



STUDY OF PRIMARY METABOLITES OF THE PLANT PHLOMOIDES

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Abstract

Phlomoides labiosa is considered a promising medicinal plant raw material for the medical and cosmetic industries. In this study, the primary metabolites present in the leaves of the plant growing in the mountainous regions of Tashkent Province were investigated. The content of water-soluble vitamins was determined by HPLC using a gradient elution mode. As a result, vitamins B₂, B₆, B₉, PP (B₃), and C were identified, with relatively high levels of ascorbic acid and riboflavin being recorded. Macro- and microelements were quantitatively analyzed using ICP-MS (inductively coupled plasma mass spectrometry). The analysis revealed the content of macroelements (K, Ca, Mg, Na) and microelements (Fe, Zn, Cu, Mn, Co, Ni, Cr, Pb, Cd) in the plant raw material. The obtained results were expressed in mg per 10 g of plant raw material. These findings confirm the biological value of the plant.

Keywords: Phlomoides labiosa, HPLC, vitamins, macro- and microelements, metabolites.





Introduction

Phlomoides labiosa is a perennial herbaceous plant belonging to the family Lamiaceae. Its natural distribution range covers Central Asia and Afghanistan. In Uzbekistan, it is found in the Samarkand, Bukhara, and Surkhandarya regions. The Lamiaceae family ranks sixth in terms of the number of species among flowering plants and comprises approximately 230 genera and about 7,000 species. [1]. *Phlomoides labiosa* is a perennial plant with a taproot system. The root is long, thickened, and has a fibrous structure. The stem is erect, reaching a height of 30–60 cm, and in cross-section it can vary from rounded to quadrangular. The entire plant is covered with simple and stellate hairs. [2]. Worldwide, scientific studies are being conducted on the isolation of triterpenoids, iridoids, flavonoids, phenylethanoid glycosides, phenolic acids, and other biologically active compounds from plants of the genus *Phlomoides*. Representatives of this genus have been found to possess cytotoxic, anticancer, anti-inflammatory, antibacterial, antimicrobial, and antioxidant properties. [3,4]. From the aerial parts of *Phlomoides labiosa* Bunge, the iridoid glycosides floridoside C, 8-O-acetylharpagide, and the methyl ester of shanzhiside have been isolated. [5]. The mineral composition of *Phlomoides speciosa*, *Phlomoides isochila*, and *Phlomoides nuda* growing in the Namangan region was studied using neutron activation analysis, and 35 elements were identified. High contents of potassium, calcium, chlorine, and magnesium were recorded in these species, along with the presence of trace elements such as iron, manganese, zinc, and copper. [6,7]. The presence of alkaloids, essential oils, tannins, ascorbic acid, saponins, microelements, Omega-3 fatty acids, iridoids, and flavonoids in *Phlomoides* determines its high medicinal properties. [8]. In folk medicine, some species of the genus *Phlomoides* have been used in the treatment of rheumatism, bone fractures, bleeding, inflammatory diseases, and allergic conditions. [9].

Therefore, the aim of this study is to investigate the primary metabolites in the leaves of *Phlomoides labiosa* growing in Uzbekistan using modern physicochemical research methods.

Experimental Part

The samples of *Phlomoides labiosa* were collected in April 2024 in the Gazalkent district of Tashkent region during the late spring period. The plant raw material was dried under natural air conditions in a well-ventilated room protected from direct sunlight.

The content of water-soluble vitamins was determined by high-performance liquid chromatography (HPLC) using a gradient elution mode. Acetonitrile and buffer





solution were used as the mobile phase. Identification was carried out in the UV range at 200–400 nm. Chromatographic conditions: Chromatograph – Agilent-1200 (equipped with an autosampler) Column – Eclipse XDB C18 (reversed-phase), 5 μ m, 4.6 \times 250 mm Detector – Diode array detector (DAD), detection at 250 nm Flow rate – 0.8 mL/min Eluent – acetate buffer : acetonitrile Gradient program: 0–5 min – 96:4

- 6–8 min – 90:10
- 9–15 min – 80:20
- 15–17 min – 96:4
- Column temperature – 25°C
- Injection volume – 5 μ L

The working standard solutions were first injected into the chromatograph, followed by the prepared sample solutions. For analysis, 5 g of the plant sample was accurately weighed and placed into a 200 mL flat-bottom flask. Then, 100 mL of 10% ethanol solution was added. The mixture was heated with continuous stirring on a magnetic stirrer equipped with a reflux condenser for 1 hour, followed by stirring at room temperature for an additional 2 hours. After sedimentation, the mixture was filtered. The obtained filtrate was centrifuged at 7000 rpm for 15 minutes, and the supernatant was taken for HPLC analysis. In the quantitative analysis, standard vitamins from Sigma were used: B1 (Cat. No. 59438), B2 (Cat. No. 83885), B3 (Cat. No. 59676), B6 (Cat. No. 58560), B9 (Cat. No. 59303), PP (Cat. No. 98920), and C (Cat. No. 50817). During chromatography, working standard solutions of water-soluble vitamins were analyzed first, followed by the samples, which were measured in triplicate. The methodology for the determination of water-soluble vitamins was carried out according to reference [10].

