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Phytochemical Profiling and Nutritional Evaluation of Phlomoides Ambigua (Lamiaceae) From the Flora of Uzbekistan

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Abstract: This study presents a comprehensive phytochemical investigation of Phlomoides ambigua distributed in the flora of Uzbekistan. The contents of water-soluble vitamins and flavonoids were determined using high-performance liquid chromatography (HPLC), while the total protein content was evaluated by the Kjeldahl method. The results revealed significant variation in vitamin and flavonoid profiles, indicating species-specific biochemical differences. In particular, several vitamins and phenolic compounds were detected at relatively high concentrations, confirming the biological activity of the plant. The relatively high protein content further demonstrates its nutritional value. These findings provide a scientific basis for the pharmacological and nutritional potential of this species.

Keywords: Phlomoides ambigua, phytochemical analysis, HPLC, flavonoids, protein content.

Introduction: Genus Phlomoides one of the largest polymorphic genera belonging to the Lamiaceae family, the range of species of the genus extends to Central Europe and the Russian Far East, and the main centers of species diversity are China, Southern Europe and Central Asia, the mountainous regions of Iran (Iran and Afghanistan) and the Mediterranean regions. There are 150-170 species of the genus in the world, 67 species in Central Asia, 43 species in the flora of Uzbekistan (Salmaki et al., 2012; Gulomov et al., 2022).

Despite their numerous medicinal properties, the chemical composition of species belonging to the Phlomoides genus distributed in Uzbekistan remains insufficiently studied. In recent years, however, research on the phytochemistry of this genus has intensified considerably. In particular, species of Phlomoides are known to be rich in flavonoids, phenolic compounds, essential oils, and proteinaceous substances (Khaydarova, 2022).

During 2018–2020, the macro- and microelement composition of Phlomoides speciosa, Phlomoides isochila, and Phlomoides nuda collected from the Namangan region was investigated (Khaidarova et al., 2021). Furthermore, physico-chemical analysis of the aerial parts of Phlomoides canescens distributed in the Fergana Valley revealed the quantitative contents of nitrogen, total protein, amino acids, and flavonoids. Notably, significant levels of essential amino acids such as glutamine (3.38 mg/g), tryptophan (2.39 mg/g), threonine (1.56 mg/g), and histidine (1.50 mg/g) were identified (Rahimova et al., 2021).

In addition, studies on the aerial parts of Phlomoides nuda and Phlomoides speciosa using physico-chemical methods determined both qualitative and quantitative

compositions of vitamins and amino acids. In P. nuda, glutamine (2.05 mg/g), proline (1.95 mg/g), and histidine (1.90 mg/g) were detected, whereas P. speciosa showed high levels of asparagine (4.38 mg/g), glycine (4.38 mg/g), and tyrosine (3.94 mg/g) (Rahimova et al., 2023).

Recent investigations have focused on Phlomoides sogdiana and Phlomoides anisochila. The results demonstrated that P. sogdiana possesses the highest protein content and amino acid concentration among the studied species. Moreover, the content of B-group vitamins was also maximal in P. sogdiana, reaching 32.802821 mg/g, whereas in P. anisochila it was 19.484143 mg/g (Kholbutayeva et al., 2026).

The next phytochemical analyses are focused on Phlomoides ambigua (Popov ex Pazij & Vved.) Adylov, Kamelin & Makhm., which occurs on rocky and stony–gravel slopes in the middle mountain belt at elevations of 1900–2900 m. This species is mainly distributed in Central Asia, including the Pamir-Alay (the Turkestan and Malkuzar ranges), the Nurota Mountains (Koytash Mountains), and the Hissar Range (the Jitjik River area). Phlomoides ambigua is recognized as a national endemic species (Fig.1).

The objective of the study was to determine the vitamin, flavonoid, and protein composition of the species within the genus.

