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BIOLOGICALLY ACTIVE SUBSTANCES OF THE PLANT PHOLOMOIDES SPECIOSA

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Phlomoides speciosa, algin acid, flavonoids, chalcones, aurons, anthraquinones, anthocyanins, xanthones

Annotation
The article describes qualitative reactions of the content of biologically active substances of the Phlomoides speciosa plant. For the first time, starch and alginic acid were isolated from an alcoholic extract of the aerial part of the plant using column chromatography on active carbon.

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Introduction. In the literary sources, 170 species of the Phlomoides genus are mentioned in the flora of the earth, and 59 species in the flora of Central Asia. Of these, 43 species are found in the flora of Uzbekistan, 41 in the flora of Iran, 37 in the flora of Kyrgyzstan, 30 in the flora of Kazakhstan, 27 in the flora of Tajikistan, and 12 in the flora of Turkmenistan. There are 8 species of the Phlomoides genus in Namangan region of Uzbekistan. These plant species are ephemeral plants [1].

Carbohydrates, alkaloids, flavonoids, iridoids, essential oils, saponins, coumarins, ascorbic acid, and microelements such as iron, magnesium, copper, zinc, manganese, nickel, and titanium were found in Phlomoides speciosa and Phlomoides angreni species of the Phlomoides Moench genus [2] ***Phlomoides Speciosa*** is a handsome looking phlomoides. Perennial herb with thick, tangled hairs. Height up to 40-50 cm. The leaves are pinnately divided. Ball-shaped, head-shaped. It blooms from the end of April. The flowers are arranged in clusters. The calyx is bell-shaped, with sharp teeth, 20-25 mm long. The flower is yellow, 40-50 mm long, the upper lip is often red. The plant is found mainly in the Tyan-Shan and Pamir-Alai mountain ranges in stony places and along roadsides.

Phlomoides speciosa contains 0.35% alkaloids, stachydrine flavonoids, alkaloids in the stem, 0.75% essential oil in the aerial parts, 0.12% alkaloids, flavonoids and coumarins in the leaves, saponins in the stems, and alkaloids in the fruits. It grows sufficiently in the Fergana Valley [3, 4].

Research results: In May 2022, Phlomoides speciosa was collected from the foothills of the Poromon pass in the Yangikurgan district of Namangan region for analysis of the plant's properties.

The biologically active compounds of Phlomoides speciosa were determined by qualitative tests. The most reliable quality reactions were selected for the analysis of the plant's properties, and extracts were obtained from the plant using water and different concentrations of water-ethanol solutions. The extracts were then analyzed based on literary sources.

The results of experiments on biologically active compounds of Phlomoides speciosa extract are presented in Table 1. The extract of Phlomoides speciosa contained phenolic compounds such as flavonoids, saponins, tannins, and alkaloids.



Figure 1.*Phlomoides Speciosa*

Results of determining the group of biologically active compounds in Phlomoides speciosa plant extract based on quality analysis

