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ISOLATION AND STUDY OF THE TOTAL FLAVONOIDS IN YOUNG WALNUT LEAVES *(JUGLANS REGIA L.)*

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Abstract. The article presents a scientific work on the search for the isolation of the sum of flavonoids from young leaves of the walnut family *Juglans regia L.* from the gardens of the Bostandyk district of the Republic of Uzbekistan. Some physicochemical properties of the isolated flavonoids were studied by qualitative and quantitative methods. In the process of considering various literary sources on the content of flavonoids in nuts, it is known that of all the variety of phenolic compounds, flavonoids prevail in walnuts, which have a wide range of biological action in medicine. Namely, young leaves have medicinal value. The results allow the development to be used for implementation in production in order to increase the effectiveness of drugs.

Keywords: flavonoids, extract, leaves, walnuts, *Juglans regia L.,* biological activity.

Introduction. The importance of flavonoids in plants is very great. Based on the available data, it is believed that flavonoids participate in various oxidation-reduction processes in plant cells, are antioxidants, protect plants from the adverse effects of UV rays and low temperatures, participate in the development and manifestation of phytoimmunity, in the process of double fertilization in higher plants, in the manifestation of various colors of flowers and fruits, which attracts the attention of insects and thereby promotes pollination and fertilization of plants.

The use of flavonoids in medicine is due to the wide range of their biological action, distribution and almost complete absence of toxicity. Flavonoids have a wide range of biological activity: antisclerotic, antispasmodic, antiinflammatory, antiulcer, wound healing, cardiovascular, choleretic, antiviral and antimicrobial, hypoazotemic, hypotensive, diuretic [1 - 3]. They exhibit vasodilatory, cardiotonic, sedative, estrogenic, radioprotective and antitumor effects [1 - 3].

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In plants, flavonoids accumulate in various parts: buds, flowers, leaves, grass, fruits, roots.

About 40% of plant flavonoids are flavonol derivatives, about 20% are flavone derivatives, about 10% are catechins, anthocyanidins, flavanones, aurones, chalcones [4].

The biological role of flavonoids in the life of plants of the *Juglans regia* family has not been sufficiently studied, the most famous of which is the walnut, or royal nut. The fruits of the nut are widely used as a food product. The bark and shell of the fruit are used to make dyes. The leaves have medicinal value. Flavonoids are found in abundance in young leaves of the walnut *(J. regia L.)* and significantly less in old leaves [5].

Therefore, the aim of our work was to isolate the total flavonoid compounds of young walnut leaves growing in the conditions of the region of Uzbekistan, and to study them using quantitative and qualitative methods.

Materials and methods. Walnuts are still valued in Uzbekistan today as a food product and medicine, consumed raw and in various confectionery products.

The leaves of the walnut tree bloom in April-May, which is what was collected for harvesting.

To conduct the analysis, aqueous-alcoholic extracts of the studied raw materials were obtained in a ratio of 1:30 in 80% ethyl alcohol for qualitative (thin-layer chromatography) and quantitative assessment (spectrophotometry), which are more reliable methods.

The flavonoid extract was separated into components using column chromatography and sorbents – cellulose (paper). Elution of flavonoid substances from the column (or paper) in the form of aglycones was carried out with a mixture of chloroform and ethanol, with increasing concentration of alcohol.

Flavonoids were identified based on their physicochemical properties and comparison with literature data.

Most flavonoids are solid crystalline substances, odorless, colorless or yellow-brown, with a certain melting point. Flavones (apigenin, luteolin), flavonols (kaempferol, quercetin, myricetin), chalcones (2`-hydroxychalcone), aurones (for example, sulfuretin) are colored yellow; catechins (flavan-3-ol), flavans (2-phenylchroman), flavanones (naringenin), isoflavones are colorless [1, 6, 7].

In an acidic environment they are red, in an alkaline environment they are blue. Flavonoids fluoresce in UV light, and in chromatograms flavones, flavonol-3-glycosides, flavanones, chalcones are detected as brown spots, flavonols and their 7-glycosides are yellow or yellow-green [1].

To determine flavonoids, we carried out some qualitative reactions.

