QO‘QON DAVLAT PEDAGOGIKA INSTITUTI ILMIY XABARLARI
(2026-yil 2-son)

TABIIY FANLAR

NATURAL SCIENCES

UDK 54.056

STUDY OF ISOLATED FLAVONOIDS IN YOUNG LEAVES OF WORMWOOD (*ARTEMISIA ABSINTHIUM L.*)

https://doi.org/10.70728/a.series.v08.i02.021

Ismailova Gulzira Orinbayevna
PhD, Associate Professor, Department of Organic and Biological Chemistry
Pharmaceutical Education and Research Institute,
Tashkent, Republic of Uzbekistan
E-mail: ismailova.gulzira@mail.ru
Allaniyazova Zukhra Durisbergen qizi
Intern-researcher, Institute of Biophysics and Biochemistry at the National
University of Uzbekistan named after Mirzo Ulugbek,
Tashkent, Republic of Uzbekistan

Abstract. This article describes a study on the isolation and chemical analysis of flavonoids in young leaves of Artemisia absinthium L., a medicinal plant native to the Republic of Uzbekistan. Three new flavonoid compounds with a broad range of biological activity in medicine were isolated. Their qualitative composition (cyanidin assay, reaction with alkalis, and chromatographic methods) and quantitative content (spectrophotometric method) were studied. Their physicochemical properties were compared with published data. The results will allow for the development to be implemented in production to improve the effectiveness of medicinal products. Further research into the component composition and pharmacological properties of their extracts appears promising.

Key words: flavonoids, isolation, leaves, wormwood, *Artemisia absinthium L.*, biological activity.

Introduction. The importance of flavonoids in plants is enormous. Based on available data, flavonoids are believed to participate in various redox processes in plant cells, act as antioxidants, protect plants from the adverse effects of UV rays and low temperatures, participate in the development and expression of phytoimmunity, participate in the process of double fertilization in higher plants, and contribute to the diverse coloration of flowers and fruits, which attracts insects and thereby facilitates pollination and fertilization of plants.

The use of flavonoids in medicine is due to their broad range of biological effects: antisclerotic, antispasmodic, anti-inflammatory, antiulcer, wound-healing, cardiovascular, choleretic, antiviral and antimicrobial, hypoazotemic, hypotensive, and diuretic. They exhibit vasodilatory, cardiotonic, sedative, estrogenic, radioprotective and antitumor effects[1,2,3].

Flavonoids accumulate in various parts of plants: buds, flowers, leaves, grass, fruits, and roots.

The biological role of flavonoids in the life of *Artemisia absinthium* plants themselves has been poorly studied. Therefore, the aim of this study was to determine the flavonoid content, qualitative composition, and quantitative content in young leaves of *Artemisia absinthium L.* (Asteraceae family), growing in the Uzbekistan region.

The above-ground part of wormwood during the flowering period, the leaves before flowering contain flavonoids[4].

Research Methodology. Bitter wormwood is currently valued in Uzbekistan in folk medicine as a medicinal remedy, consumed as a tincture, and is sometimes used in cooking as a spice.

It blooms in August–September. The leaves are alternate, with pinnately divided to linear, pointed lobes. The lower leaves are long-petiolate and rounded, while the stem leaves are sessile, tapering toward the inflorescence. The leaves turn black over time, initially being bicolor. Young leaves were collected for harvesting in August 2024 in the Tashkent region, Bostanlyk district, and Ugam-Chatkal National Park. They were dried in a well-ventilated area.

Three types of flavonoids were isolated using sequential extraction and gradient extraction methods in silica gel and polyamide columns: **(1)** artemisetin (3,3′,4′,6,7-pentamethoxy-5-hydroxyflavone); **(2)** isorhamnetin (3,4',5,7-tetra-hydroxy-3'-methoxyflavones); **(3)** narcissin (3,12-didehydro-2′H-[1,3]dioxolo[4′,5′:9,10]galantane-1α,2β-diol).

The structure of the isolated flavonoids was established using chromatographic and spectral methods, as well as comparison with known samples. Purification of the substances was performed using polyamide column chromatography, thin-layer chromatography,

[1] Федосеева, Г.М., Мирович, В.М., Горячкина, Е.Г., Переломова, М.В. (2009). Фитохимический анализ растительного сырья, содержащего флавоноиды. Методическое пособие по фармакогнозии, 3-51.

[2] Bolling, B.W., McKay, D.L., Blumberg, J.B. (2011). The phytochemical composition and antioxidant actions of tree nuts. Nutrition Research Reviews, (24), 244-275.

