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IDENTIFICATION OF FLAVONOIDS IN THE SUBSTANCE OBTAINED FROM THE PLANT *RHUS GLABRA* USING MODERN ANALYTICAL METHODS

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Annotation: In recent years, increasing attention has been directed toward biologically active compounds of plant origin due to their natural descent, high pharmacological potential, and relatively low incidence of adverse effects. Among these compounds, flavonoids occupy a special place because of their pronounced antioxidant, anti-inflammatory, antimicrobial, and capillary-protective properties. In this context, the "Glabtan" substance obtained from the plant *Rhus glabra* (R. glabra) has attracted considerable scientific interest as a promising natural source of flavonoids, particularly rutin and quercetin.

Preliminary investigations of the "Glabtan" substance were carried out using spectrophotometric analysis, which confirmed the presence of total flavonoids in the studied samples. Although spectrophotometry is widely used because of its simplicity, rapidity, and cost-effectiveness, this method has certain limitations. In particular, it does not provide sufficient selectivity for the identification of individual flavonoid compounds and cannot accurately determine the quantitative content of each specific component within a complex plant matrix.

To overcome these limitations and achieve more precise analytical characterization, high-performance liquid chromatography (HPLC) was selected as the main analytical technique for flavonoid identification and quantification. HPLC is considered one of the most reliable and sensitive modern analytical methods for the separation, identification, and quantitative determination of biologically active substances in plant-derived materials. The method enables accurate differentiation of structurally related flavonoids, determination of their retention times, and comparison with standard reference compounds such as rutin and quercetin.

The application of HPLC analysis allowed not only confirmation of the presence of individual flavonoids in the "Glabtan" substance, but also determination of their quantitative content with high accuracy and reproducibility. The obtained results demonstrate the suitability of the selected chromatographic method for the standardization and quality control of flavonoid-containing substances derived from *R. glabra*. Furthermore, the study highlights the перспективность of this plant as a valuable natural source of pharmacologically active flavonoids for the development of herbal medicinal products and phytopharmaceutical preparations.

Keywords: *Rhus glabra*, Glabtan, standardization, Berghof. HPLC, spectrophotometry, substance , rutin.

Introduction. At present, one of the major challenges in the pharmaceutical sector of the Republic of Uzbekistan is the insufficient supply of affordable and high-quality medicines produced from locally available natural raw materials. The growing dependence on imported pharmaceutical products has increased the need to explore domestic medicinal plant resources as alternative sources for the development of effective and economically accessible drugs. In this regard, special attention is being paid to biologically active compounds isolated from wild and

cultivated medicinal plants that remain insufficiently studied from phytochemical and pharmacological perspectives.

Uzbekistan possesses rich and diverse flora with significant reserves of medicinal plants widely used in traditional medicine for centuries. Despite their extensive application in folk healing practices, many of these plants have not yet been fully introduced into official medicine or scientifically standardized according to modern pharmaceutical requirements. Therefore, the comprehensive investigation of such plants, including their chemical composition, biological activity, and therapeutic potential, represents an important and promising direction in contemporary pharmaceutical research.

One of these valuable medicinal plants is *Rhus glabra* (R. glabra), commonly known as smooth sumac or "soccum totum." This plant grows as a small shrub or tree and is widely distributed in thickets, along roadsides, and in open uncultivated areas. *R. glabra* is highly adaptable to various climatic and soil conditions, demonstrating strong resistance to environmental stress factors, which makes it a convenient and sustainable source of medicinal raw material. Its ability to grow under diverse ecological conditions also increases its industrial and pharmaceutical significance as a renewable natural resource.

In traditional medicine throughout different regions of the world, the bark, roots, leaves, and fruits of *R. glabra* have long been used for the treatment of numerous diseases and pathological conditions. Folk healers have utilized preparations obtained from this plant for the management of colds, fever, diarrhea, dysentery, sore throat, oral inflammatory diseases, rectal bleeding, urinary bladder inflammation, burns, and various skin disorders. In addition, decoctions and extracts of *R. glabra* have been traditionally applied because of their antiseptic, anti-inflammatory, wound-healing, and astringent properties.

