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SPECTROPHOTOMETRIC DETERMINATION OF FLAVONOIDS *Scrophularia nodosa* L., WIDESPREAD IN THE SHAHBUZ REGION OF THE NAKHCHIVAN AUTONOMOUS REPUBLIC

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СПЕКТРОФОТОМЕТРИЧЕСКОЕ ОПРЕДЕЛЕНИЕ ФЛАВОНОИДОВ ВИДА *Scrophularia nodosa* L., ШИРОКО РАСПРОСТРАНЕННОГО В ШАХБУЗСКОМ РАЙОНЕ НАХЧЫВАНСКОЙ АВТОНОМНОЙ РЕСПУБЛИКИ

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Abstract. Shahbuz region, considered the “flora museum” of the Nakhchivan Autonomous Republic, occupies a special place in the Autonomous Republic due to the richness of its biodiversity, especially the diversity of flora species. In fact, this richness is due to the many species contained in the flora of the region, including wild vegetables, honey and nectar plants, medicinal plants, as well as essences, alkaloids, saponins, flavonoids, glycosides, grafting agents, etc. Medicinal plants are of particular importance in this wealth. Some plant raw materials contain bioactive compounds with a wide range of effects, and the study and application of various substances in these mixtures, especially flavonoids, has always been the focus of scientific attention. Particularly, the high biological activity of flavonoids, their broader pharmacological effects and abundance in the plant kingdom should be considered as the main reasons for conducting research. A number of flavonoids have antibacterial effects. Thanks to their antioxidant properties, flavonoids protect the body from harmful environmental influences. In particular, the use of these substances in medicine and cosmetics, the pharmaceutical and food industries leads to a further increase in interest in them. Considering that the species Scrophularia nodosa L., widespread in the Nakhchivan Autonomous Region, including the Shahbuz region, is rich in flavonoids, qualitative analyzes were carried out to prove the presence of flavonoids in *Scrophularia nodosa*, and the amount of flavonoids was determined by the spectrophotometric method. As a result of laboratory studies, the total amount of flavonoids, determined by spectrophotometric method in the form of Scrophularia nodosa L., was 5,58%.

Аннотация. Район Шахбуз, считающийся «музеем флоры» Нахчыванской Автономной Республики, занимает особое место в Автономной Республике из-за богатства своего биоразнообразия, особенно разнообразия видов флоры. Фактически, это богатство обусловлено множеством видов, содержащихся во флоре региона, включая дикие овощи, медоносные и нектароносные растения, лекарственные растения, а также эссенции, алкалоиды, сапонины, флавоноиды, гликозиды, прививочные средства и т. д. Лекарственные

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растения имеют особое значение в этом богатстве. Некоторые виды растительного сырья содержат биоактивные соединения с широким спектром действия, и изучение и применение различных веществ в этих смесях, особенно флавоноидов, всегда было в центре внимания ученых. В частности, высокую биологическую активность флавоноидов, их более широкое фармакологическое действие и обилие в растительном мире следует рассматривать как основные причины проведения исследований. Ряд флавоноидов обладают антибактериальным действием. Благодаря своим антиоксидантным свойствам флавоноиды защищают организм от вредных воздействий окружающей среды. В частности, использование этих веществ в медицине и косметике, фармацевтической и пищевой промышленности приводит к дальнейшему росту интереса к ним. Учитывая, что вид *Scrophularia nodosa* L., широко распространенный в Нахчыванской автономной области, включая Шахбузский район, богат флавоноидами, были проведены качественные анализы, доказывающие наличие флавоноидов в *Scrophularia nodosa*, и количество флавоноидов было определено спектрофотометрическим методом. В результате лабораторных исследований общее количество флавоноидов, определенное спектрофотометрическим методом в *Scrophularia nodosa* L., составило 5,58%.

Keywords: Scrophularia nodosa L., qualitative analyses, flavonoids, spectrophotometry.

Ключевые слова: Scrophularia nodosa L., качественные анализы, флавоноиды, спектрофотометрия.

