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ANALYSIS OF THE FLAVONOID CONTENT IN THE COMPOSITION OF THE "GEPAGAL" MIXTURE

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Abstract: In this article, the flavonoid composition and quantitative indicators of the "Gepagal" substance, prepared from locally sourced medicinal plant raw materials, were thoroughly analyzed. During the research, the aim was to determine the content of the main bioactive compounds present in the substance — namely rutin, quercetin, dihydroquercetin, sennoside, and luteolin. For this purpose, the High-Performance Liquid Chromatography (HPLC) method was employed, which is distinguished by its accuracy, sensitivity, and reliability in analytical studies. The experimental results showed that each of the identified flavonoids was present in a measurable amount within the "Gepagal" substance; however, the luteolin content was found to be considerably higher compared to the other flavonoids. This finding indicates that luteolin serves as one of the principal active components of the preparation and plays a significant role in determining its biological activity and pharmacological effects. Furthermore, the obtained results are of great importance for improving the quality of pharmaceuticals derived from local medicinal plant materials and for scientifically substantiating their standardization process, thereby contributing to the development of effective and safe plant-based medicines.

Keywords: "GEPAGAL" substance, flavonoids, High-Performance Liquid Chromatography (HPLC).

Introduction

At present, the global demand for medicinal plant-based products, including various herbal mixtures, plant extracts, pharmaceuticals, and biologically active supplements (BAS), is increasing year by year. Accordingly, extensive scientific research is being carried out worldwide to develop highly effective, natural-origin preparations that have mild effects on the human body and exhibit minimal or no side effects, as well as to study their composition in depth and introduce them widely into medical practice. Modern pharmaceutical science pays special attention to the comprehensive study of the chemical composition, pharmacological activity, and therapeutic potential of medicinal plants, with the goal of creating new, environmentally friendly, safe, and effective drugs. As a result, plant-based preparations are now widely used not only in traditional medicine but also in modern clinical practice [1,2]. In recent years, the use of medicinal plant raw materials in the treatment and prevention of diseases, as well as in the production of pharmaceuticals, therapeutic cosmetics, and biologically active supplements, has expanded significantly. This trend reflects the growing global interest in natural bioactive compounds within the fields of pharmacy and biotechnology.

Preparations and biologically active supplements derived from natural plant sources have several important advantages over synthetic analogues. Firstly, they retain a natural complex of biologically active substances beneficial to the human body, which enables them to exert multidirectional and complex therapeutic effects. Consequently, such preparations are highly effective in balancing physiological functions, strengthening the immune system, and accelerating recovery processes. Moreover, due to their biological compatibility with the human

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body, the risk of allergic reactions or adverse effects when using natural-origin compounds is significantly lower than that of synthetic drugs. Therefore, these preparations are considered safe for long-term use and are also suitable for preventive health care purposes.

From this perspective, medicinal plant-based pharmaceuticals and biologically active supplements play an essential role not only in pharmaceutical practice but also in promoting a healthy lifestyle. Expanding their production is regarded as one of the promising directions for the development of the national pharmaceutical industry [3,4]. Currently, herbal mixtures prepared from medicinal plants possess a broad therapeutic spectrum and have proven effective in the treatment of various diseases, including those affecting the internal organs, cardiovascular system, nervous system, and digestive system [3]. Particularly, scientific studies have demonstrated the exceptional importance of such herbal formulations in eliminating pathological disorders associated with liver and biliary tract functions. Therefore, developing highly effective, natural, and safe herbal preparations based on locally available plant materials for the treatment of liver diseases has become a pressing scientific priority. The development and production of such preparations will not only help improve public health, but also promote the growth of the domestic pharmaceutical industry by expanding the range of affordable, high-quality, import-substituting medicines [4,5].

Research Objective: To conduct a quantitative analysis of the flavonoid content in the “GEPAGAL” dry extract mixture using the High-Performance Liquid Chromatography (HPLC) method.

Materials and methods. The quantitative analysis of flavonoids in the dry extract mixture of “GEPAGAL” was carried out using a high-performance liquid chromatograph (HPLC), Agilent 1200 Series (Agilent Technologies, USA), equipped with a gradient pump and a spectrophotometric detector, operated with Chemstation 09.03.a software. The separation was performed on a Zorbax Eclipse C18 column (4.6 × 150 mm, 5 µm particle size).

