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PHOTOCHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF PLANT *PHLOMIS TUBEROSA*

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Abstract. The article presents the results of a study on the chemical composition of the above-ground part of *Phlomis tuberosa*, collected in the Turkestan region. One of the main objectives in the study of medicinal plants is the identification of biologically active compounds, which determine their potential for pharmaceutical application. This work describes qualitative and quantitative analysis methods using *Phlomis tuberosa* as an example and highlights their importance in identifying pharmacologically significant substances. *Results.* High-performance liquid chromatography (HPLC) was used to identify flavonoids, vitamins, and other phytochemical compounds. The quercetin content in the raw material was found to be 0.182 mg/g. The extract was also shown to contain naringin and phloridzin. Among the detected vitamins, vitamin C was found in the highest concentration. All data were obtained using certified standards and modern equipment, confirming their relevance for pharmaceutical development. *Scientific novelty.* A method for accurately determining biologically active compounds in *Phlomis tuberosa* was developed. Certified standards were used to identify the key components. The application of chromatographic techniques expanded the phytochemical profile of the plant. This approach is based on modern analytical tools and validated protocols. *Practical significance.* The obtained results contribute to the standardization of plant raw materials and the development of herbal medicines with consistent chemical

composition and proven biological activity. Accurate identification of active substances ensures the safety, efficacy, and reproducibility of pharmaceutical products.

Key words: *Phlomis tuberosa*, medicinal plant raw materials, biologically active substances, flavonoids, quercetin, HPLC, vitamin, phytochemical analysis

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PHLOMIS TUBEROSA ӨСІМДІК ШИКІЗАТЫНЫҢ ФИТОХИМИЯЛЫҚ ҚҰРАМЫ ЖӘНЕ БИОЛОГИЯЛЫҚ БЕЛСЕНДІЛІГІ

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Аннотация. Мақалада Түркістан өңірінен жиналған *Phlomis tuberosa* (түйнекті зопник) өсімдігінің жер үсті бөлігінің химиялық құрамы зерттеліп, фармацевтикалық мақсатта қолдану мүмкіндігін анықтауға бағытталған нәтижелер ұсынылған. Дәрілік өсімдіктерді зерттеудегі маңызды бағыттардың бірі – олардың құрамындағы биологиялық белсенді қосылыстарды анықтау және оларды сапалық әрі сандық тұрғыдан сипаттау болып табылады. Бұл зерттеу жұмысында *Phlomis tuberosa* мысалында фитохимиялық құрамды анықтау әдістері қарастырылып, олардың ғылыми және тәжірибелік маңызы сипатталған. **Нәтижелер.** Флавоноидтар, дәрумендер мен басқа да фитохимиялық заттарды анықтау үшін жоғары эффективті сұйықтық хроматографиясы (ЖЭХС) қолданылды. Жүргізілген талдаулар нәтижесінде шикізат құрамында 0,182 мг/г мөлшерінде кверцетин анықталды. Сонымен қатар, экстракттан нарингин мен флоризидин табылды. Дәрумендердің ішінен С дәруменінің мөлшері ең жоғары деңгейде тіркелді. Барлық мәліметтер заманауи аналитикалық құрылғылар және сертификатталған стандарттар көмегімен алынды, бұл алынған нәтижелердің дәлдігі мен сенімділігін қамтамасыз етеді. **Ғылыми жаңалығы.** *Phlomis tuberosa* құрамындағы негізгі биологиялық белсенді қосылыстар алғаш рет нақты әдістемелермен толық анықталды. Хроматографиялық зерттеулер фитохимиялық профильді кеңейтуге мүмкіндік берді. **Практикалық маңыздылығы.** Жүргізілген зерттеу нәтижелері *Phlomis tuberosa* өсімдігінен алынған фитошикізатты стандарттау, сондай-ақ оның негізінде сапалы, біртекті және биологиялық белсенділігі дәлелденген фитопрепараттар өндіру үшін ғылыми негіз бола алады. Белсенді қосылыстардың нақты мөлшерін анықтау фармацевтикалық

өнімдердің қауіпсіздігі мен тиімділігін қамтамасыз етуге мүмкіндік береді. Бұл мәліметтер фитопрепараттардың өндірісіндегі қайталанатын химиялық құрам мен биологиялық белсенділікке қол жеткізуге, сонымен қатар оларды тіркеу және сертификаттау үдерісін жеңілдетуге көмектеседі.