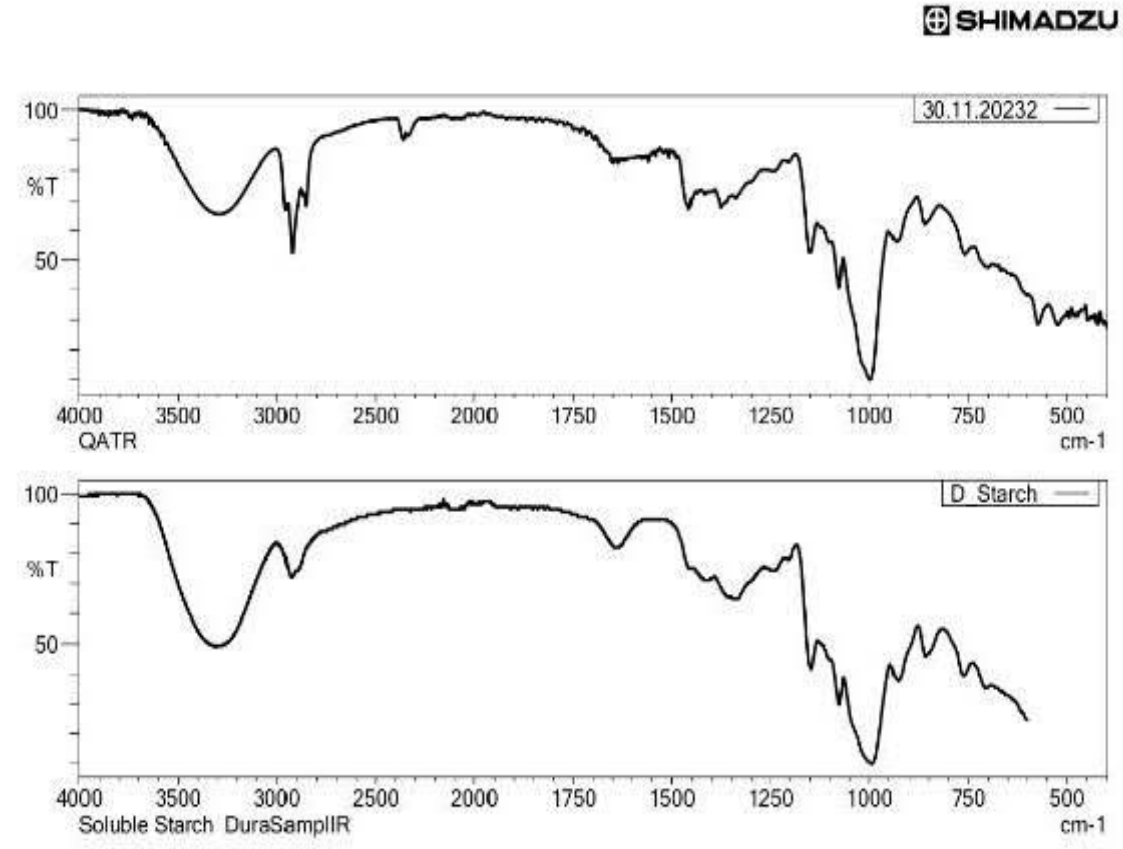
Table 1.

Quality reaction type	**The result of the reaction**	**Extrogen**			
		Water	**50% Alchohol**	**75% Alchohol**	**96% Alchohol**
Flavonoids					
Bryant's cyanidin test	Separation into 2 phases occurred. The aqueous phase is more stained	++	++	+++	+++
		The superiority of glycosides over aglycones			

Iron (III)-chloride solution	Formation of black-green color	+	+	++	++
Concentrated HCl and heat Zn powder	Red color solution	+	+	++	++
Heating with ammonia	Golden yellow color	+	+	++	++
		Flavone, the predominance of flavonones			
With a 10% solution of alkali	With the formation of a yellow color	++	++	++	++
Tannin substances					
With protein solution	Formation of turbidity	+++	++	++	+
Saponins					
Foaming	Stable and abundant foaming	++	+++	+++	+++
HCl and NaOH in test tubes	Foaming	Continuous foaming in both tubes Triterpene saponins			
Alkaloids					
Reactive Dragendorff (precipitating reagent)	Orange-brown precipitate	++	++	++	++

Note: *"The number of the "+" sign indicates the intensity of staining, the height of the precipitate and foam.*

This is a scientific experiment involving the analysis of biological substances. 20 grams of plant material was taken and 50% ethanol was added to it in a 10:1 ratio with CaCO3. The mixture was left for 20 minutes before being filtered. The filtrate was then boiled down and the ethanol was separated from the water using a water bath. The resulting solution was then activated with charcoal and left to cool. After 20 minutes in the freezer, white sediment was observed in the solution. This sediment was separated from the liquid and was shown to react positively to starch. Additionally, iodine was added to the solution and the resulting colour was similar to potato starch. The sample was then analysed using IQ spectroscopy and it was confirmed that the sediment was indeed starch.