The mobile phase consisted of a gradient of 0.3% phosphoric acid (A) and acetonitrile (B). Detection was conducted at a wavelength of 370 nm, corresponding to the λmax of luteolin. The flow rate of the eluent was 1 mL/min, with an injection volume of 10 µL. The column temperature was maintained at 40°C, and the total analysis time was 25 minutes [6,7].

To identify luteolin in the dry extract mixture, a working standard solution of luteolin was also subjected to chromatographic analysis. The retention time of luteolin was found to be 11.99 minutes.

Preparation of the Test Solution.

To prepare the test solution, 1000 mg of the substance was accurately weighed and transferred into a 100 mL volumetric flask. Then, 60 mL of 70% ethyl alcohol was added. After the substance was completely dissolved, the volume of the solution was brought up to the mark with 70% ethyl alcohol. The resulting solution was mixed thoroughly and kept in a dark place. After 40 minutes, the solution was filtered through “white ribbon” filter paper.

Table 1

Gradient mode

Time / min	**Mobile phase A, %**	**Mobile phase B, %**

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3,00	80,0	20,0
5,00	70,0	30,0
7,00	70,0	30,0
10,00	50,0	50,0
14,00	50,0	50,0
16,00	30,0	70,0
20,00	30,0	70,0
22,00	80,0	20,0
25,00	stop	

Preparation of Luteolin Working Standard Solution 0An accurately weighed 0.010 g (10 mg) of luteolin working standard was dissolved in 70% ethyl alcohol in a 100 mL volumetric flask, and the volume was made up to the mark with the same solvent.

During the initial stage of the study, the working standard solutions were first injected into the chromatograph, followed by the test sample solutions.

This procedure and the corresponding chromatograms are shown in Figure 1.

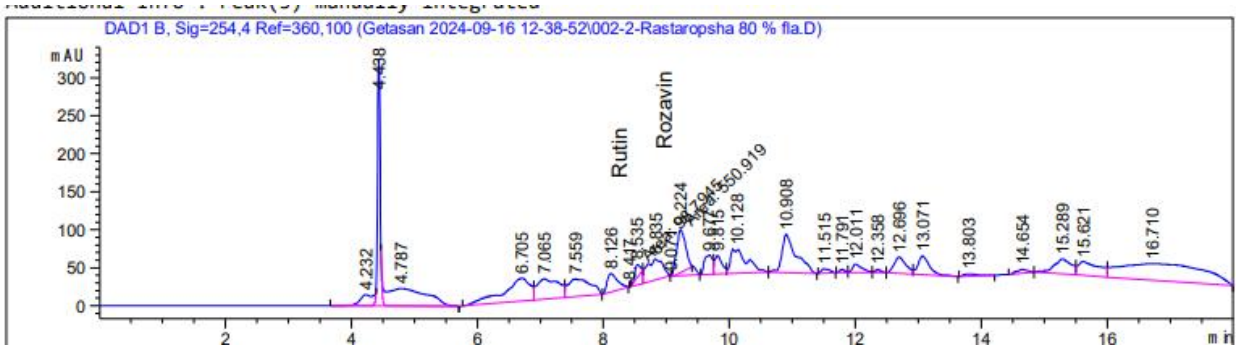

Figure 1. Chromatogram of the Luteolin Standard Solution

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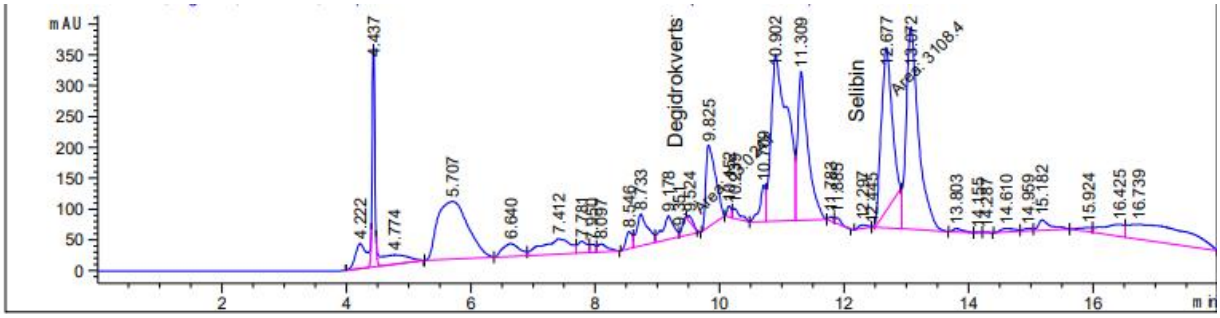