Түйін сөздер: *Phlomis tuberosa*, дәрілік өсімдік шикізаты, биологиялық белсенді заттар, флавоноидтар, кверцетин, ЖЭСХ, витамин, фитохимиялық талдау

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ФИТОХИМИЧЕСКИЙ СОСТАВ И БИОЛОГИЧЕСКАЯ АКТИВНОСТЬ РАСТИТЕЛЬНОГО СЫРЬЯ *PHLOMIS TUBEROSA*

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Аннотация. В статье представлены результаты комплексного исследования химического состава надземной части растения *Phlomis tuberosa*, собранного в Туркестанском регионе. Одной из приоритетных задач при изучении лекарственного растительного сырья является выявление и количественная оценка биологически активных соединений, определяющих его потенциал для фармацевтического применения. В данной работе подробно описаны методы качественного и количественного анализа фитохимических веществ, включая флавоноиды, витамины и другие метаболиты, с использованием современных аналитических подходов. **Результаты.** Определение состава проводилось методом высокоэффективной жидкостной хроматографии (ВЭЖХ), что обеспечило высокую точность и надёжность полученных данных. В ходе анализа установлено, что содержание кверцетина в растительном сырье составляет 0,182 мг/г. Кроме того, были идентифицированы нарингин и флоридзин. Среди витаминов наибольшее содержание зафиксировано у витамина С. Все исследования выполнены с применением сертифицированных стандартов и современного оборудования. **Научная новизна.** Впервые для *Phlomis tuberosa* был применён комплексный подход к анализу биологически активных веществ, что позволило расширить фитохимический профиль растения и уточнить его фармакологический потенциал. **Практическая значимость.** Полученные данные способствуют стандартизации сырья и разработке эффективных и безопасных фитопрепаратов с воспроизводимым составом и стабильной биологической

активностью. Идентификация биологически активных соединений и определение их количественного содержания позволяет обеспечить контроль качества на всех этапах – от заготовки сырья до выпуска готового продукта. Данный подход особенно важен для обеспечения стабильности действия препаратов и повышения доверия со стороны медицинского сообщества и потребителей.

Ключевые слова: *Phlomis tuberosa*, лекарственное растительное сырьё, биологически активные вещества, флавоноиды, кверцетин, ВЭЖХ, витамин, фитохимический анализ

Introduction. About 15-20% of modern phytopreparations are produced in domestic factories, and the rest are imported from abroad. Accordingly, this leads to an increase in the price of drugs obtained by synthesis on the market. However, drugs obtained by synthesis cause problems with their excretion from the human body. This is inefficient for public health and the economy of the state. Therefore, determining the composition and activity of medicinal plants that have long been used in folk medicine, obtaining new biologically active complexes and bringing them to the market are important and relevant topics for folk medicine and pharmaceutical production.

The emergence of many branches of organic chemistry and various industries is directly related to the study of substances secreted by plants.

The plant raw material of *Phlomis tuberosa* which is widespread in Kazakhstan, is of great theoretical and practical interest. In this regard, it determines the relevance of conducting a phytochemical examination of the composition of the plant raw material of *Phlomis tuberosa*, conducting quantitative and qualitative analysis of plant raw materials. The purpose of this study is to determine the total composition of flavonoids and other biologically active compounds in the tuberous plant of Kazakhstan. The results of this study may be useful in medicine, food industry and phytopharmaceuticals (Van Der Kooy, et al., 2009).

Phlomis tuberosa L., also known as *Phlomoides tuberosa* is a perennial herbaceous plant belonging to the *Lamiaceae* family. The *Phlomis* genus includes about 140 species distributed in Europe, Asia and Africa, and about 50 species are found in the territory of the former USSR. The *Lamiaceae* family belongs to the Lamiales order. The plant is widespread in the European part of Russia, Western and Eastern Siberia, the Caucasus, the Far East, Northern Europe, Armenia, Kurdistan, Iran, Mongolia, Western China (Kulzha). In Kazakhstan, some species of *Phlomis* are found in the North Kazakhstan, East Kazakhstan, and Turkestan regions. *Phlomis tuberosa* is found on steppe slopes, steppe meadows, arid lands, shrubby and semi-desert areas (Pavlov, 1964).

