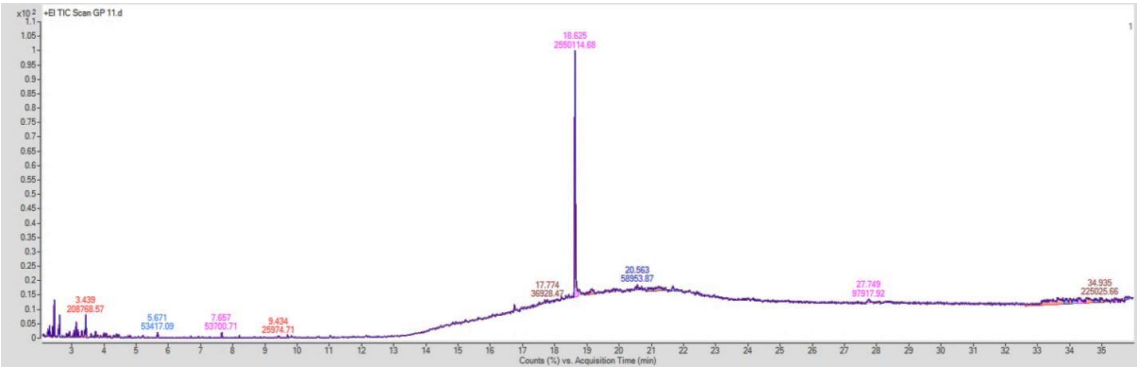

Figure 11. GC/MS chromatogram of extraction from petroleum ether (sample No 5) from *G. pentaphyllum* rhizomes (fresh raw materials)

A qualitative analysis of sample No 5 revealed 9 compounds (Figure 11, Table 5), of which the structure of 4 organic compounds from various groups has been established: hydrocarbons, terpenes, steroids and flavonoids. The data on the "peak area" and the content of substances in the sample showed that the dominant compounds were not

identified. It should also be noted that the content of the steroid compound is *14-.beta.-H-pregna* in the sample is 5.394% and the flavonoid *4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy* – 5,615%.

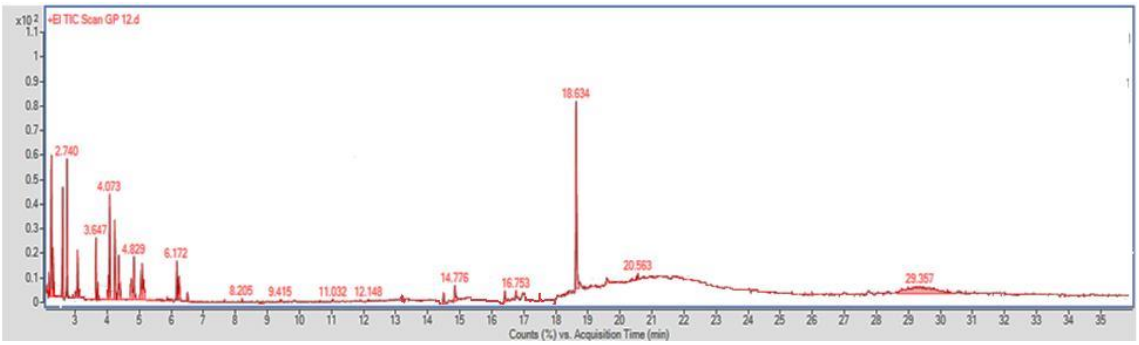

Figure 12. GC/MS chromatogram of hexane extraction (sample No 6) from *G. pentaphyllum* rhizomes (fresh raw materials)

A qualitative analysis of sample No 6 revealed 14 compounds (Figure 12, Table 5), of which the structure was established in 13 substances belonging to the group of hydrocarbons (cyclic, acyclic, dicarboxylic acids and their derivatives), among which the hydrocarbons are the dominant of the identified substances in the sample: *meso-2,5-dimethyl-3,4-hexanediol* (6338399 and 3.918%), *nonane*, 2,2,4,4,6,8,8-heptamethyl- (5254752 and 3.248%), *nonane, 3-methyl-5-propyl* (4663109 and 2.882%) and *carbonic acid, eicosylvinyl ether* (3866900 and 2.390%).