Figure 2. Chromatogram of the Flavonoid Solution in the Composition of "GEPAGAL" Dry Extracts

The amount of flavonoids in the composition of the "GEPAGAL" substance was calculated using the following formula: $X = \frac{S \quad a \quad V_{синов} \quad P \quad 1000}{Sst \quad V_{стд} \quad a_{синов} \quad 100} = \frac{S_{синов} \quad a_{стд} \quad V_{синов} \quad P \quad 10}{S_{стд} \quad V_{стд} \quad a_{синов}}$

Bu yerda:

S_{std} – area of the main peaks on the chromatogram of the luteolin working standard solution;mAU;

S_{sinov} – area of the main peaks on the chromatogram of the luteolin test solution, mAU;

a_{std} – weight of the luteolin working standard sample, g;

a_{sinov} – weight of the luteolin test sample, g;

P – purity of the standard sample, %.

Table 2.

Metrological characteristics of the results of determining the luteolin content in the "GEPAGAL" substance

№	X%	$X_{o'rt}$	S^2	S	S_x	t(95%,4)	ΔX	$\Delta X_{o'rt}$	E,%	E,% o'rt
1	2,2445	2,2422	0,00005991	0,0069989	0,0033412	2,59	0,0211824	0,0088213	0,89	0,41
2	2,2424									
3	2,2442									
4	2,2405									
5	2,2395									

Determination of Flavonoids in the Composition of the "GEPAGAL" Substance by HPLC Method

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A high-performance liquid chromatography (HPLC) method was developed to determine the flavonoid content in the “GEPAGAL” substance. The luteolin content was found to be 2.19%, while the average error relative to luteolin was 0.36%. In addition to luteolin, several other flavonoids such as scenirozide, solidrosin, dihydroquercetin, rutin, and quercetin were also identified in the composition of the “GEPAGAL” substance using the same HPLC method.
Chromatographic conditions:

- Chromatograph: Agilent 1200 (equipped with an autosampler)
- Column: Eclipse XDB C18 (reversed-phase), 5 µm, 4.6 × 250 mm
- Detector: Diode Array Detector (DAD) – identification performed at 254 nm, 272 nm, and 276 nm
- Flow rate: 0.8 mL/min
- Eluent: Phosphate buffer : Acetonitrile gradient
  - 0–5 min → 95:5
  - 6–12 min → 70:30
  - 12–13 min → 50:50
  - 13–15 min → 95:5
- Thermostat temperature: 30°C
- Injection volume: 10 µL

Initially, standard working solutions were injected into the chromatograph, followed by the prepared test solutions under the same conditions.

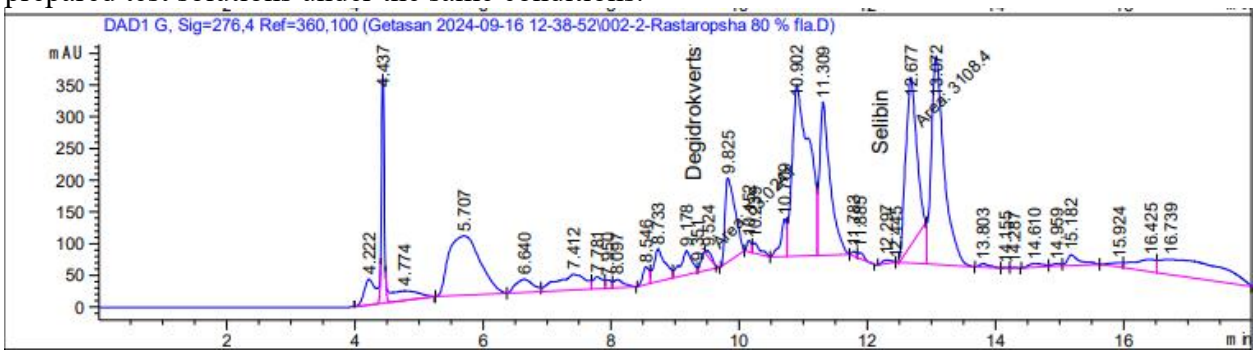

Figure 3. Chromatogram of the Flavonoid Solution in the Composition of the “GEPAGAL” Substance

The results of the metrological analysis of the values obtained using the developed method for determining the amount of senirozide in the composition of the “GEPAGAL” substance are presented in Table 3.

Table 3.