The cyanidin test (Synod test), based on their reduction by atomic hydrogen in an acidic medium in the presence of magnesium or zinc, was carried out using concentrated

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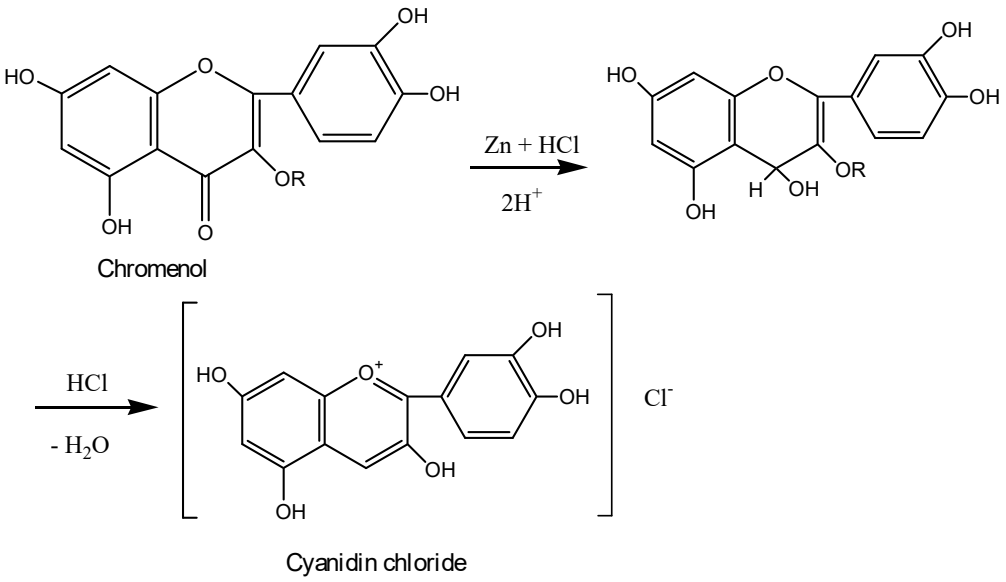
hydrochloric acid and metallic zinc shavings. The released hydrogen acts on the flavonoid molecule to form an oxonium compound, which produces a color from orange (flavones) to red-violet (flavonols, flavanones, flavanonols), caused by the formation of anthocyanidins (for example, cyanidin, pelargonidin, delphinidin) (scheme 1):

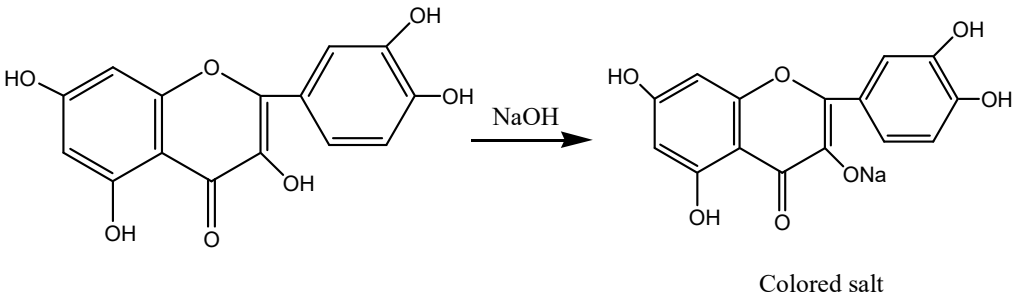


Scheme 1. **Reaction of the cyanidin test for the determination of flavonoids.**

Chalcones, aurones and isoflavones do not give color in the cyanidin reaction.

Therefore, an additional reaction with alkalis was carried out, forming colored

salts: flavones and flavonols, chalcones and aurones - yellow; catechins, flavanones, isoflavones - red (scheme 2):



Scheme 2. **Alkaline reaction for determination of flavonoids.**

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During chromatographic separation of the sum of flavonoids on paper with a mixture of n-butanol - acetic acid - water (4:1:5), the R_f values were about 0.4 and 0.65, respectively, under UV rays, yellow spots were revealed, and after developing the chromatograms with an alcohol solution of Na hydroxide - an orange glow (after developing in ammonia vapor - an orange-brown glow).

The quantitative content of the isolated total flavonoids was determined by a spectrophotometric method based on color reactions, the ability of flavonoids to absorb light in the UV region of the spectrum and to fluoresce. Two absorption maxima characteristic of flavonoids, in particular flavonols, are found at about 260 nm and 360 nm, which is confirmed by a bathochromic shift of the long-wave band in the presence of $AlCl_3$, as well as by data from differential spectra with an absorption maximum of 410 – 412 nm.