Modern scientific studies have demonstrated that the therapeutic activity of *R. glabra* is associated with the presence of various groups of biologically active compounds, including flavonoids, tannins, phenolic acids, organic acids, and essential oils. Among these compounds, flavonoids are considered particularly important due to their antioxidant, antimicrobial, anti-inflammatory, and capillary-protective effects. Rutin and quercetin, identified in *R. glabra*, are known to play a significant role in reducing oxidative stress, strengthening vascular walls, and preventing inflammatory processes.

Considering the abundance of this plant in Uzbekistan and its rich phytochemical composition, *R. glabra* represents a promising source for the development of new phytopharmaceutical substances and herbal medicinal preparations. Therefore, detailed investigation of its chemical constituents using modern analytical methods, including high-performance liquid chromatography (HPLC), is of great scientific and practical importance. Such studies contribute not only to the standardization and quality control of plant-derived substances but also to the expansion of the national pharmaceutical industry through the creation of effective medicines based on local natural resources.

Flavonoids belong to a group of naturally occurring biologically active compounds of high pharmacological importance, and their antioxidant, anti-inflammatory, antimicrobial, and membrane-protective properties have been extensively studied. In recent years, interest in and demand for biologically active substances derived from natural sources have been steadily increasing. This is primarily explained by their natural origin, biological compatibility, and lower incidence of adverse effects compared to synthetic drugs.

From this perspective, the investigation of flavonoids in the substance Glabtan obtained from the plant *Rhus glabra* is of significant scientific importance. In previous studies, flavonoids such as

rutin and quercetin contained in this substance were analyzed using spectrophotometric methods. However, this method does not fully allow for the precise quantification of the individual components and their exact proportions within the flavonoid composition.

Therefore, the use of high-performance liquid chromatography (HPLC) is considered appropriate for the accurate and reliable quantitative determination of flavonoids. The HPLC method is distinguished by its ability to efficiently separate complex mixtures, determine even low concentrations of flavonoids with high accuracy, and ensure a rapid and reliable analytical process.

In the present study, the HPLC method was used to determine the amount of rutin in the Glabtan substance, and important scientific results were obtained for evaluating its chemical composition and standardization parameters [5,6].

Aim of the study: To develop an HPLC method for the determination of rutin content in the Glabtan substance obtained from *Rhus glabra*.

Materials and methods: The HPLC analysis of flavonoids was performed using an LC-2030 liquid chromatograph (SHIMADZU, Japan). During the analysis, the “LabSolutions” software and a spectrophotometric detector were employed.

Chromatographic conditions: The separation process was carried out using a Zorbax Eclipse C18 column (4.6 × 150 mm, particle size 5 µm). A mixture of 0.3% phosphoric acid and methanol was used as the mobile phase. Detection was performed at a wavelength of 330 nm, which corresponds to the maximum absorption (λmax) of both rutin and quercetin. The flow rate of the eluent was 1 mL/min, the injection volume was 10 µL, the column temperature was maintained at 40°C, and the total analysis time was 30 minutes. For identification purposes, chromatographic analysis of standard samples of rutin and quercetin was performed in parallel.

Preparation of rutin standard solution: 0.0055 g (accurately weighed) of rutin (RS) was transferred into a 100 mL volumetric flask and dissolved in 40 mL of methanol. The solution was then diluted to volume with methanol.

Preparation of the test sample solution: 1.0 g (accurately weighed) of Glabtan substance was transferred into a 100 mL volumetric flask and dissolved in 40 mL of methanol. The solution was then diluted to volume with methanol. The obtained solution was filtered through a Millipore filter with a pore size of 0.45 µm.

Both standard and test solutions were chromatographed three times, and the rutin content in the substance was determined. The obtained results are presented in Figures 1 and 2 and in the table.