Metrological Characteristics of the Results of Senirozide Content Determination

№	X%	X_a	S^2	S	S_x	t (95%,4)	ΔX	$\Delta X_{o'rt}$	E,%	$E,\%_{o'rt}$
1	1,4925	1,4915	0,00039 811	0,01977 96	0,00881 29	2,58	0,06013 42	0,03100 17	2,95	1,23
2	1,4920									

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3	1,4895									
4	1,4950									
5	1,4885									

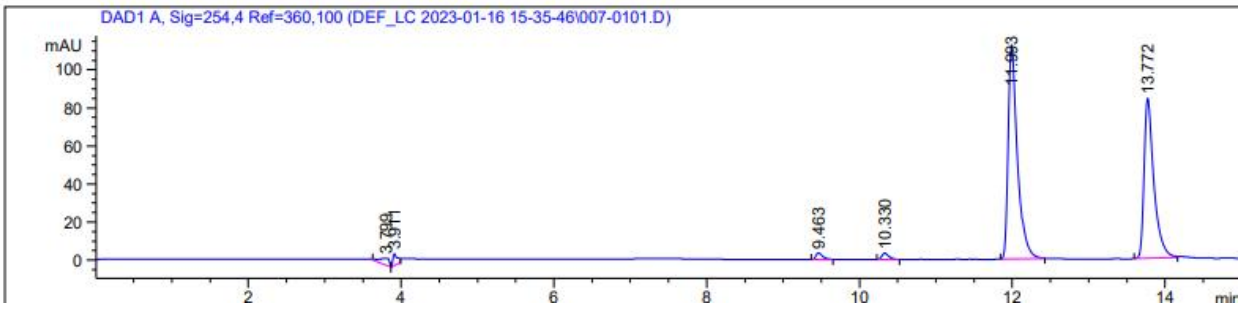

Figure 4. Chromatogram of the Senirozide Solution in the Composition of the "GEPAGAL" Substance

The results of the metrological analysis of the values obtained using the developed method for determining the dihydroquercetin content in the composition of the "GEPAGAL" substance are presented in Table 4.

Table 4

Metrological Characteristics of the Results of Dihydroquercetin Content Determination

№	X%	$X_{o'rt}$	S^2	S	S_x	t(95%,4)	ΔX	$\Delta X_{o'rt}$	E,%	E,% $_{o'rt}$
1	1,5860	1,5925	0,00021210	0,0121053	0,0059879	2,58	0,0397712	0,0170313	2,23	1,07
2	1,5950									
3	1,5929									
4	1,5920									
5	1,5967									

As can be seen from the data presented in Table 4, the flavonoid dihydroquercetin is present in a sufficient amount in the composition of the isolated "Gepagal" dry extract mixture.

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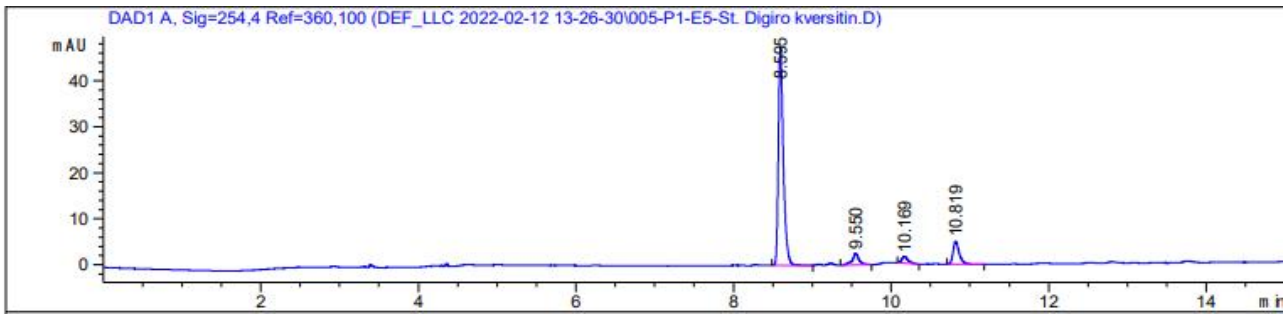

Figure 5. Chromatogram of the Dihydroquercetin Standard Solution

The analysis of the data presented in Table 4 shows that the flavonoid dihydroquercetin was detected in a significant amount in the composition of the Gepagal dry extract mixture. This result confirms that the given flavonoid is sufficiently accumulated within the extract. The presence of dihydroquercetin is of particular importance, as it serves as a factor that enhances the antioxidant, anti-inflammatory, and capillary-strengthening properties of the preparation. Therefore, the numerical indicators presented in Table 4 scientifically substantiate the richness of the Gepagal mixture in bioactive compounds and its high pharmacological activity.