	Score	Library	Name	Comment
1	942	4 - ATR-Organic2	D_Starch	Soluble Starch DuraSamplIR
2	942	4 - ATR-Organic2	D_Starch	Soluble Starch DuraSamplIR
3	926	174 - ATR-Polymer2	D_Tencel	Tencel(LENZING Coorporation) DuraSamplIR-II
4	915	43 - A_FoodAdditives2	A_Microfibrillated Cellulose_100G-4	Microfibrillated Cellulose(Product name:CELISH FD-100GCSales origin;Daicel Chemical Industries Ltd.)@DuraSamplIR2(diamond)
5	914	41 - A_FoodAdditives2	A_Microcrystalline Cellulose_102-4	Microcrystalline Cellulose(Product name:VIVAPUR102CSales origin;TOAKASEI CO.,LTD.)@DuraSamplIR2(diamond)
6	914	2 - ATR-Organic2	D_Sucrose	Sucrose DuraSamplIR
7	914	2 - ATR-Organic2	D_Sucrose	Sucrose DuraSamplIR
8	913	19 - A_FoodAdditives2	A_Carboxymethyl Cellulose Calcium-4	Carboxymethyl Cellulose Calcium(Product name;E.C.G-FACSales origin;Gotoku CHEMICAL CO.,LTD.)@DuraSamplIR2(diamond)
9	912	31 - ATR-Organic2	D_PropyleneGlycol	PropyleneGlycol DuraSamplIR
10	912	11 - ATR-Polymer2	D_Cellulose4	Bemberg(Cupra) DuraSamplIR-I
11	912	31 - ATR-Organic2	D_PropyleneGlycol	PropyleneGlycol DuraSamplIR

Figure 2. IQ spectrum of starch.

On the coal column, a light brown layer was formed, which was separated and cleaned with petroleum. After drying, the matter was treated and an IQ spectrum was obtained. By comparing the spectrum with the information on the base spectrum, it was determined that the substance was algene acid (figure 3).

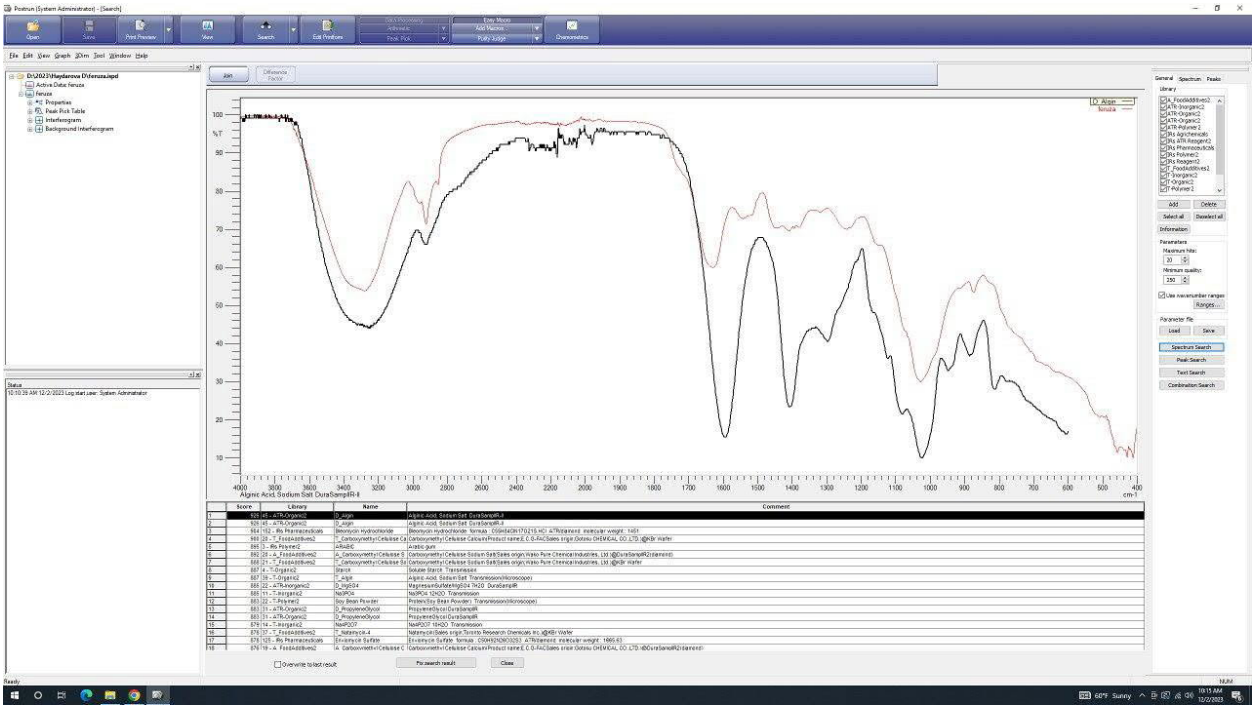


Figure 3. IQ spectrum of algene acid.