Historically, *Phlomis* has been used in various cultures. In ancient Greece and Rome, the plant was used to treat wounds and inflammation. The Latin name of the plant - *Phlomis* - comes from the word "flame", which is due to the fact that in ancient times the leaves of the plant were used as oil lamps. It is also used as an aromatic plant in perfumery, in Mongolia, where tea is brewed from its leaves, local healers, having learned about the beneficial properties of *Phlomis tuberosa*, prepared infusions and tinctures from its various parts to treat chronic diarrhea and lung diseases (Fathali et

al., 2024). *Phlomis tuberosa* is widely used in folk medicine and has anti-inflammatory, antibacterial, antiviral, regenerating, antioxidant, tonic and hypoallergenic effects. For medicinal purposes, the underground part of *Phlomis tuberosa* is used - stems, leaves, buds and flowers. The optimal period for the preparation is the period of mass flowering of the plant, which, depending on the region of growth, is from May to September. At the same time, the raw material is rich in a large concentration of biologically active substances, including flavonoids, iridoids, tannins and essential oils (Echishvili, et al., 2017). Scientific studies confirm that iridoids in *Phlomis tuberosa* affect the process of blood formation, accelerating it and effectively combating anemia (Kruglaya, 2008). The extracts of the raw material contain a number of bioactive compounds, such as flavonoids, phenolic acids and alkaloids, which have a strong antiinflammatory effect. These substances reduce the synthesis of pro-inflammatory cytokines and inhibit the activity of enzymes involved in inflammatory processes. Thus, drugs based on *Phlomis tuberosa* are effectively used to treat inflammatory diseases. In medicine, extracts and individual compounds of *Phlomis tuberosa* are used in the form of ointments and creams to treat skin diseases such as eczema and dermatitis, and plant tinctures and decoctions to strengthen the immune system and treat colds. These plant-based drugs have antiseptic and immunostimulating effects, which makes them useful for the prevention and treatment of infections (Vagabova et al., 2017).

The development of drugs based on *Phlomis tuberosa* can help reduce dependence on synthetic drugs and develop domestic production of phytopharmaceuticals. In particular, extracts from this plant can be used to produce liquid dosage forms (tinctures, syrups, decoctions), solid dosage forms (tablets, capsules), and soft dosage forms for external application (ointments, creams, gels) for the treatment of various diseases.

Methods and materials. *Phlomis tuberosa* was collected in the summer of 2023. The first step in the analysis of the obtained medicinal plant raw materials is to conduct quantitative and qualitative analysis.

Quantitative indicators of plant raw materials: moisture content, ash content, extractive substances content.

The moisture content of medicinal plant raw materials is the mass loss due to hygroscopic moisture and volatile substances, determined during drying of the raw materials to a constant weight. The moisture content of plant raw materials should not exceed the norm, since high humidity reduces its quality during storage. The methods of the 11th edition of the MF of the Republic of Kazakhstan were used to determine the moisture content of raw materials. A sample of medicinal plant raw materials was crushed to a particle size of about 10 mm, mixed, and two samples weighing 3 and 5 g were obtained, with a measurement error of 0.01 g. Each sample was placed in a pre-dried box, weighed together with the lid, and placed in a drying cabinet heated to 100-105°C. The drying time was counted from the moment the temperature in the drying cabinet reached 100-105°C again. The initial weighing of the herbs was carried out after 2 hours. Drying was carried out to a constant mass. If the difference between the two weights after 30 minutes of drying in a desiccator and 30 minutes of cooling did not exceed 0.01 g, a constant mass was considered to be achieved (Tulegenova, 2009).

Total ash is the incombustible residue obtained as a result of burning medicinal plant raw materials and heating them to a constant weight at 500°C. The average ash residue was 2%. Determining the total ash content is an important parameter in the analysis of raw materials, since it indicates the content of mineral substances that affect their quality and nutritional value. The methods of the MF RK, XI edition, were used to determine the total ash content. After the raw materials were burned, the ash remaining was placed in a muffle furnace and left for additional combustion at high temperature. The burnt ash was removed from the muffle furnace, cooled, weighed to constant weight, and any carbon deposits were removed with hydrogen peroxide, concentrated nitric acid, or ammonium nitrate solution. The final ash mass was compared with the initial mass, and the difference in mass indicated the amount of organic components burnt. This process allowed the total mineral content of the plant materials to be estimated (Ministerstvo zdavookhraneniya SSSR, 1987). Analysis of plant extracts by HPLC.

Sample preparation and analysis methods: 10 µl of the extract were collected and analyzed by highperformance liquid chromatography (HPLC) on a liquid chromatograph (Shimadzu LC-40).