It was found that the flavonoid isolated from walnut leaves determines the nature of the absorption curve of the aqueous-alcoholic extract from walnut leaves, meaning it is a diagnostic substance for this type of raw material. Taking into account the fact that the absorption maxima of the solution of the isolated flavonoid and the aqueous-alcoholic extract of walnut leaves are in the region of 412 nm (differential variant), it is advisable to determine the content of the sum of flavonoids in terms of the isolated flavonoid at a wavelength of 412 nm.

During the development of the method, it was determined that the optimal parameters are: 80% ethyl alcohol, raw material-extractant ratio of 1:30, extraction time of 30 min, analytical wavelength at 412 nm (differential option).

Results and discussion. Samples - young leaves of walnut (*Juglans regia L.)* were collected during the flowering period in April 2024 in the gardens of the Bostandyk district of the Republic of Uzbekistan.

Drying of the leaves was carried out naturally under a canopy without access to direct sunlight, since slow drying, especially in the sun, leads to the destruction of flavonoids. The end of drying was checked by the fragility of the leaves. The raw materials were stored in packaged form without access to direct sunlight.

Extraction of flavonoids from walnut leaves was performed with an alcohol solution. The resulting alcohol extracts were then evaporated to an aqueous residue, diluted with hot water, and lipophilic substances (resins, fatty oils, chlorophyll) were removed from the aqueous phase using a separatory funnel, adding dichloroethane or carbon tetrachloride.
Individual substances were isolated from walnut leaves using column chromatography on silica gel L 40/100 under gradient elution conditions with a mixture of chloroform-ethanol solvents in different ratios. 60 g of air-dried raw walnut leaves were extracted with 70% ethyl alcohol, first carrying out two extractions at room temperature for 24 h, and then by heating in a boiling water bath for 30 min, the degree of grinding of the raw material was 2 mm. The combined aqueous-alcoholic extract was evaporated under vacuum to a volume of 50 ml, mixed with 20 g of silica gel L 40/100 and dried. The dried powder (dry extract + silica gel) was applied to a layer of silica gel (diameter 8 cm, height 5 cm). The chromatographic column (Bionis, France) was eluted with chloroform and a mixture of

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chloroform and ethanol in various ratios (99:1, 93:7, 85:15, 80:20, 75:25, 70:30, 60:40, 50:50, 40:60) at 400C. The separation of substances was monitored by TLC analysis.
TLC was performed using chromatographic plates (Sorbfil PTSKh-AF-A-UF, Russia), 0.02 ml of aqueous-alcoholic extracts of walnut leaves obtained in 40, 70 and 96% ethyl alcohol, tincture of walnut leaves were applied with a micropipette. 0.01 ml of witness solutions - a standard sample (SS) of rutin (quercetin-3-O-rutinoside or sophorin) were applied nearby with a micropipette. The determination was carried out in the system n-butanol - glacial acetic acid - water (4:1:2) and chloroform - ethanol - water (26:16:3). The chromatographic plate was placed in a chamber, which was pre-saturated for 60 min with a mixture of solvents and chromatographed in an ascending manner.
The resulting chromatogram was viewed in daylight, in a UV lamp (Biostep, Vizualizator HP-Uvis NxG, France) at λ=406 nm, and also treated with a 3% alcohol solution of aluminum chloride ($AlCl_3$).

For quantitative determination of flavonoids in walnut leaves, the method of differential spectrophotometry was used, based on the reaction of complex formation of flavonoids with a solution of aluminum chloride. Registration of UV spectra was carried out using a spectrophotometer ("UV-5100", Metash, China). The calculation of the sum of flavonoids was carried out using the specific absorption index of the rutin complex with a 3% alcohol solution of aluminum chloride.

The determination of the sum of flavonoids in walnut leaves *(Juglans regia L.)* was carried out by differential spectrophotometry at a wavelength of 412 nm, the content of the sum of flavonoids, X in percent, and absolutely dry raw materials were calculated by the formula in the absence of a standard sample of rutin, using the theoretical value of 240:

$$X = \frac{D * 30 * 50 * 100}{m * 240 * (100 - W)}$$

where, D - optical density of the test solution;

m - mass of raw material, g;

240 - specific absorption index (E 1%/1 cm) of the State Standard Sample of rutin at 412 nm;

W - loss in mass on drying, %.