During our research, a part of the composition of the plant's volatile component was also identified. 27 substances were found from the upper part of the Phlomoides speciosa plant. The ether extract was tested at the Biorganic Chemistry Institute laboratory of O'z FA and the following spectra were

obtained (figure 4).

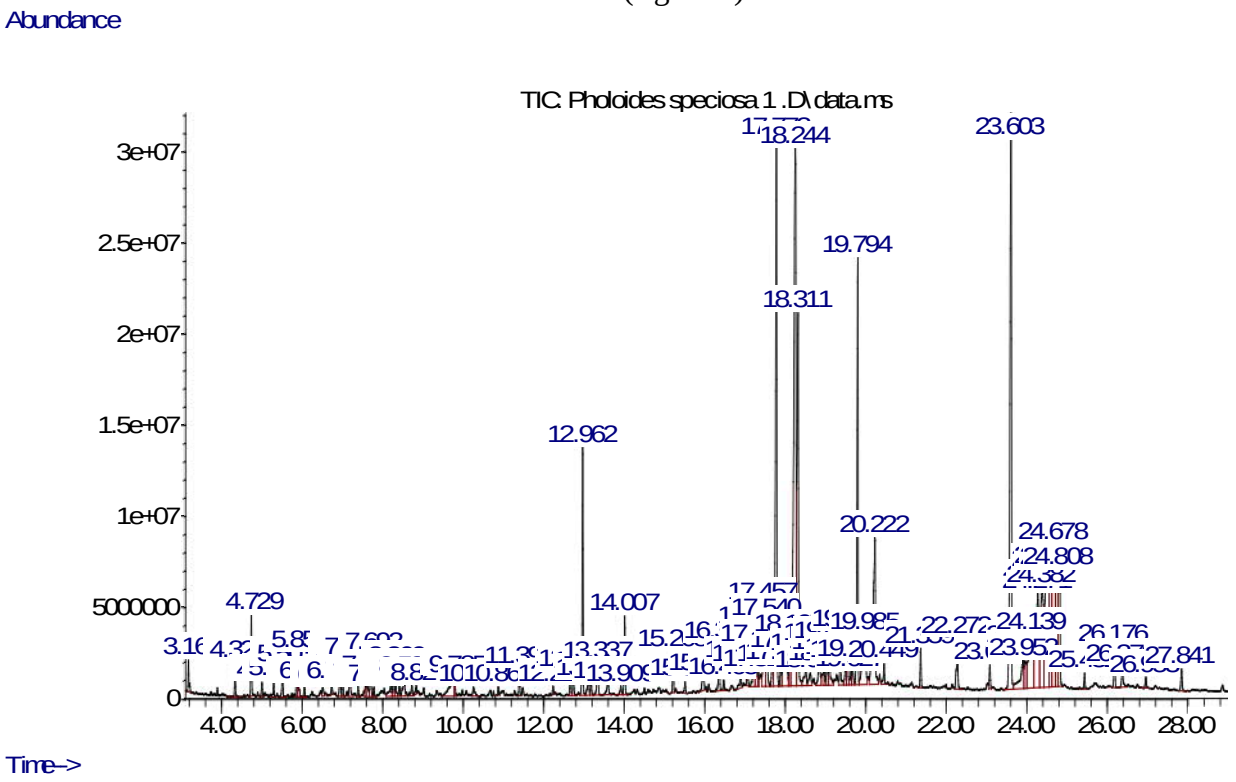


Figure 4. GX-MS spectrum of ether extract obtained from the upper part of Phlomoides speciosa plant.

Table 2. Compounds and their percentage content in the ether extract of Phlomoides speciosa.

Table 2.