[3] Ismailova, G.O., Yuldashev, N.M., Akbarhodjaeva, Kh.N., Shertaev, M.M., Ziyamutdinova, Z.K. (2021). Biologically Active Natural 2'-Hydroxychalcones. Russian Journal of Bioorganic Chemistry, (47 (3)), 660–669. DOI.org/10.1134/S1068162021030080

[4] Ржевский, С.Г., Гудкова, А.А., Агафонов, В.А., Верлина, А.А. (2019). Сравнительное исследование химического состава *Artemisia armeniaca Lam., Artemisia latifolia Lideb.* и *Artemisia absinthium L.* Вестник ВГУ. Серия: химия. биология, (2), 109-116.

preparative paper chromatography, and fractional crystallization. The identity of the isolated compounds was verified using two-dimensional paper chromatography.

This method is based on the complexation of flavonoid compounds with metal solutions, particularly trivalent metals. Total flavonoid content was calculated as rutin.

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

The flavonoid extract was separated into its components using column chromatography and cellulose (paper) as sorbents. Flavonoid substances were eluted from the column (or paper) as aglycones using a mixture of chloroform and ethanol, with increasing alcohol concentrations.

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

The isolated flavonoids were odorless, yellow-brown, crystalline solids with a defined melting point[1,5,6].

In an acidic environment, they appear red; in an alkaline environment, they appear blue. Flavonoids fluoresce under UV light, appearing as brown spots on chromatograms[1].

Several qualitative reactions were conducted to determine flavonoids.

The cyanidin test (Synod's test), based on their reduction by atomic hydrogen in an acidic medium in the presence of magnesium or zinc, was conducted using concentrated hydrochloric acid and metallic zinc turnings. The released hydrogen reacts with the flavonoid molecule to form an oxonium compound, which produces an orange color (flavones), due to the formation of anthocyanidins (for example, cyanidin, pelargonidin, delphinidin) (Fig. 1):

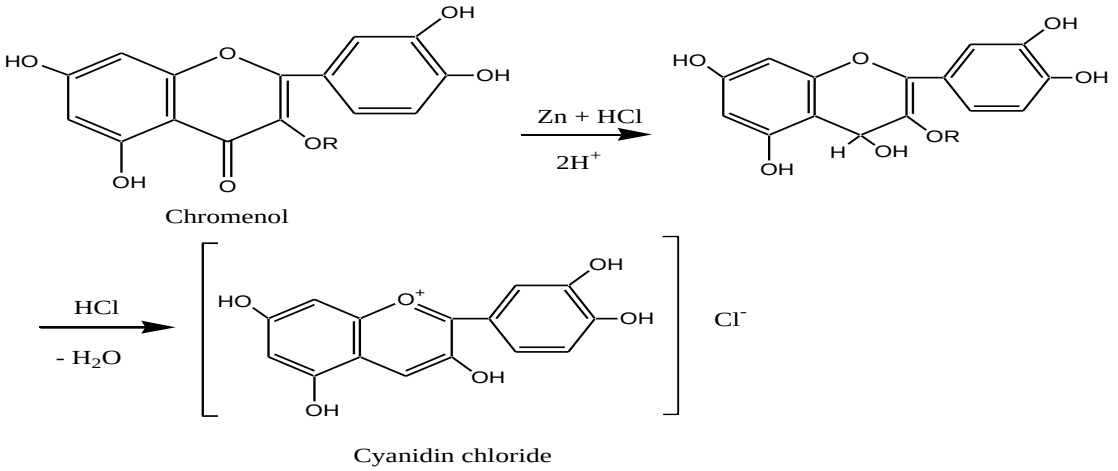
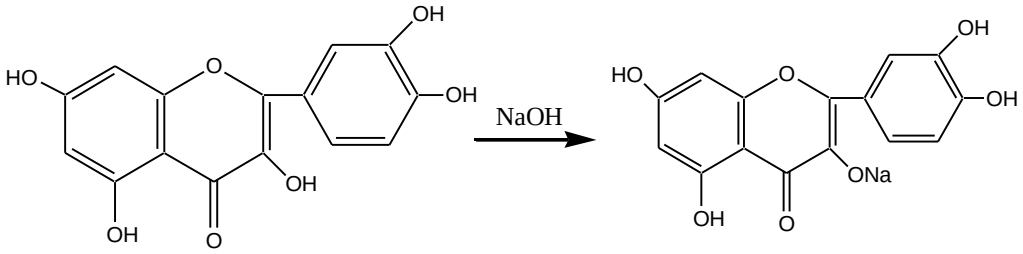


Figure 1. Reaction of the cyanidin test for the determination of flavonoids

[5] Ismailova, G.O., Mavlyanov, S.M., Kamaev, F.G. (2012). Synthesis of Structural Fragments of Natural Flavonoids and Flavolignans from 2'-Hydroxychalcones. Russian Journal of Bioorganic Chemistry, (38 (3)), 335-337. DOI:10.1134/S1068162012030065

[6] Исмаилова, Г.О., Каримова, Ш.Ф., Зиямутдинова, З.К., Баходирова, М.А. (2016). Распространённые природные халконы. Альманах современной науки и образования, (10 (112)), 36-45.