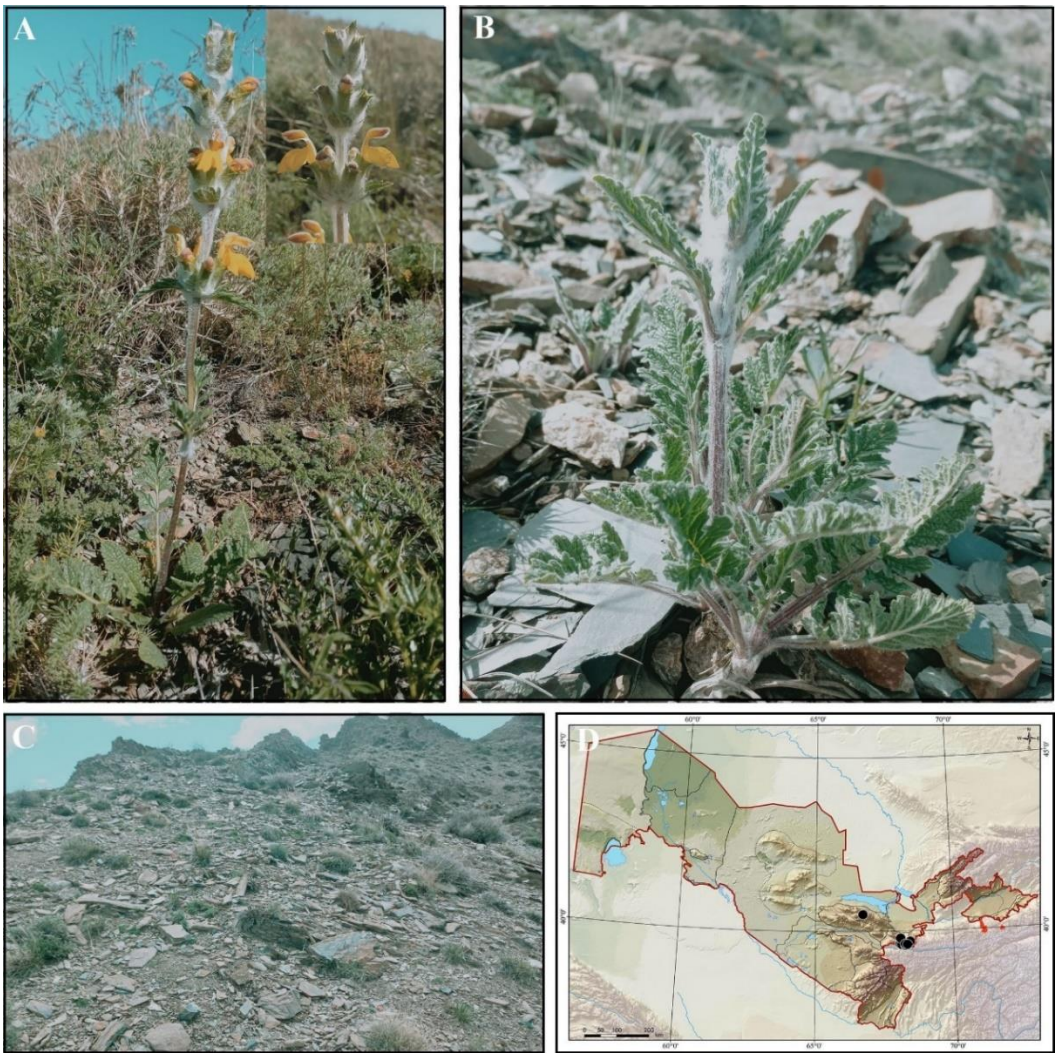

Figure 1. A-B) P. ambigua (By photo R.Gulomov); C) Ecology; D) distribution areas

METHODS

Vitamin extraction. For analysis, 1.0 g of the finely ground plant sample was extracted with 40% ethanol. The extraction was carried out under reflux conditions for 1 hour, followed by continuous stirring at room temperature for 2 hours. The residue was re-extracted twice with 40% ethanol, and the filtrates were combined and brought to a final volume of 100 mL. The obtained solution was centrifuged at 7000 rpm for 10 minutes, and the supernatant was collected for analysis.