According to APG IV data as of June 2024, there are 2,350 known species belonging to 60 genera of the family Scrophulariaceae Juss. As a result of research in the late 20th century and early 21st century, many genera of the family *Scrophulariaceae* Juss were included in other families included in the suborder *Lamiaceae*, most notably *Plantaginaceae* and *Orobanchaceae*. Currently, in the Nakhchivan Autonomous Republic there are 44 species of this family, included in 6 genera [1]. A. Askerov It is shown that in the flora of Azerbaijan there are 30 species of the genus. It is known that 17 species of the genus are widespread in the Nakhchivan Autonomous Republic [9]. 12 species of the genus *Scrophularia* L. were discovered in the Shahbuz region:

Scrophularia atropatana Grossh. This is a biennial glabrous plant. The stems are straight, quadrangular, simple, purple in color. The leaves are petiolate, ovate or almost round. The crown is dark red-brown.

Scrophularia nachitchevanica Grossh. Biennial green plant, hairy or slightly powdery. The body is flat and simple. The leaves are lance-shaped or lance-shaped-rectangular. The group of flowers (young) has the shape of a pyramidal broom or rectangle. The crown is black-brown-red.

Scrophularia chrysantha Jaub et Spach. Biennial plant. The stem is thickened, quadrangular, hollow inside, slightly pubescent. The leaves are alternate, the lower ones are long-petiolate, the upper ones are almost sessile, heart-shaped, triangular and obtuse. The flowers are numerous, the corolla is 6-7 mm long, yellow.

Scrophularia ilvensis C. Koch. Annual, glabrous, stem erect, quadrangular, simple, usually purple. Leaves vary from short-petiolate, ovate (bottom) to oval-oblong and oblong-lanceolate. The flowers are collected in a semi-umbrella, the crown is matte red.

Scrophularia olympica Boiss. It is a perennial, bare plant. The stem is numerous, simple or with few branches, purple in color. The stem is numerous, simple or with few branches, purple in color. The lower leaves are alternate, others alternate, sometimes entire and serrated, ovate or ovate-

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oblong, swallow-toothed, lyre-shaped, pinnately dissected or pinnately dissected. The crown is yellowish, the upper part is purple.

Scrophularia orientalis L. Perennial, stem glabrous or very sparsely pubescent. The leaves are slightly twisted, ovate or oblong-lanceolate. The crown is spherical, yellowish-green on the outside, purple at the base, sometimes purple-striped.

Scrophularia rupestis Boiss. Perennial, stem straight, slightly quadrangular. The leaves are oblong-ovate. The flowers are yellow, the upper lip is dark red, 5-6 mm long. The flower group is broom-shaped.

Scrophularia thesioides Boise & Buhse. Perennial, glabrous, stem thin, straight, sometimes curved, purple in color. The leaves are divided into 1-3 pairs of long, narrow-linear and pointed lobes up to 1 mm wide. The inflorescence is paniculate, the crown is purple.

Scrophularia alata Gilib. var. *glandulosa* Karjag. A perennial glabrous plant, the stem is straight, four-lobed, narrow-leaved. Leaves with winged petioles, oval-rectangular, acute or obtuse, round or slightly heart-shaped. The calyx is about 6-8 mm long, red-brown, greenish, tube-shaped.

Scrophularia variegata Bieb. Perennial, glabrous or with very short glandular white hairs, the stem is reddish, straight, simple or sometimes branched. The leaves are oblong, deeply pinnately dissected or pinnately dissected. The flower crown is variegated, yellowish, with a brownish-red upper lip.

Scrophularia umbrosa Dumort. Perennial, erect, tetrahedral stem, leaf petioles filmy, edged and bare. The leaf blades are oblong-ovate. The crown is green, purple-yellow or brown, the middle part of the lower lip is slightly narrow.

Scrophularia nodosa L. It is a perennial glabrous plant 50-125 cm high, sometimes with sparse glandular hairs in the floral group and tuberous thickened rhizomes. The trunk is sharp, quadrangular, erect. The leaves are ovate, wide heart-shaped at the base, the edges are pointed, sawtooth, and have stems 1-2.5 cm long. Inflorescences lanceolate or linear-lanceolate. Pedicels are linear, 5-10 times shorter than the pedicel. The calyx is bare, the segments are broadly ovate, obtuse, the edges have a narrow white border. The crown is dark, 7-9 mm long, olive or brownish-green, the lower part of the tube and fold are predominantly green, the upper part and posterior part are brownish-red, the lobes of the upper lip are twice as long as the lateral lobes of the lower lip. Blooms from May to August. Found in humid areas of forests in the middle zone. It is also found in Batabat, Kuku, Keceldag of Shahbuz district [3].