Determination of Macro- and Microelements and Heavy Metals

The content of macro- and microelements and heavy metals in the aerial parts of the plant was determined as follows. An accurately weighed sample of 0.1000 g (100 mg) was placed into Teflon microwave digestion vessels. Then, 3 mL of concentrated HNO₃ and 2 mL of pure H₂O₂ were added. The vessels were sealed and placed in a Berghof microwave digestion system (Speed Wave Xpert or equivalent). The digestion was performed under controlled temperature and pressure conditions. The minimum temperature was 50 °C and the maximum temperature was 230 °C, with a maximum pressure of 40 bar. The wet digestion process lasted 35–45 minutes. After cooling the vessels to room temperature, the resulting solution was transferred to a 50 or 100 mL volumetric flask and brought to volume with bidistilled water. The



solution was thoroughly mixed and placed into an autosampler tube. The mineralized solutions were quantitatively analyzed for macro- and microelements using a Perkin Elmer inductively coupled plasma mass spectrometer (ICP-MS) model Nexion 2000 (or an equivalent instrument). Sample mass and dilution factors were entered into the system, which automatically recalculated the results and provided the accuracy level along with the deviation (error) values [11].

Results and Discussion

After shade-drying the leaves of *Phlomis labiosa* using air ventilation, the content of water-soluble vitamins was analyzed. The results showed that the largest proportion belongs to vitamin B3 (niacin) – 12.9%, indicating its dominance among other vitamins in the plant. Vitamin B3 is directly involved in protein metabolism, the release of energy from carbohydrates and fats, as well as in the synthesis of nucleic and fatty acids, cholesterol, acetylcholine, and mitochondrial enzymes. It also plays a key role in the conversion of vitamin B9 into tetrahydrofolic acid. In addition, a significant amount of vitamin C (ascorbic acid) was detected – 11.8%. Ascorbic acid plays an important role in cellular metabolism and is involved in the maintenance and restoration of connective tissues. Deficiency of ascorbic acid in the body disrupts the structure of bone cells and can lead to diseases of the chest cavity. Furthermore, ascorbic acid is not synthesized or stored in the human body. [12]. Vitamin B2 (riboflavin) was detected at a level of 9.26%, indicating its significant presence in the aerial parts of the plant. Riboflavin plays an important role in plant growth and development and is essential for energy production in the metabolism of proteins, fats, and carbohydrates [13]. Smaller amounts of vitamin B9 (folic acid) – 4.88% and vitamin B6 (pyridoxine) – 3.94% were also identified. However, vitamin B1 (thiamine) was not detected (0%) in the analyzed aerial part samples. The absence of thiamine may be associated with its biosynthesis and accumulation characteristics in plant organs. Thus, the aerial parts of *Phlomis labiosa* are characterized by a high content of vitamins B3, C, and B2, while vitamins B6 and B9 are present in lower amounts, and vitamin B1 is absent.