Additionally, a reaction was carried out with alkalis, forming colored salts - yellow (Fig. 2):



Chromenol Colored salt

Figure 2. Alkaline reaction for the determination of flavonoids

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

The quantitative content of isolated total flavonoids was determined using a spectrophotometric method based on color reactions, the ability of flavonoids to absorb light in the UV spectrum, and fluorescence. Characteristic flavonoids, particularly flavones, are detected at a wavelength of 365 nm, as confirmed by a bathochromic shift of the long-wavelength band in the presence of $AlCl_3$.

It was found that the flavonoid isolated from wormwood leaves determines the absorption curve of the aqueous-alcoholic extract of wormwood leaves, thus serving as a diagnostic substance for this type of raw material. Considering the fact that the absorption maxima of the solution, the isolated flavonoid, and the aqueous-alcoholic extract of wormwood leaves are all in the 365 nm region (differential variant), it is advisable to determine the total flavonoid content based on the isolated flavonoid at a wavelength of 412 nm. During the development of the methodology, it was determined that the optimal parameters are: 80% ethyl alcohol, raw material-extractant ratio of 1:30, extraction time of 30 minutes.

Results and discussion. Young leaves of wormwood (*Artemisia absinthium L.*) were collected before flowering in August 2024.

The leaves were dried naturally under a canopy, out of direct sunlight, as slow drying, especially in the sun, leads to the destruction of flavonoids. Drying completion was determined by leaf fragility. The raw materials were stored in a packaged container out of direct sunlight.

Flavonoids were extracted from the wormwood leaves using an alcohol solution. The resulting alcohol extracts were then evaporated to an aqueous residue, diluted with hot

water, and lipophilic substances (resins, fatty oils, and chlorophyll) were removed from the aqueous phase using a separatory funnel and dichloroethane or carbon tetrachloride.

The isolation of individual substances from the leaves of wormwood was carried out using column chromatography on silica gel 5/40 μm under gradient elution conditions with a chloroform-ethanol solvent mixture in a ratio of 9:1. Air-dried raw material (60 g) of leaves was 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 minutes, 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 5/40 μm 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, ethyl acetate and *n*-butanol at 40ºC.

Elution with chloroform and subsequent crystallization from ethanol yielded **compound 1**. Separation of the ethyl acetate fraction in a polyamide column using chloroform-alcohol mixtures with a gradient increase in chloroform concentration yielded **compound 2**, which was obtained by elution with a chloroform-ethanol mixture in a 1:19 ratio. Chromatography of the butanol fraction in a polyamide column using an ethanol-water mixture in a 2:8 ratio yielded **compound 3**.

The separation of the substances was monitored using paper chromatography (PC) and thin-layer chromatography (TLC).

TLC was performed using chromatographic plates (Sorbfil PTSKh-AF-A-UF, Russia) with a chloroform-ethanol (9:1) solvent system. A micropipette was used to add 0.02 ml of aqueous-alcoholic extracts of wormwood leaves obtained in 40, 70, and 96% ethanol, as well as tincture of wormwood leaves. A micropipette was used to add 0.01 ml of reference solutions – a standard sample (SS) of rutin (quercetin-3-*O*-rutinoside). The chromatographic plate was placed in a chamber pre-saturated with the solvent mixture for 60 min and chromatographed in an ascending manner.

For biochromatography (Filtrak FN-1, 3, 5, 11), the following solvent system was used: *n*-butanol-glacial acetic acid-water (4:1:2).

The resulting chromatogram was viewed in daylight, under a UV lamp (Biostep, Vizualizator HP-Uvis NxG, France) at λ = 406 nm, and also treated with a 1% alcoholic solution of aluminum chloride ($AlCl_3$).

To quantify flavonoids in wormwood leaves, differential spectrophotometry was used, based on the complexation reaction of flavonoids with aluminum chloride. UV spectra were recorded using a spectrophotometer (UV-5100, Metash, China). Total flavonoid content was calculated using the specific absorption coefficient of the rutin complex with a 1% alcoholic solution of aluminum chloride.

The determination of the sum of flavonoids in the leaves of wormwood 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 according to 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 the raw material, g;

240 - specific absorption coefficient (E $^{1\%}/_{1}$ cm) of the State Standard Sample of rutin at 412 nm;

W - loss on drying, %.

Tables 1 and 2 present data on the flavonoid content in samples of young leaves of wormwood (*Artemisia absinthium L.*).