The plant contains carbohydrates and related compounds: dulcite, organic acids: fatty and malic, iridoids: aucubin, harpagide, saponins, vitamins: vitamin C, phenylcarboxylic acids: cinnamic acid, flavonoids. Carbohydrates are widespread in the underground parts of the plant: sucrose, aucubin from iridoids, catalpol, methylcatalpol, harpagide. Along with them, there are saponins, alkaloids, nitrogenous compounds: choline, phenylcarboxylic acids and their derivatives: cinnamon, ferul, caffeine, vanillin, coumarins and flavonoids. The plant contains the alkaloid scrofularine, making it particularly toxic to horses and ruminants. The plant is widely used in folk medicine. The plant can have anti-inflammatory, wound-healing, diaphoretic and antipruritic effects, as well as laxative, diuretic, emetic and antiparasitic properties. The therapeutic effect of *Scrophularia nodosa* L. has been the subject of several clinical studies conducted by Chinese scientists. These studies showed that the plant has a powerful analgesic effect with virtually no side effects The plant extract reduces swelling, infiltration and proliferation of activated T-lymphocytes in joint tissues, and suppresses inflammatory factors. Thus, it has been confirmed that Scrophularia nodosa can be a powerful analgesic and anti-inflammatory agent [5, 7].

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Material and methodology of the study

The species Scrophularia nodosa L. taken for the study was collected in the vicinity of the village of Kuku, Shahbuz district. The collected plant was left to dry after being cleared of foreign impurities. Part of the dried plant is crushed in a mortar and pestle. The crushed part of the plant was used to isolate flavonoids according to the analytical procedure.

Initial weight was determined by weighing the collected plant material after collection. The weighed plant material was allowed to dry and, once dry, it was weighed again.

After determining the difference between both masses, the amount of moisture in the raw material was calculated. The percentage loss of moisture from the raw material during drying (x) was calculated using the following formula:

$$x = \frac{(m - m_1)100}{m}$$

Here m -is the mass of the raw material sample in grams before drying, m1- is the mass of the raw material sample after drying. The weight of 300 grams of wet plant mass taken for the experiment after drying was 259 grams. According to the results of the experiments, the plant *Scrophularia nodosa* L. had a loss of moisture.

$$x = \frac{(300 - 269)100}{300} = 13{,}67\%$$

Flavonoids are more abundant in various plant organs, especially in above-ground organs (flowers, leaves). Moreover, the flowers are richer in them. In plants, flavonoids are found in the form of glycosides and in free form. Under the influence of enzymes, they are broken down into sugars and aglycones. To determine the resulting flavonoids, qualitative reactions were carried out; for comparison, a 0.1% alcohol solution of rutin was used during the work [8].

Reaction with alkali. When adding 1-2 drops of a 10% alcohol solution of sodium hydroxide or potassium hydroxide to 1 ml of extract, the natural color of the solution intensifies.

Reaction with aluminum chloride (AlCl3). To 1 ml of the resulting solution add 1 ml of a 2% alcohol (C2H5OH) solution of aluminum chloride, the solution turns yellow.

Cyanidin reaction or Shinoda reaction. To 1 ml of the resulting solution, add 2-3 drops of hydrogen chloride (HCl) solution and a certain amount of magnesium (Mg) residues; as a result of reduction with magnesium, the solution turns red or orange.

Reaction with iron-3 chloride ($FeCl_3$). When 2 drops of 1% ferric chloride solution are added to 1 ml of extract solution (extract), the color of the solution turns brown or green [2, 6].

Determination of the amount of flavonoids in the herb *Scrophularia nodosa* L. by spectrophotometric method. The spectrophotometric method of analysis plays a key role in determining the amount of biologically active substances in plants. The spectrophotometric method depends on the wavelength and absorption intensity of the incident light. With spectrophotometric analysis, even if the solution concentration is low, the accuracy of the analysis is high. The accuracy and sensitivity of the results obtained make this method widely used. Spectrophotometry is widely used in studying the composition of various compounds, in the qualitative and quantitative determination of substances.