Analysis conditions: sample volume 10 µl, sample injection temperature 40°C. Separation was performed using a C18 chromatographic column with a length of 25 cm, an internal diameter of 4.6 mm and a film thickness of 5 µm at a constant flow rate of acetonitrile and 1% acetic acid in water at 1 ml/min in different ratios. Shimadzu LabSolutions software was used to control the liquid chromatography system, record and process the obtained results and data. Data processing included determining retention times and peak areas.

The working principle of the vitamin A determination method.

Determination of vitamin A content by high-performance liquid chromatography. By the method of quantifying vitamin A in food products using high-performance liquid chromatography (HPLC), a food sample is saponified to release retinol from The Matrix. After saponification, retinol is extracted with an organic solvent. This method allows you to determine the content of all-trans-retinol and 13-CIS-retinol in various food products. (Figure 2). Determination of the mass concentration of vitamins and carotenoids is based on measuring the Peak area (or height) with the appropriate detection wavelength for each compound after the introduction of analyzed samples and calibration solutions into the chromatographic system (Zhilinskaya et al., 2018).

Determination of the content of vitamin C in dietary supplements.

Based on the direct determination of the content of ascorbic acid (AC), without taking into account the oxidized form of vitamin C - dehydroascorbic acid (DAC).

Detection by visual titration using digital oxidation of AC with 2,6-dichlorophenolindophenolate sodium solution is the simplest and most accessible for daily analysis purposes. However, this method is only used for research objects that produce light-colored extracts. In other cases, the method of Potentiometric titration, spectrophotometric and fluorometric methods of analysis applied to any research objects (GOST P EH 14130–2010).

To account for the total concentration of vitamin C, dehydroascorbic acid is reduced

to ascorbic acid using a reducing agent such as L-cysteine. The resulting solution is analyzed by high-performance liquid chromatography (HPLC) using spectrophotometric detection at a wavelength of 265 Nm. This method allows you to determine the total amount of vitamin C (Ascorbic and dehydroascorbic acids) in food products and biologically active additives .

The principle of operation of the method for determining the content of vitamin D.

"Determination of the content of cholecalciferol (vitamin D) and ergocalciferol (vitamin d₂) by highperformance liquid chromatography" establishes a method for the quantitative determination of vitamins d₂ and D in food products using HPLC. This method involves saponification, extraction, purification, and chromatographic analysis steps to accurately measure the amount of these vitamins (Federal'nyy tsentr gossanepidnadzora, 2004)

Determination of the amount of vitamin E.

The procedure" determination of the content of vitamin E (Alpha-, Beta-, Gamma - and Deltatocopherols) by high-performance liquid chromatography " establishes a method for quantifying various forms of vitamin E in food products using high-performance liquid chromatography (HPLC). The method includes the following stages: sample preparation, during which a food sample is saponified to remove tocopherols from The Matrix; extraction, in which tocopherols are extracted with an organic solvent after saponification; purification of the extract, removal of impurities that interfere with the analysis; chromatographic analysis of the purified extract by HPLC using a suitable detector, usually spectrophotometric. This method allows you to accurately and reliably determine the content of various forms of vitamin E in food products, which is important for assessing their nutritional value and ensuring safe consumption (GOST EN 12822-2014.).

Results and Discussion. The moisture content of the studied plant raw materials is 5.88%, ash content is 7.46%, and the content of extractives is 34.04%. These indicators allow us to determine the amount of biologically active substances in the plant. In particular, flavonoids were found in the amount of 1.12%, carbohydrates - 0.216%, free organic acids - 0.571%, coumarins - 0.02%, tannins - 1.0%, and alkaloids - 1.03%. Flavonoids are widespread compounds in plants and have antioxidant, anti-inflammatory, antiviral, and antitumor properties. Carbohydrates play an important role as a source of energy, and free organic acids participate in metabolic processes. Coumarins improve blood clotting, tannins have an antiseptic effect, and alkaloids affect the nervous system. Thus, the high content of biologically active substances in the studied plant raw materials increases its pharmacological value and can serve as the basis for its use for medicinal purposes.

The extract of plant raw materials was analyzed by HPLC method, and the presence of Naringin and Phloridzin was determined.

Table 2 - Presence of naringin and phloridzin in the extract

Retention time	Area	Height	Concentration	Unit	Name
17,431	51639	3162	24,973	mg/l	Naringin
19,522	195304	9852	84,048	mg/l	Phloridzin

Naringin is a plant flavonoid that is often found in citrus fruits. It gives citrus fruits a sour taste. Taking naringin has a beneficial effect on the body: it improves metabolism, has an antioxidant effect, helps prevent atherosclerosis, reduces the amount of cholesterol in the body, affects the immune system, restores the composition of the intestinal microflora. In addition, naringin helps to increase the elasticity of blood vessels, strengthens delicate capillaries and prevents bleeding (Wang et al., 2006: 247-253).

