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**OBTAINING PHYTOSUBSTANCES FROM ZINNIA ELEGANS JACQ.
HERB AND DETERMINATION OF THEIR FLAVONOID CONTENT**

Actuality. The growing interest in phytopharmaceuticals is driven by their safety profile, biological activity, and synergistic effects of biologically active substances. *Zinnia elegans* Jacq., a representative of the Asteraceae family, is a promising source of flavonoids with potential antioxidant, hepatoprotective, and anti-inflammatory properties. Therefore, optimizing extraction conditions to obtain flavonoid-enriched phytosubstances is of practical and scientific relevance.

The purpose of the study was to obtain phytosubstances from the herb of *Zinnia elegans* Jacq. using different ethanol concentrations and to determine the qualitative composition and quantitative content of flavonoids.

Material and methods. Eight series of phytosubstances were obtained by maceration with stirring using ethanol concentrations ranging from 10% to 80% at a raw material-to-extractant ratio of 1:10. The total flavonoid content was determined spectrophotometrically at 408 nm (as rutin equivalent), while the qualitative and quantitative composition of individual flavonoids in the extract with the highest yield was analyzed using high performance liquid chromatography (HPLC).

Research results. The study confirmed that ethanol concentration significantly affects the extraction efficiency of flavonoids from *Zinnia elegans* Jacq. herb. The highest total flavonoid content (9,16%) was obtained using 70% ethanol, while higher concentrations reduced extraction efficiency. HPLC analysis of the 70% ethanol extract identified five individual flavonoids: rutin, isoquercitrin, astragalin, silybin, and rhamnetin, with a total content of 7,87 mg/g. Rhamnetin predominated (4,57 mg/g), confirming its pharmacological significance.

Conclusion. The findings demonstrate that 70% ethanol provides optimal conditions for extracting flavonoids from *Zinnia elegans* Jacq. herb. The presence of rhamnetin as a dominant compound highlights the potential of this species as a promising source of biologically active flavonoids for further phytopharmaceutical development.

Key words: *Zinnia elegans* Jacq., herb, maceration with stirring, flavonoids, spectrophotometric method, HPLC.

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ОТРИМАННЯ ФІТОСУБСТАНЦІЙ ІЗ ТРАВИ *ZINNIA ELEGANS* JACQ.
ТА ВИЗНАЧЕННЯ В НИХ ВМІСТУ ФЛАВОНОЇДІВ

Актуальність. Зростання інтересу до фітопрепаратів зумовлене їхньою безпечністю, біологічною активністю та синергічною дією біологічно активних речовин. *Zinnia elegans* Jacq., представник родини Asteraceae, є перспективним джерелом флавоноїдів з вираженими антиоксидантними, гепатопротекторними та протизапальними властивостями. Тому оптимізація умов екстрагування для отримання фітосубстанцій, збагачених флавоноїдами, має як практичне, так і наукове значення.

Мета дослідження – отримати фітосубстанції із трави *Zinnia elegans* Jacq. з використанням різних концентрацій етанолу, визначити якісний склад і кількісний вміст флавоноїдів.

Матеріал і методи. Вісім серій фітосубстанцій отримано методом мацерації з перемішуванням, з використанням етанолу в концентраціях від 10 до 80% у співвідношенні сировини до екстрагенту 1:10. Вміст суми флавоноїдів визначали спектрофотометричним методом за довжини хвилі 408 нм (у перерахунку на рутин), якісний склад і кількісний вміст окремих сполук в екстракті з найвищим виходом досліджували методом вискоєфективної рідинної хроматографії (ВЕРХ).

Результати дослідження. Встановлено, що концентрація етанолу істотно впливає на ефективність екстракції флавоноїдів із трави *Zinnia elegans* Jacq. Найвищий вміст суми флавоноїдів (9,16%) отримано з використанням 70% етанолу, тоді як подальше підвищення концентрації знижувало ефективність екстрагування. Методом вискоєфективної рідинної хроматографії у фітосубстанції (70% етанол) ідентифіковано п'ять індивідуальних флавоноїдів: рутин, ізокверцитрин, астрагалін, силібінін і рамнетин, із загальним вмістом 7,87 мг/г. Переважав рамнетин (4,57 мг/г), що свідчить про його фармакологічну значущість.

Висновок. Оптимальною концентрацією для вилучення флавоноїдів із трави *Zinnia elegans* Jacq. є 70% етанол. Переважання рамнетину підтверджує перспективність виду як джерела біологічно активних флавоноїдів для подальшого розроблення фітопрепаратів.

Ключові слова: *Zinnia elegans* Jacq., трава, мацерація з перемішуванням, флавоноїди, спектрофотометричний метод, ВЕРХ.

Introduction. Actuality. Ensuring effective and high-quality prevention, as well as comprehensive treatment of various diseases, remains closely associated with the development and implementation of phytopharmaceuticals containing plant-derived raw materials. Medicinal plants have long attracted human interest due to their therapeutic potential (Budniak, 2023; Storozhuk, 2024).