Discussion and conclusions of the study

The plant under study (*Scrophularia nodosa* L.) is crushed and passed through a sieve with a pore size of 1 mm.1 g of sifted pomegranate is carefully weighed from the raw material and poured into a 150 ml flask. 30 ml of 50% ethyl alcohol is added to the raw material. The flask is then

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connected to reflux and simultaneously heated in a hot water bath for 30 minutes. During this time, the flask is periodically shaken to moisten the particles of raw materials adhering to the walls of the flask. After 30 minutes, the hot extract is filtered through a cotton swab into a 100 ml flask. During filtering, care should be taken to ensure that raw material particles do not fall on the cotton wool. After completion of filtration, the cotton wool used for filtration is placed back into the extraction flask and 30 ml of 50% alcohol (C_2H_5OH) is added to it. The extraction process is repeated twice in the same way. After each process, the resulting extract is filtered into a flask containing the same extract. After mixing all the extracts obtained, the entire solution is filtered through a paper filter into a flask of the same size. The filter paper is washed with 50% alcohol. After the total solution has cooled to room temperature, add 50% alcohol to the mark on the flask.

After solution A is prepared, we continue to work. To do this, add 1 ml of solution A to a 25 ml volumetric flask and add 2 ml of a 2% solution of aluminum chloride in 95% ethyl alcohol. Then we bring the volume of the solution in the flask to the required value by adding 95% ethyl alcohol. After waiting 40 minutes, we measure the optical density of the resulting solution using a spectrophotometer (Boeco Germany S-220 Spectrophotometer) at a wavelength of 415 mm, in a cuvette with a layer thickness of 10 mm.

To prepare a reference solution, pour 1 ml of extract into a 25 ml volumetric flask and add 1 drop of concentrated acetic acid. Then 95% alcohol is added to the solution to the size of the flask and mixed A standard solution of rutin is prepared in the same way and its optical density is measured under the same conditions.

After completing the measurements, determine the amount of flavonoids in plant raw materials as a percentage relative to rutin using the following formula:

$$X = \frac{D\ m_0\ 100\ 100\ 100}{D_0\ m\ 100\ (100\text{-}W)}$$

Here D- is the optical density of the solution under study; D_0 – optical density of standard rutin solution; m – mass of raw materials in grams; m_0- is the mass of the standard rutin sample in grams; W – percentage weight loss during drying.

It should be noted that the quantitative determination of the spectrophotometric optical density of the solution should not be carried out less than 6 times. The calculation is made based on the average value of the results [4]. The following table presents the results of spectrophotometric measurements of the Scrophularia nodosa L. plant extract and the standard rutin solution (Table).

Table

RESULTS OF SPECTROPHOTOMETRIC MEASUREMENTS OF *Scrophularia nodosa* L. EXTRACT AND STANDARD RUTIN SOLUTION

Number of measurements	*Scrophularia nodosa L extract.*		*Standard solution of rutin*	
	Optical density,D	*Solution viscosity,C*	*Optical density,D0*	*Solution viscosity,C*
1	0,342	0,921	0,353	0,952
2	0,332	0,891	0,359	0,975
3	0,337	0,896	0,350	0,961
4	0,328	0,885	0,345	0,937
5	0,329	0,910	0,347	0,941
6	0,338	0,908	0,351	0,936

By substituting the calculation results into formula 1, it is possible to calculate the percentage of flavonoids in the raw material of *Scrophularia nodosa* L. compared to rutin.

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D=(0,344+0,332+0,337+0,328+0,339+0,338):6=0.336; M 0=0.05; D 0=(0,353+0,359+0,355+0.342+0,346+0,341):6=0.349; m =1

Indicators:D= 0,336, D0= 0,349, m= 1,0 m0= 0,05,W= 13,6

$$X = \frac{0{,}336\ 0.05\ 100\ 100\ 100}{0{,}349\ 1\ 100\ (100\text{-}13{,}67)} = 5{,}58\%$$

From the obtained result, it is known that the percentage of flavonoids in the raw material of *Scrophularia nodosa* L. is 5.58% compared to rutin**.**

Results

After positive results of the identification reactions of flavonoids in the raw materials of Scrophularia nodosa L., their quantity was determined. As a result of a spectrophotometric study, it was found that the leaves and flowers of *Scrophularia nodosa* L. contain 5.51% of the total flavonoids..

It has been established that the plant Scrophularia nodosa, which is widespread in the Shahbuz region and has a rich raw material base, is a valuable alternative natural source for purchasing flavonoids individually. It is possible to continue scientific research in the direction of obtaining medicines based on flavonoids.

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