Floridzin is an important phytochemical component, first isolated from the bark of an apple tree. Numerous studies have shown that floridzine has several pharmacological properties, such as an antihyperglycemic effect, acts as a natural antioxidant, eliminates free radicals, increases the activity of antioxidant enzymes, and slows down the oxidation process. It also performs a hepatoprotective function, has anti-inflammatory and antibacterial effects. He was also active in fighting cancer and improving heart function (Chiryapkin et al., 2023).

Determination of the content of flavonoids in the composition of the *Phlomis tuberosa* plant according to GOST 57990-2017 using the HPLC method. (Figure 1).

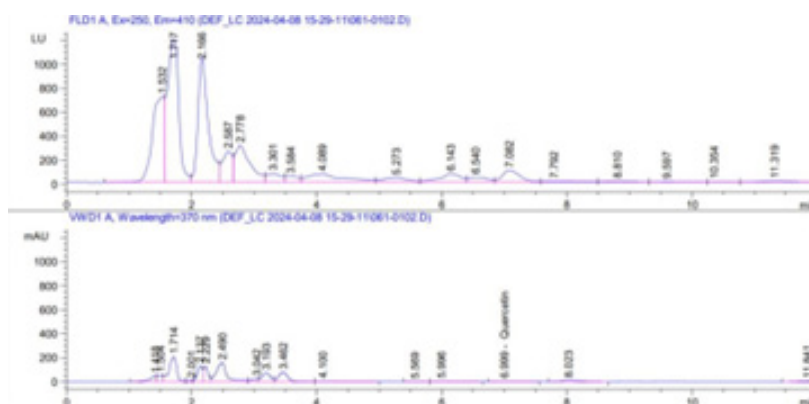


Figure 1 - Chromatographic indication of quercetin from plant raw materials of the *Phlomis tuberosa*

Analysis of flavonoids in the plant *Phlomis tuberosa* revealed that the amount of quercetin was 0.182 mg/g. Quercetin is a widely distributed flavonoid in plants, known for its antioxidant, anti-inflammatory, antiviral and antitumor properties. The presence of quercetin in plant raw materials increases its pharmacological properties and may serve as the basis for its use in medicinal purposes. Quercetin is a potent antiviral compound that can inhibit the early stages of viral infection, inhibit viral replication and reduce inflammation caused by viral infection. Due to its antiviral activity, quercetin helps to strengthen immunity against viruses that cause common diseases. Quercetin is a potent antioxidant, antiviral, anti-inflammatory, antitumor, anti-allergic and immunomodulatory (Rupasinghe & Yasmin, 2010: 251-257).

The chromatograms obtained during the study clearly separated peaks of all-trans-retinol and 13-cis-retinol (vitamin A), as well as ascorbic acid (vitamin C) in the studied samples are shown in Figures 2 and 3. Analysis of the chromatograms showed that the

HPLC method allows for the effective separation and quantitative determination of the indicated forms of vitamins. The results obtained indicate the presence of both all-trans-retinol and 13-cis-retinol in the studied samples, which confirms the effectiveness of this selected method for the analysis of various isomers of vitamin A. Furthermore, for vitamin C (ascorbic acid), the method based on direct determination without taking into account its oxidized form, dehydroascorbic acid, showed high specificity and sensitivity.

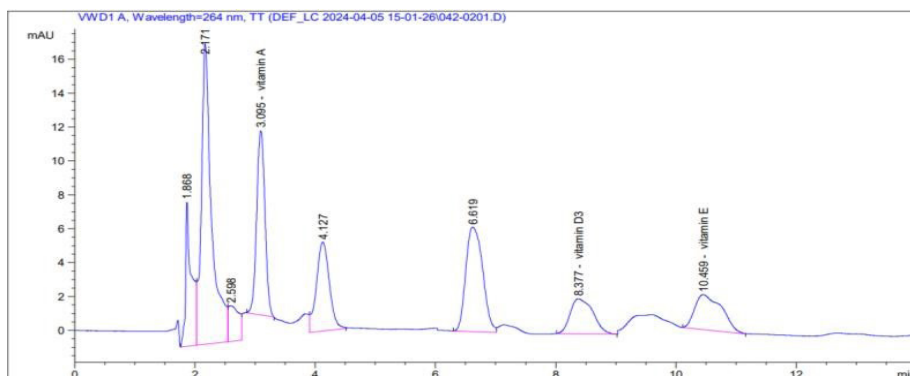


Figure 2 - Chromatographic indication of vitamin A obtained from plant raw materials of the *Phlomis tuberosa*