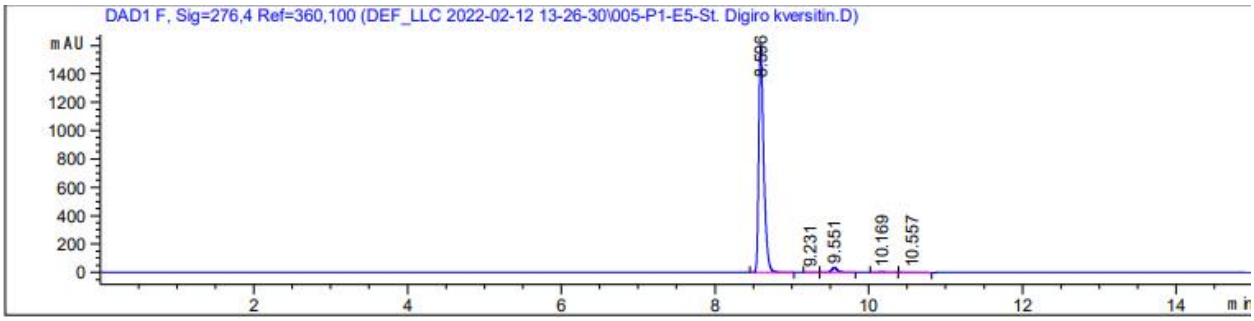

Figure 6. Chromatogram of Dihydroquercetin in the Composition of the "GEPAGAL" Substance

As shown in Figures 5 and 6, the retention time of dihydroquercetin is 9.559 minutes, during which it exhibits its maximum peak intensity.

The results of the metrological analysis of the values obtained for the determination of rutin content in the composition of the "GEPAGAL" substance are presented in Table 5.

Table 5

Metrological Characteristics of the Results of Rutin Content Determination

№	X%	X_a	S^2	S	S_x	t(95%,4)	ΔX	$\Delta X_{o'rt}$	E,%	E,% $_{o'rt}$
1	1,7327	1,7330	0,00079171	0,0300161	0,0121329	2,58	0,0763714	0,0321738	3,49	1,83
2	1,7329									
3	1,7331									

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4	1,7334									
5	1,7331									

Based on the data presented in Table 5, the presence of rutin in the composition can also be observed. Therefore, the existence of this compound in the "GEPAGAL" mixture plays an important role in enhancing the pharmacological efficacy of the preparation and broadening its therapeutic activity spectrum. The numerical indicators given in Table 5 serve as a significant scientific basis confirming the richness of the "GEPAGAL" extract mixture in bioactive substances, the balance between natural components, and their synergistic effects. Moreover, the obtained results verify that this preparation possesses high-quality phytocomponents and can be considered an effective source of biologically active compounds.