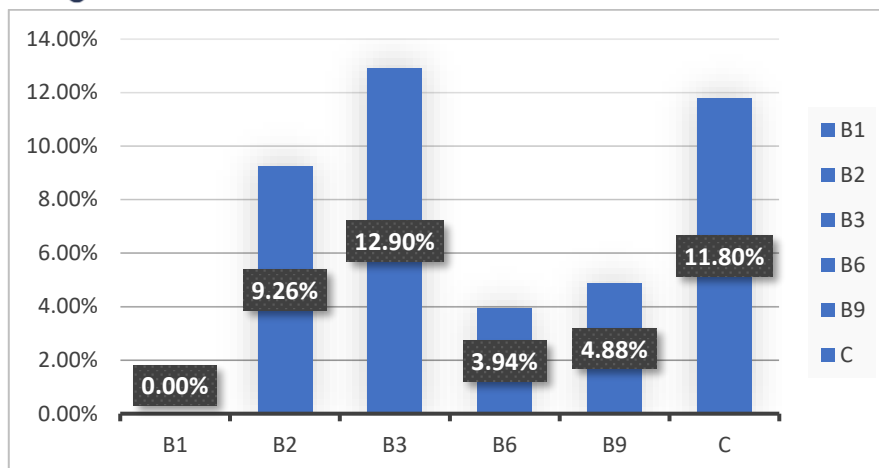


Figure 1. Percentage Composition of Water-Soluble Vitamins in *Phlomis labiosa*

The composition of heavy metals, as well as macro- and microelements, was studied using the ICP-MS method (inductively coupled plasma mass spectrometry, Nexion 2000). The results of the sample analysis include the quantitative content of macro- and microelements, as well as heavy metals. These data are presented in micrograms and recalculated per 10 g of the sample.

Table 1. Content of Macroelements in *Phlomis labiosa*

Element	Content ($\mu\text{g}/10\text{ g}$)
Ca	144,952
Mg	0,019
K	169,471
Na	0,003
P	12,369
S	2,858

The macroelement composition includes calcium, magnesium, potassium, sodium, phosphorus, and sulfur. The highest amounts were found for calcium (144.952 $\mu\text{g}/10\text{ g}$) and potassium (169.471 $\mu\text{g}/10\text{ g}$), indicating their significant presence in the mineral composition of the studied plant. The content of phosphorus was 12.369 $\mu\text{g}/10\text{ g}$, and sulfur was 2.858 $\mu\text{g}/10\text{ g}$, while magnesium (0.019 $\mu\text{g}/10\text{ g}$) and sodium (0.003 $\mu\text{g}/10\text{ g}$) were detected in trace amounts. These data are presented in Table 1.



Table 2. Microelement Composition of *Phlomis labiosa*

Element	Content ($\mu\text{g}/10\text{ g}$)
Fe	5,145
Cu	0,030
Zn	0,399
Mn	2,179
Co	0,058
Ni	0,169
Se	0,186
Mo	0,229
Al	0,413
Ba	0,225
Li	0,121
Rb	0,778
Cs	0,024

The microelement composition includes iron, copper, zinc, manganese, cobalt, nickel, selenium, molybdenum, as well as a number of conditionally biogenic elements. The highest contents were observed for iron (5.145 $\mu\text{g}/10\text{ g}$) and manganese (2.719 $\mu\text{g}/10\text{ g}$), which are important for enzymatic and redox processes. The content of zinc was 0.399 $\mu\text{g}/10\text{ g}$, copper – 0.030 $\mu\text{g}/10\text{ g}$, cobalt – 0.058 $\mu\text{g}/10\text{ g}$, nickel – 0.1695 $\mu\text{g}/10\text{ g}$, selenium – 0.186 $\mu\text{g}/10\text{ g}$, and molybdenum – 0.229 $\mu\text{g}/10\text{ g}$. The levels of aluminum (0.413 $\mu\text{g}/10\text{ g}$), barium (0.225 $\mu\text{g}/10\text{ g}$), lithium (0.121 $\mu\text{g}/10\text{ g}$), rubidium (0.778 $\mu\text{g}/10\text{ g}$), and cesium (0.024 $\mu\text{g}/10\text{ g}$) were very low and do not exceed typical values for biological objects. These data are presented in Table 2.

Table 3. Heavy Metal Composition of *Phlomis labiosa*

Element	Content ($\mu\text{g}/10\text{ g}$)
Pb	0,023
Cd	0,569
Hg	0
As	0
Cr	0,103
Sb	0



The content of heavy metals in the sample was very low. The concentrations of lead (Pb), cadmium (Cd), and chromium (Cr) were found to be 0.023 µg/10 g, 0.569 µg/10 g, and 0.103 µg/10 g, respectively, while mercury (Hg), arsenic (As), and antimony (Sb) were not detected in the sample. The analysis results indicate that there is no significant toxic load in the studied material, suggesting that it is environmentally safe. These data are presented in Table 3.

Conclusion

The study of primary metabolites in the leaves of *Phlomis labiosa* revealed that the aerial parts of the plant possess a rich and well-balanced biochemical composition. Water-soluble vitamins B₃, C, and B₂ were found to be dominant. Mineral composition analysis demonstrated high levels of calcium and potassium, as well as the presence of essential microelements, while heavy metals were detected only in very low concentrations. The obtained data confirm the biological value and environmental safety of *Phlomis labiosa*, indicating its potential for application in pharmaceutical and nutraceutical practices. Furthermore, these findings substantiate the need for more in-depth studies of its biological activity.

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