In this experiment, it was found that ascorbic acid (vitamin C) is found in high quantities in plant raw materials as the main biologically active form. Ascorbic acid plays many important functions in the human body. Vitamin C strengthens the immune system, accelerates the healing process of wounds, affects the synthesis of a number of hormones, regulates blood formation and normalizes capillary permeability. In addition, it regulates metabolism, removes toxins and has other beneficial effects on the body. Thus, the high content of ascorbic acid in plant raw materials makes it valuable for human health, as it participates in many physiological processes and strengthens overall health.

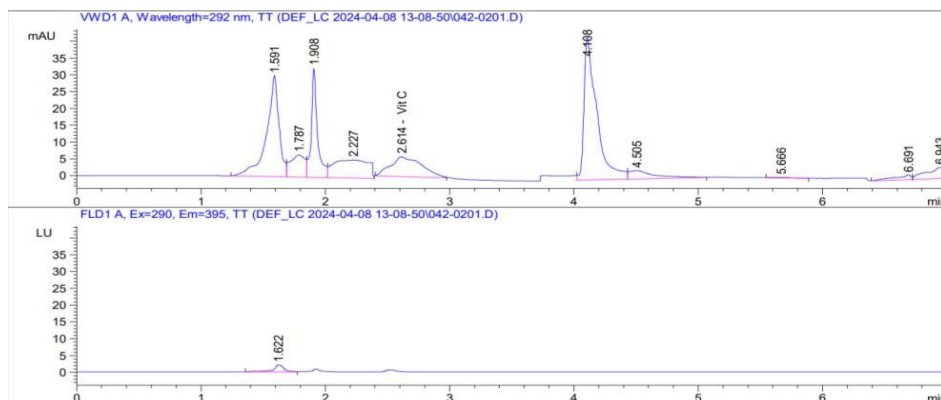


Figure 3 - Chromatographic indication of vitamin C from plant raw materials of the *Phlomis tuberosa*

Table 2 - Quantitative indicators of vitamins contained in the *Phlomis tuberosa*

№	Name	Number	Unit of measurement
1	Vitamin C	47,35	mg/100g
2	Vitamin A	6,74	mcg/g
3	Vitamin D	2,226	mcg/g
4	Vitamin E	17,838	mcg

The results of the analysis of vitamins contained in the *Phlomis tuberosa* plant showed that the composition of the selected plant raw materials, the content of vitamin C found in the amount of 47.35 mg/100g, the *Phlomis tuberosa* plant raw materials are rich in ascorbic acid, which has a positive effect on the immune, digestive, respiratory systems. It also ensures the normal structure of blood vessels, plays an important role in the good development of metabolism. Vitamin C is necessary for the normal performance of a number of vital functions, such as cell integrity, reparative processes, immune reactivity and growth.

Conclusion. The content and quantitative and qualitative composition of moisture, ash content and extractive substances of the *Phlomis tuberosa* plant are determined. When checking the flavonoids contained in the studied plant, the quercetin content showed 0.182 mg/g. They play an important role in the Prevention of various infectious diseases due to their antiviral activity. Also, using the HPLC method, it was found that the plant raw materials contain Naringin and Phloridzin. Their presence opens up prospects for the development of phytotherapeutic drugs that can be used not only to prevent infectious diseases, but also to improve the general condition of the body. By studying vitamins A, D, E and C, it was found that the medicinal plant *Phlomis tuberosa* has a high vitamin C content. Due to the pronounced antioxidant properties of vitamin C, the medicinal plant helps to strengthen the immune system and treat skin diseases. Based on the components identified from plant raw materials, one can also consider the possibility of creating drugs with immunostimulating, antiseptic effects. This allows them to be used in the treatment and Prevention of not only colds, but also various skin diseases, such as eczema and dermatitis.

In conclusion, the study of the composition of *Phlomis tuberosa* confirms that it is a valuable raw material for the creation of new herbal preparations with proven medicinal properties. This opens up wide opportunities for the use of this plant in medical practice and phytopharmaceuticals.

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