Working standard solutions with a concentration of 1 mg/mL were prepared for each vitamin (50 mg of standard substance was dissolved in 50 mL of 40% ethanol). Quantitative determination was performed using the external standard method.

Chromatographic conditions: Instrument: Agilent 1200 (with autosampler); Column: Eclipse XDB C18 (4.6 × 250

mm, 5 µm); Detector: DAD, 250 nm; Flow rate: 0.8 mL/min; Injection volume: 5 µL; Column temperature: 25°C; Mobile phase: acetate buffer–acetonitrile (gradient mode).

Gradient program: 0–5 min – 96:4; 6–8 min – 90:10; 9–15 min – 80:20; 15–17 min – 96:4.

For the extraction of flavonoids. The dried aerial parts of Phlomoides ambigua were used as the study material. A precisely weighed 1.0 g sample was extracted with 70% ethanol. The extraction was carried out at 70°C for 1 hour using a magnetic stirrer under reflux conditions. The mixture was then allowed to stand at room temperature for 2 hours. The residue was re-extracted twice with 70% ethanol, and the combined filtrates were brought to a final volume of 100 mL. The resulting solution was centrifuged at 6000 rpm for 20 minutes, and the clear supernatant was collected for analysis.

Chromatographic analysis was performed under the following conditions:

Instrument: Agilent 1200 (with autosampler); Column: Eclipse XDB C18 (4.6 × 250 mm, 5 µm); Detection: diode-array detector (254 and 276 nm); Flow rate: 0.8 mL/min; Injection volume: 10 µL; Column temperature: 30°C; Mobile phase: phosphate buffer–acetonitrile system (gradient mode).

Quantitative determination was carried out based on peak area using the external standard method.

For the extraction of proteins. The total protein content was evaluated based on nitrogen determination using the Kjeldahl method. The principle of the method is the decomposition of organic matter in the presence of concentrated sulfuric acid, conversion of the resulting ammonium salts into ammonia, distillation of ammonia into an acid solution, and its subsequent quantitative determination by titration.

For analysis, an accurately weighed portion of the previously ground and homogenized plant sample was taken with an error not exceeding 0.1%. The sample was transferred into a Kjeldahl flask, and the procedure was carried out according to the methodological guidelines.

The mass fraction of nitrogen (X, %) was calculated using the following formula:

$$X = ((V_0 - V_1) \times K \times 0.0014 \times 100) / M$$

where: V_0 – volume of 0.1 mol/L NaOH used to titrate 0.05 mol/L H_2SO_4 in the blank experiment (mL); V_1 – volume of 0.1 mol/L NaOH used for titration of the test solution (mL); K – correction factor; 0.0014 – nitrogen equivalent corresponding to 1 mL of 0.05 mol/L sulfuric acid; M – sample mass (g).

RESULTS AND DISCUSSION

The analysis of the results showed that the applied high-performance liquid chromatography (HPLC) method ensured reliable separation and reproducible determination of vitamins. The acetate buffer–acetonitrile system created an effective separation medium for vitamins of a polar nature. The content of water-soluble vitamins (in µg/g) (Tab.1; Fig. 2):

Table. 1. Comparison results of the vitamin composition and content of the species

Vitamin	*P. ambigua*	*P. nuda* (Rahimova et al., 2023)	*P. speciose* (Rahimova et al., 2023)	*P.anisochila* (Kholbutayeva et al.,2026)	*P.sogdiana* (Kholbutayeva et al., 2026)
Concentration, µg/g					
B-1	3.69	1,877	1,0382	1,023151	1,023151
B-2	19.55	0,019	1,4440	9,00832	9,00832
B-6	28.85	1,267	1,8146	4,082808	4,082808

B-9	85.79	0,628	0,024	1,172807	1,172807
PP, B-3	2.09	0,204	0,1668	0,890171	0,890171
C	6.95	1,524	2,0784	4,963739	4,963739