Table 1 presents data on the flavonoid content in samples of young walnut leaves *(Juglans regia L.)*, April 2024.

Table 1.

Content of total flavonoids in samples of young walnut leaves *(Juglans regia L.)*

General chemical structure of flavonoids	Total flavonoids, mg/100 g	Optical density, D

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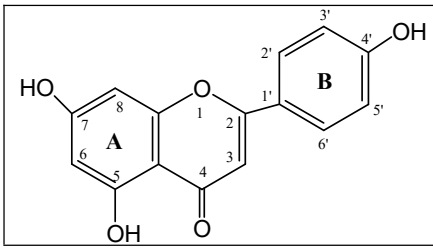	2,73 ± 744,5	0,7220

Conclusion. The conducted comparative chromatographic study allowed to reveal the presence of flavonoids in water-alcohol extracts from young walnut leaves. Using column chromatography, the sum of flavonoids identified on the basis of UV lamp and spectrophotometry data was isolated from walnut leaves. In this spectrum of the studied sample, upon addition of an alcoholic solution of $AlCl_3$, a bathochromic shift of the long-wave band is detected, which indicates the contribution of many flavonoids to the absorption curve of the UV spectra. Under the conditions of differential spectrophotometry, the absorption maximum is observed in the region of 410 – 412 nm. This gives grounds for applying the method of determining the content of the sum of flavonoids by differential spectrophotometry at a wavelength of 412 nm to other types of flavonoid derivatives for walnut leaves.

Based on a comparative study of the electronic spectra of aqueous-alcoholic extracts of walnut leaves, a method for quantitative determination of the sum of flavonoids has been developed, which consists of using 80% ethyl alcohol, extraction for 60 minutes in a ratio of "raw material-extractant" - 1:30 at an analytical wavelength of 412 nm.
It was determined that the content of the sum of flavonoids for the studied samples varies within 22.73 ± 744.5 mg/100 g.
Thus, young walnut leaves are a promising source of medicinal plant raw materials and can serve as a source of biologically active compounds - flavonoids.

References:

1. Федосеева Г.М., Мирович В.М., Горячкина Е.Г., Переломова М.В. «Фитохимический анализ растительного сырья, содержащего флавоноиды». Методическое пособие по фармакогнозии. – Иркутск. – 2009. – С. 3-51.
2. Bolling B.W., McKay D. L., Blumberg J.B. The phytochemical composition and antioxidant actions of tree nuts // Nutrition Research Reviews. – 2011. – 24. – P. 244-275.
3. Ismailova G.O., Yuldashev N.M., Akbarhodjaeva Kh.N., Shertaev M.M., Ziyamutdinova Z.K. Biologically Active Natural 2'-Hydroxychalcones // Russian Journal of Bioorganic Chemistry. – 2021. – Vol. 47. – No. 3. – P. 660–669. DOI.org/10.1134/S1068162021030080
4. Tiwari B.K., Brunton N.P., Brennan C.S. Handbook of Plant Food Phytochemicals: Sources, Stability and Extraction. – John Wiley and sons Ltd. – 2013. [in English].
5. Жилин С.Г. Семейство ореховые (Juglandaceae). [Электронный ресурс]. – Режим доступа: URL: https://organiclawn.ru/books/book_03_51/chapter_2/hamamelididae/juglandales/juglandaceae/

ISSN: 2692-5206
AMERICAN ACADEMIC PUBLISHER
INTERNATIONAL JOURNAL OF MEDICAL SCIENCES

6. Ismailova G.O., Mavlyanov S.M., Kamaev F.G. Synthesis of Structural Fragments of Natural Flavonoids and Flavolignans from 2'-Hydroxychalcones // Russian Journal of Bioorganic Chemistry. – 2012. – Vol. 38. – No. 3. – P. 335-337. DOI:10.1134/S1068162012030065
7. Исмаилова Г.О., Каримова Ш.Ф., Зиямутдинова З.К., Баходирова М.А. Распространённые природные халконы. // Журнал «Альманах современной науки и образования». – 2016. – №10 (112). – Тамбов: Грамота. – С. 36-45.