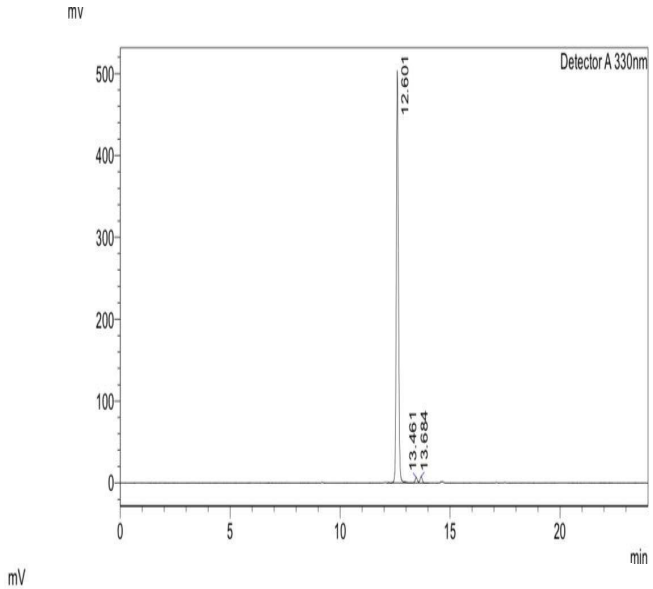


Figure 1. Chromatogram of rutin standard solution.

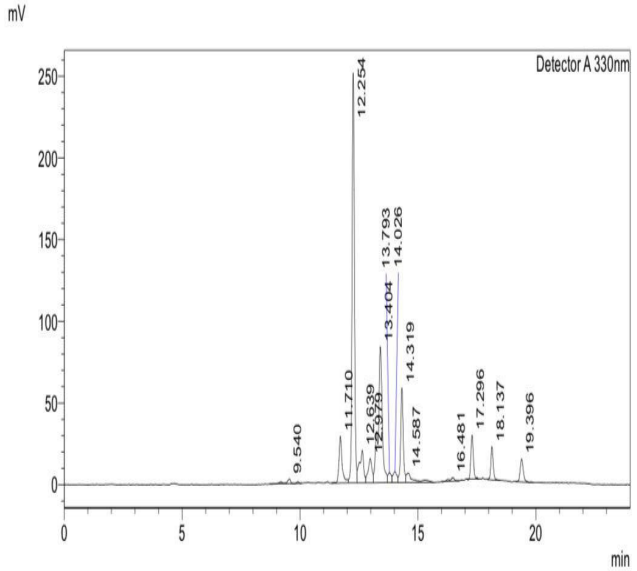


Figure 2. Chromatogram of Glabtan substance.

Table 1

Results of rutin content determination in the Glabtan substance by HPLC and metrological characteristics of the method.

X_i (mg/g)	X_{mi} d g	S^2	S	ΔX	ΔX_{mid}	E %	E_{mid} %
4,35	4,33	0,00037	0,0192	0,0534	0,0239	1,24	0,55
4,34							
4,30							
4,33							

4,32							

Results: During the study, the determination of rutin content in the "Glabtan" substance obtained from *R. glabra* was shown not merely as a numerical assessment, but as a "precision science," where each chromatographic peak revealed its own identity. Using High-Performance Liquid Chromatography (HPLC), each peak disclosed its chemical character, clearly defining the position of rutin within the flavonoid profile.

According to the obtained results, the average rutin content in the Glabtan substance was 4.33 mg/g**,** while the average relative error was ±0.55%**,** demonstrating a very narrow margin of analytical uncertainty. The chromatographic profile confirmed the high sensitivity and reliability of the method, where each component was distinctly separated and expressed as a clear analytical signal.

Recalculation of the chromatographic data showed that the rutin content constituted 0.43%**.** This result demonstrated that the HPLC method is not only a numerical analytical tool, but also a system that "teaches precision," transforming natural compounds into clearly interpretable scientific evidence. Each peak was not a random signal, but a structured confirmation of analytical order.

Conclusion: The study resulted in the development of an HPLC method for determining the rutin content in the "Glabtan" substance obtained from *R. glabra*. This method enables the standardization of the substance based on rutin content and serves as a reliable criterion of analytical precision, allowing natural compounds to be accurately expressed in quantitative scientific terms.

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