A comparative qualitative analysis of the biochemical profile of extracts from *G. pentaphyllum* rhizomes (fresh raw materials) using solvents: chloroform, petroleum ether and hexane showed that the largest amount of substances passes into hexane fractions. However, it turned out that there were no compounds out of the identified 20 that were found in all three samples. No matches were found in samples No 4 and No 6, No 5 and No 6 and No 4 and No 5 – 2 compounds of *14-beta.-H-pregna* (16,753 min), *4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy* (19,139 - 19,147 min) and 18 substances were detected only singly in different samples.

A comparative qualitative analysis of the biochemical profile of extracts from *G. pentaphyllum* rhizomes using fresh and dried raw materials also turned out to be ambiguous. Thus, in extracts from chloroform, 9 substances were isolated from dry raw materials and 3 were recognized, 11 were isolated from fresh raw materials and 5 were recognized. The identified substances belonged to different classes of organic compounds. Thus, in dry raw materials, the structure was found in compounds related to steroid compounds (*14-beta.-H-pregna*), flavonoids (*4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy*) and heterocyclic compounds (*2,2,7-trimethyl-2,3-dihydro-1-benzofuran*). In fresh raw materials (sample No 4), the following were identified: alkyl carboxylic acid derivative (*pentanoic acid, 5-hydroxy-, 2,4-di-tert-butylphenyl esters*), heterocyclic (*2,2,7-trimethyl-2,3-dihydro-1-benzofuran*) and steroid compound (*14-beta.-H-pregna*), as well as the flavonoid *4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7-dihydroxy*. Thus, it can be said that the identified compounds of sample No 1 are repeated in sample No 4.

In extracts from petroleum ether, 8 substances were isolated from dry raw materials and 2 were recognized, 9 were isolated from fresh raw materials and 4 compounds were also recognized, among which there were no repetitions.

In hexane extracts from dry *G. pentaphyllum* raw materials, 15 substances were isolated and 7 were recognized, 14 were isolated from fresh raw materials and 13 were recognized. The identified substances belonged to different classes of organic compounds, among which there were no repetitions.

4. Conclusion

Fingerprints of substances in a plant object were analyzed using TLC and HETLC methods and three compounds were identified: flavonoid nature - rutin, quercetin and saponin nature - β-escin, which was first established in *G. pentaphyllum* raw materials.

Metabolomic screening of dry and fresh G. pentaphyllum rhizomes by gas chromatography showed the component composition of various groups of substances. In total, 27 compounds were identified in the samples, of which terpenoids (monoterpenes, sesquiterpenes, diterpenes, their derivatives and some representatives of aromatic compounds) were detected for the first time: *eucalyptol*, *alpha.-terpenyl acetate*, *D-Limonene*, *isocariophyllene*, *p-cymene*, *L-menthone*, *3,5-ditret-butylphenyl)methanol*, *dehydrosabinene*, *naphthalene*, *decahydro-1,6-dimethyl*, *naphthalene*, *decahydro-1,5-dimethyl-*, *eugenol*; flavonoid - *4H-1-Benzopyran-4-one,2-(3,4-dihydroxy-phenyl)-6,8-di-.beta.-D-glucopyranosyl-5,7- dihydroxy*; steroid compound *14-beta.-H-pregna*; alkyl carboxylic acid derivatives - *pentanoic acid, 5-hydroxy-, 2,4-di-t-butylphenyl esters* and *carbonic acid, eicosylvinyl ether*; heterocyclic compound - *2,2,7-trimethyl-2,3-dihydro-1-benzofuran*.

The component composition of the recognized substances in the rhizomes of *G. pentaphyllum* suggests the possibility of their use as an additional source of raw materials for the producing plant. The identified components may also cause cytotoxic, antibacterial, adaptogenic and other effects that require further detailed preclinical and clinical studies.

Funding

This work was supported by the Bashkir State Medical University Strategic Academic Leadership Program (PRIORITY-2030).

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