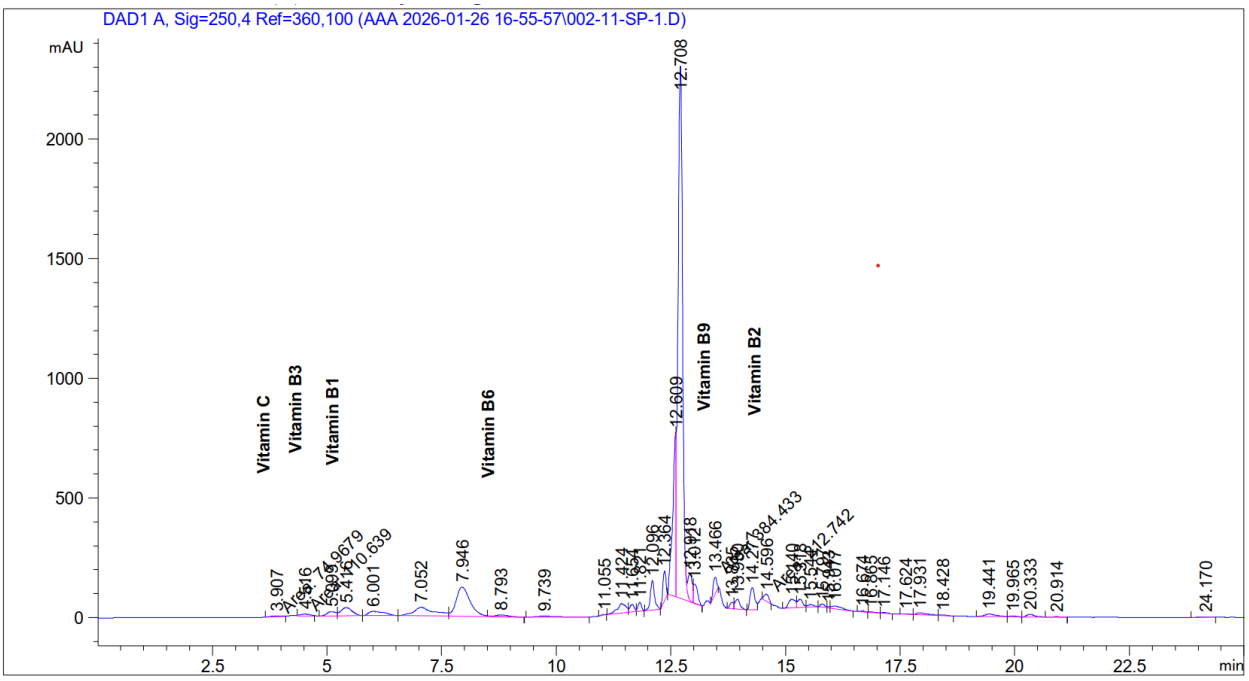

Figure 2. Vitamin chromatogram

The results of the HPLC analysis showed that salidroside (29.51 mg/g) was identified as the dominant component in the P. ambigua sample. This compound belongs to the group of phenolic glycosides and is characterized by antioxidant and adaptogenic properties. Quercetin was recorded at a concentration of 2.18 mg/g.

The variability in flavonoid composition may be associated with genetic factors, environmental conditions, soil–climatic characteristics, and the phenological stage. The applied reversed-phase HPLC method enabled efficient separation of components and reliable quantitative evaluation (Tab.2; Fig. 3).