The use of medicinal plants in healthcare has a centuries-old tradition (Stoiko & Kurylo, 2018). It is widely recognized that biologically active compounds in plants occur in naturally balanced proportions. Plant-based medicines influence the human body through a synergistic combination of bioactive substances and essential microelements.

One of the principal advantages of phytotherapy is its low incidence of adverse effects, even with prolonged administration of herbal preparations (Slobodianiuk, 2022; Storozhuk, 2024).

In this regard, particular attention has been focused on the extraction and investigation of phytosubstances derived from *Zinnia elegans* Jacq., a promising representative of the Asteraceae family.

Zinnia elegans Jacq. is an annual herbaceous species with a bushy growth habit and well-developed foliage, typically attaining a height of 30–90 cm, although individual specimens may reach up to 120 cm. The plant exhibits a prolonged flowering period, extending from early summer to autumn in temperate regions. Growth may, however, decelerate under conditions of excessive heat, particularly when the plant is affected by fungal infections.

The species demonstrates considerable morphological variability in its floral structures, producing single, semi-double, and double flower forms with a broad color spectrum, including shades of red, orange, yellow, pink, lavender, crimson, green and white.

Leaves are arranged oppositely, ovate to lanceolate in shape, closely clasping the stems, and may reach up to approximately 12,5 cm in length. Due to its wide range of flower colors, prolonged blooming period, and adaptability to temperate climates, *Zinnia elegans* Jacq. possesses significant ornamental value and remains one of the most cultivated representatives of the Asteraceae family (*Zinnia elegans*, https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a618&utm_source=chatgpt.com#AllImages).

Zinnia elegans Jacq. is among the most widely distributed species in the southern and eastern regions of the United States, including Texas, Florida, Georgia, South Carolina, Alabama, and Louisiana, as well as along the western coast, particularly in California (*Zinnia elegans*, <https://bonap.net/MapGallery/County/Zinnia%20elegans.png>).

The species thrives in fertile, evenly moist, and well-drained soils under conditions of adequate sunlight.

Seed sowing is typically carried out directly in open soil after the last spring frosts (*Zinnia elegans*, https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a618&utm_source=chatgpt.com#AllImages).

In Ukraine, *Zinnia elegans* Jacq. has been introduced as an ornamental plant (Tulub, 2022).

Due to its prolonged flowering period, bright inflorescence colors, and low maintenance requirements, this species is widely used for ornamental landscaping and decorative planting (*Zinnia elegans*, https://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?kempercode=a618&utm_source=chatgpt.com#AllImages).

In the inflorescences of *Zinnia elegans* Jacq., acetylated diglucosides of cyanidin and pelargonidin have been identified (Gomaa, 2018; Burlec, 2019).

Another important class of secondary metabolites in this species is terpenoids, which are present in the essential oil. It has been established that the essential oil extracted from the inflorescences of *Zinnia elegans* Jacq. contains germacrene D and *p*-cymene as major constituents (Burlec, 2019).

Several sesquiterpenes, including cyniolide, germacrene D, and cynaflorin III, have been identified in the aerial parts and roots of the plant (Gomaa, 2018; Burlec, 2019).

The seeds of *Zinnia elegans* Jacq. are characterized by a relatively high oil content (approximately 28%). It has been determined that saturated fatty acids account for about 29%, while the amount of monounsaturated fatty acids is 1.6 times higher, reaching up to 48% (Azimova, 2012; Burlec, 2019).

Moreover, acetylene compounds and alkaloids derived from nicotine have also been detected in *Zinnia elegans* Jacq. (Burlec, 2019). Among the fatty acid profile of this species, unsaturated fatty acids predominate. The highest concentrations were observed in the flowers, while the lowest were found in the leaves.

Ethnopharmacological data indicate that infusions prepared from various parts of *Zinnia elegans* Jacq. have traditionally been used for analgesic purposes (Bieski, 2012). In traditional medicine, this species is employed for its hepatoprotective, antiviral, antibacterial, antiparasitic, antifungal, and analgesic properties. In Latin American countries, the leaves and roots of *Zinnia elegans* Jacq. are used in the treatment of stomach pain, malaria, hepatitis, cough, helminthiasis, and convulsions. Extracts from this plant are also applied externally as poultices for burns, furunculosis, and ulcers (Tulub, 2022).

Scientific studies have reported antioxidant, hepatoprotective, antifungal, and antimalarial activities of this species (Hafiza, 2002; Mohamed, 2015; Gomaa, 2018). Burlec et al. demonstrated that the methanolic extract of *Zinnia elegans* Jacq. contains a substantial amount of flavonoids and exhibits higher antioxidant activity than extracts obtained using more lipophilic solvents such as chloroform or hexane. These findings suggest that the antioxidant potential is primarily associated with the presence of polyphenolic compounds (Burlec, 2018).