No:	Essential oil composition	The percentage of the substance in the essential oil (%)	No:	Essential oil composition	The percentage of the substance in the essential oil (%)
1.	α – pinene	0.62	17.	1,2-Benzenedicarboxylic acid, bi (2-methylpropyl) ester	19.50
2.	D – Limonene	0.84	18.	n-Hexadecanoic acid	3.99
3.	γ -Terpinene	0.67	19.	Octadecane, 1-chloro-	0.66
4.	Phenol, 2,6-bi (1,1-dimethylethyl)	1.34	20.	4a, 7-methano-4aH-naphth [1,8a-b]oxirene, octahydro-4,4,8,8-tetramethyl-	0.94
5.	Nonadecane	1.24	21.	Hexadecane, 1-chloro-	0.67
6.	Azulene, 1,2,3,3a,4,5,6,7-octahydro u-1,4-dimethyl-7-(1-methylethenyl) -, [1R- (1.alpha,	0.86	22.	9-Octadecanoic acid (S)-, methyl ether	1.69

	3a.beta, 4.alpha. , 7.beta.)] -				
7.	Hexadecane	0.65	23.	Methyl stearate	9.38
8.	Hexadecane	0.87	24.	Octadecanoic acid	4.35
9.	Phthalic acid, monodecyl ether	1.07	25.	Hexacosane	1.91

As a result of the tests, the highest amounts of the following compounds were identified: 1,2-Benzene dicarboxylic acid, di(2-methylpropyl) ester - 19.50%, Di(2-ethylhexyl) phthalate - 14.23%, Methyl ester of hexadecanoic acid - 13.71%, Methyl stearate 9.38%, Heptadecane - 5.85%, Octadecanoic acid - 4.35% (Table 1).

Experimental part:

Biological active compounds were identified in the extract prepared from plants using paper and thin-layer chromatography methods. For chromatographic analysis of the plant, a spirit extract was prepared. For this purpose, 1 g of Phlomoides Speciosa plant's dried flowers were taken in a 25 ml flask and 10 ml of alchohol was added on top. The flask was cooled and boiled in water for 10 minutes. After the extract was cooled, it was filtered through a paper filter. 0.1 ml of the filtrate and the "witness" flavonoids' alchohol extract were spotted on the starting line of "Silufol" plastic film using a capillary needle or a special pipette, at a distance of 2 cm from each other, and dried in air. Then, the plastic film was placed onto a chromatographic column filled with n-butanol-acetic acid-water (4:1:5) or 15% acetic acid, for 30-40 minutes. After removing the plastic film, it was dried in air and the spots were identified under UV light.

Adding 1-2 ml of a 10% alkali solution to the Phlomoides speciosa plant caused it to turn brown, but when a 2% CuSO4 solution was added, the color turned blue. This indicates the presence of a dipeptide in the plant.

To analyze the plant further, a 50% alcohol extract of Phlomoides speciosa was divided into 3 test tubes with 5ml each. When 2ml of a 10% alkali solution of KOH was added to the first test tube, the color of the solution turned yellowish-brown. This indicates the presence of anthocyanins, chalcones, and aurones in the plant.

When the second test tube was treated with NH3, the color of the solution turned yellowish-green, indicating the presence of C=O-containing compounds such as flavonoids, pigments, anthocyanins, anthraquinones, chalcones, aurones, and xanthones.

In the third test tube, the addition of FeCl3 caused the solution to turn reddish-brown, indicating the presence of flavonoids.

Determination of essential oil content. To extract the plant's ether, the upper part of the plant weighing 200g was hydrodistilled for 2 hours using a Clevenger apparatus. The resulting distillate was extracted with dichloromethane, dried with anhydrous sodium sulfate, and stored at -4°C until analysis. The ether extract of Phlomoides speciosa was analyzed using Agilent 5977A mass-selective detector equipped with a VF-Wax CP 9205 column (100% polyethylene glycol, 30 m × 0.25 mm, film thickness 0.25 μm, Agilent Technologies, the Netherlands) using Agilent 7890B gas chromatography. The results were identified using the Wiley Registry of Mass Spectral Data (9th edition), NIST Mass Spectral Library (2011), and other catalogs.

Conclusion:

An ethanol extract was obtained from the upper part of the Phlomoides speciosa plant and anthraquinones, chalcones, aurones, flavonoids, pigments, anthocyanins, anthraquinones, flavonoid trioxysilanes, and starch were found to be present. The ethanol extract was fractionated using activated charcoal adsorbents, and starch and alginic acid were separated from the Phlomoides speciosa plant. When the volatile components of the plant were analyzed using the GSX-MC mass spectral database, 27 modes were identified. Phlomoides speciosa is a medicinal plant and can be used to produce biologically active food supplements**.**

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