Table. 2. The determined content of flavonoids (in mg/g)

Flavonoids	***P. ambigua***
	Concentration, µg/g
Dihydroquercetin	0.72
Rutin	1.11
Rosavin	3.4
Quercetin	2.18

Salidroside	29.51
Luteolin	0.98
Seneroside	1.72

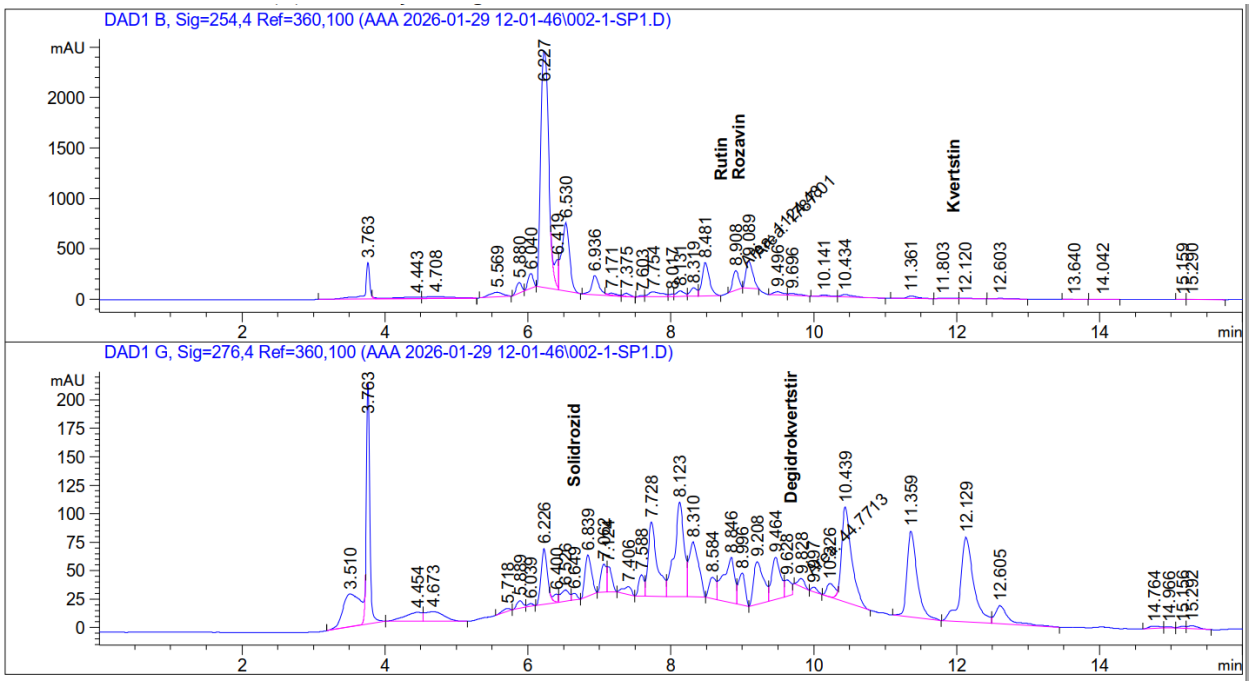

Figure 3. Flavonoids chromatogram.

According to the obtained data, the nitrogen content in Phlomoides ambigua was 2.88%, which corresponds to 18.37% total protein when calculated using a coefficient of 6.38.

The results indicate that this species possesses relatively high nutritional value. Protein content is one of the key indicators determining the biological effectiveness of plant raw materials, as proteins are integral components of enzymes, structural elements, and biologically active compounds.

The observed differences may be explained by several factors. In particular, the genetic characteristics of the plant, the vegetation period, growth conditions (soil composition, moisture level, and amount of sunlight), agrotechnical practices, and harvesting time can significantly influence the chemical composition. In addition, drying conditions and storage duration of the samples may also affect the final protein content to some extent.

CONCLUSION

The conducted study demonstrated that Phlomoides ambigua is rich in vitamins, flavonoids, and proteins. The identified phytochemical constituents confirm its significant biological and antioxidant potential. The relatively high protein content highlights its nutritional importance. Overall, the obtained results indicate that this species is a promising natural source for the development of medicinal raw materials and functional food products.

These results are of great importance for the comprehensive evaluation of the biological and pharmacological value of these species, as well as for providing a scientific basis for their potential use in the development of phytopreparations and functional food products.

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