Table 1.

Total flavonoid content in samples of young leaves of wormwood

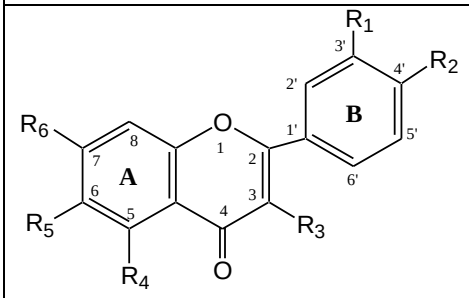
General chemical structure of isolated flavonoids	**Content in terms of rutin, SF method (%)**	**Optical density (D)**	**TLC (Rf)**
	0,56 ± 0,01	0,7220	0,4 - 0,65

Table 2.

Composition of isolated flavonoids (1 - 3) in samples of young leaves of wormwood

No.	**Compound**	**Tm (°C)**	**UV spectrum, λ (nm)**	
			Presence of substituted oxy groups	**Presence of free hydroxy groups**
1	C20H20O8	220–223	C-3′, C-4′, C-6, C-7	C-5
2	C16H12O7	302–303	C-3′	C-4′, C-3, C-5, C-7
3	C28H32O16	177–179	C-3	C-5, C-7, C-4′

According to the literature[7], all isolated and studied flavonoid compounds (**1 - 3**) from young leaves of wormwood (*Artemisia absinthium L.*) possess antimicrobial activity. They are capable of suppressing the growth zone of Staphylococcus aureus, the causative agent of diphtheria (*Corynebacterium diphtheriae*), enterococcal infection (*Enterococcus faecalis*), *Streptococcus frexner, Enterobacter cloacae* and *Proteus mirabilis*.

Conclusion. A comparative chromatographic study revealed the presence of flavonoids in aqueous-alcoholic extracts from young leaves of wormwood (*Artemisia absinthium L.*). Using column chromatography, total flavonoids were isolated from the leaves, identified based on UV lamp and spectrophotometric data. This provides grounds for applying the method for determining total flavonoid content by differential spectrophotometry at a wavelength of 412 nm to other types of flavonoid derivatives in leaves of the genus *Artemisia*.

It was determined that the total flavonoid content of the studied samples varied within 7%.

Thus, young leaves of wormwood (*Artemisia absinthium L.*) are a promising source of medicinal plant material from the flora of Uzbekistan – a source of antimicrobial agents.

Reference list:

1. Федосеева, Г.М., Мирович, В.М., Горячкина, Е.Г., Переломова, М.В. (2009). Фитохимический анализ растительного сырья, содержащего флавоноиды. Методическое пособие по фармакогнозии, 3-51.
2. Bolling, B.W., McKay, D.L., Blumberg, J.B. (2011). The phytochemical composition and antioxidant actions of tree nuts. Nutrition Research Reviews, (24), 244-275.
3. Ismailova, G.O., Yuldashev, N.M., Akbarhodjaeva, Kh.N., Shertaev, M.M., Ziyamutdinova, Z.K. (2021). Biologically Active Natural 2'-Hydroxychalcones. Russian Journal of Bioorganic Chemistry, (47 (3)), 660–669. DOI.org/10.1134/S1068162021030080
4. Ржевский, С.Г., Гудкова, А.А., Агафонов, В.А., Верлина, А.А. (2019). Сравнительное исследование химического состава *Artemisia armeniaca Lam., Artemisia latifolia Lideb.* и *Artemisia absinthium L.* Вестник ВГУ. Серия: химия. биология, (2), 109-116.
5. Ismailova, G.O., Mavlyanov, S.M., Kamaev, F.G. (2012). Synthesis of Structural Fragments of Natural Flavonoids and Flavolignans from 2'-Hydroxychalcones. Russian Journal of Bioorganic Chemistry, (38 (3)), 335-337. DOI:10.1134/S1068162012030065

[7] Шалдаева, Т.М., Высочина, Г.И. (2007). Возрастная изменчивость содержания флавоноидов в индивидуальных экземплярах растения *Artemisia dracunculus* L. при интродукции в лесостепную зону Западной Сибири. Сибирский экологический журнал, (1), 103-109.

6. Исмаилова, Г.О., Каримова, Ш.Ф., Зиямутдинова, З.К., Баходирова, М.А. (2016). Распространённые природные халконы. Альманах современной науки и образования, (10 (112)), 36-45.

7. Шалдаева, Т.М., Высочина, Г.И. (2007). Возрастная изменчивость содержания флавоноидов в индивидуальных экземплярах растения *Artemisia dracunculus* L. при интродукции в лесостепную зону Западной Сибири. Сибирский экологический журнал, (1), 103-109.