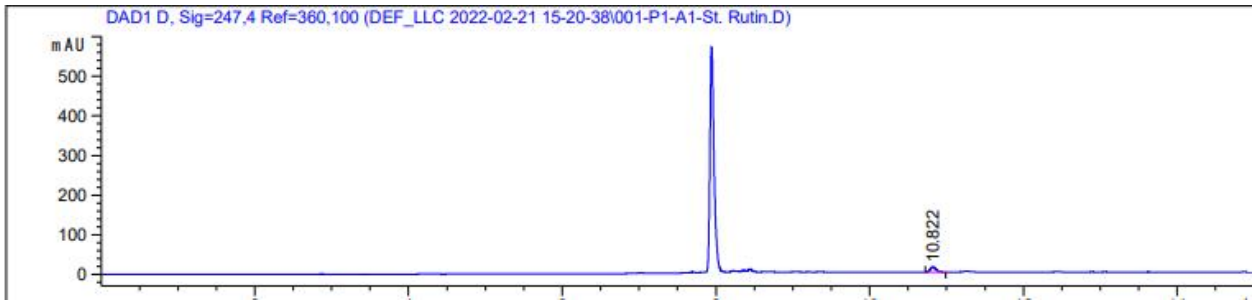

Figure 7. Chromatogram of the rutin standard solution

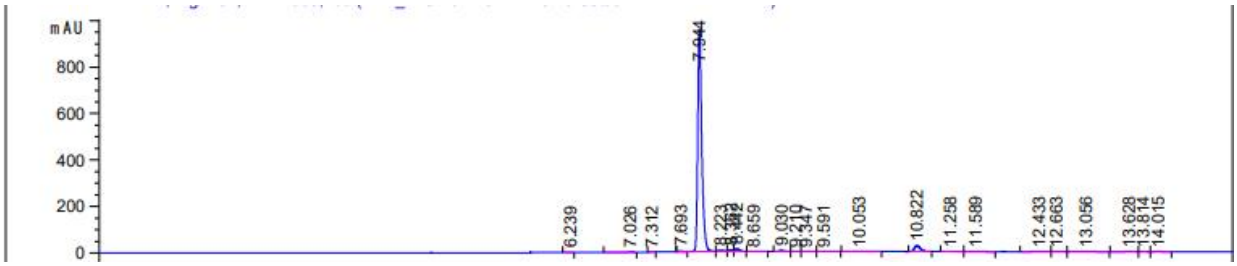

Figure 8. Chromatogram of rutin in the composition of the "GEPAGAL" substance

Results and Discussion.

To identify and quantitatively evaluate the flavonoids present in the composition of the "Gepagal" substance, the High-Performance Liquid Chromatography (HPLC) method was employed. This modern analytical technique allows for the precise identification of biologically active compounds and the determination of their relative quantities. During the study, under the selected chromatographic conditions, the retention time and peak height of the flavonoids contained in the substance were determined, and characteristic values for each compound were recorded. According to the obtained results, the following flavonoids were identified in the "GEPAGAL" substance: rosavin – 9.229 min, solidroside – 12.526 min, dihydroquercetin – 9.178 min, rutin – 8.126 min, and luteolin – 10.847 min. These findings clearly represent the chromatographic characteristics of each flavonoid component and scientifically confirm their presence in the composition of the substance. Thus, the results of the HPLC analyses

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demonstrated that “GEPAGAL” contains a complex of several bioactive flavonoids, which play a key role in determining the chemical stability and overall quality of the extract.

Conclusion: For the first time, the quantitative composition of flavonoids in the “Gepagal” substance was determined using a newly developed analytical method based on High-Performance Liquid Chromatography (HPLC). This method was applied as a modern and reliable analytical approach, allowing for the accurate and reproducible measurement of bioactive components present in the substance. As a result of the conducted analyses, the following flavonoid contents were identified in “Gepagal”: rosavin – 17.32 mg/g, solidroside – 4.74 mg/g, luteolin – 19.24 mg/g, dihydroquercetin – 41.31 mg/g, and rutin – 8.95 mg/g. These findings confirm that “Gepagal” is rich in biologically active flavonoids, which are key contributors to the pharmacological effectiveness, antioxidant potential, and overall therapeutic value of the preparation.

REFERENCES:

1. Kayumov F.S., Mamatkulov Z.U., Tuxtayev H.R. Determination of Storage Conditions and Shelf Life of ‘GEPASILIMARIN’ Herbal Tea with Hepatoprotective Effect. Farmatsevtika. 2025;4:45-51.
2. Mamatkulov Z.U., Rizayev K.S. Development of a “Dissolution” Test for “STICHMACHOLE” Capsules. Int Bull Med Sci Clin Res. 2024;4(8):10-13.
3. Abu Ali ibn Sino. The Canon of Medicine. Books 1–5. Tashkent: Turon Zamin Ziyo Publishing House; 2015.
4. Mamatqulov ZU, Rizaev KS, Fayzullaev JSh. Amino Acid Composition of the Dry Extract of ‘STIGMACHOLE – ZEA’. Uzbekistan Pharm Bull. 2024;2:33-36.
5. Qayumov FS, Mamatqulov ZU, Tuxtayev HR. Optimization of the Composition and Technology of Hepatoprotective Herbal Tea ‘GEPASILIMARIN’. Farmatsevtika. 2025;4:37-44.
6. Maksudova FX, Baratova MB, Tadjibayeva DSh. Composition and Technology Improvement of Indian Ginseng Capsules. Pharm J. Tashkent. 2021;1:74-78.
7. Jigmitov AA, Nagaslaeva OV, Nikolaeva GG, Namzalov B-SB. Development of a Method for Quantitative Determination of Total Flavonoids by UF-Spectrophotometry in the Biologically Active Supplement ‘ARURA-TAN №10’. Bull Buryat State Univ. 2012;Special Issue:81-83.