Given the diverse chemical composition and wide range of biological activities demonstrated by *Zinnia elegans* Jacq., further research on the extraction and quantitative evaluation of its bioactive constituents remains highly relevant. Among the numerous groups of secondary metabolites, flavonoids attract particular scientific interest due to their pronounced antioxidant potential and key role in the pharmacological activity of plant-derived preparations. Previous studies have established the presence of various flavonoid glycosides in the aerial parts of *Zinnia elegans* Jacq., suggesting that these compounds may serve as markers of its biological activity. Therefore, optimizing the extraction process to obtain flavonoid-enriched phyto-substances from the herb of *Zinnia elegans* Jacq. and evaluating their content under varying solvent concentrations are essential steps toward developing standardized phyto-substances of potential therapeutic value.

The aim of the study was to obtain phytosubstances from the herb of *Zinnia elegans* Jacq. by maceration using different concentrations of ethanol and to determine the flavonoid content in them.

Materials and methods of the study. The objects of the study were phytosubstances obtained from the herb of *Zinnia elegans* Jacq. by the maceration method with stirring, using ethanol of various concentrations as the extractant at a raw material-to-extractant ratio of 1:10.

The aerial part of the plant was collected during the early flowering stage in 2024 in the Lviv region. The voucher specimen № 371 is deposited at the Department of Pharmacognosy and Medical Botany, TNMU.

The dried aerial part of *Zinnia elegans* Jacq. was ground into particles of 3–5 mm using a manual mill. The crushed raw material was placed in glass containers and poured with ethanol “to the mirror level”. Maceration was carried out with periodic stirring at a temperature of 15–20 °C for 7 days. After infusion, the extract was filtered and concentrated at a temperature of 40 °C.

The quantitative content of flavonoids in the phytosubstances obtained from *Zinnia elegans* Jacq. was determined by a spectrophotometric method, expressed in terms of rutin equivalent.

A sample of 0,1 g of the *Zinnia elegans* Jacq. phytosubstance was placed into a 25 mL volumetric flask and brought to the mark with 70% ethanol (solution A). Then, 2 mL of the previously prepared solution were transferred into another 25 mL volumetric flask, followed by the addition of 1 mL of a 2% ethanolic solution of aluminum chloride. The volume of the mixture was adjusted to the mark with 70% ethanol.

The optical density of the solution was measured after 45 minutes using a Lambda 25 Perkin Elmer spectrophotometer at a wavelength of 408 nm.

The reference solution consisted of 2 mL of solution A, 1–2 drops of diluted acetic acid, and was brought to volume with 70% ethanol.

The optical density of the standard rutin solution, prepared in the same manner as the test solution, was measured in parallel (Ohar, 2025).

The total flavonoid content, recalculated as rutin, was calculated on an absolutely dry basis of the *Zinnia elegans* Jacq. phytosubstance.

The qualitative composition and quantitative content of individual flavonoids in the extract with the highest total flavonoid amount were determined using high performance liquid chromatography (HPLC) (Budniak, 2022).

A 0,05 g (accurately weighed) portion of the obtained phytosubstance from the herb of *Zinnia elegans* Jacq., which had the highest total flavonoid content, was extracted in sealed glass vials with Teflon caps using an ultrasonic bath in 10 mL of 80% ethanol at 80 °C for 5 hours. The resulting solution was centrifuged at 3000 rpm, and the supernatant was filtered through disposable membrane filters with a pore size of 0,22 µm.

Chromatographic analysis was carried out using an Agilent Technologies 1 200 liquid chromatograph (Budniak, 2021; Feshchenko, 2021).

The mobile phase consisted of acetonitrile (A) and 0,1% formic acid solution in water (B). Elution was performed in a gradient mode (table 1).

Separation was performed on a Zorbax SB-C18 chromatographic column (3,5 µm, 150 mm × 4,6 mm) (Agilent Technologies, USA) (Khvorost, 2023). The flow rate through the column was 0,25 mL/min, the thermostat temperature – 30 °C, and the injection volume – 4 µL.

Detection was carried out at the wavelengths of 280 nm and 365 nm using a diode-array detector, with signal recording and absorption spectra registration in the range of 210–700 nm (Pyrzynska & Sentkowska, 2019).

Identification and quantification of individual flavonoid compounds were performed using standard flavonoid solutions (kaempferol, rutin, quercetin, isoquercitrin, luteolin, naringin, apigenin, neohesperidin, naringenin, astragalol, fisetin, rhamnetin, silybin, baicalin, and casticin). Calibration was performed using the external standard method. The content of individual flavonoid compounds was expressed in mg/g.

The study results were analyzed using mathematical statistics methods in Microsoft Excel. Statistical evaluation of the obtained data was carried out following the guidelines of the State Pharmacopeia of Ukraine (SPhU).

Research results and their discussion. Eight series of phytosubstances were obtained from the herb of *Zinnia elegans* Jacq. using the maceration method with stirring and ethanol (10, 20, 30, 40, 50, 60, 70, and 80%) as the extractant at a raw material to extractant ratio of 1:10.

The total flavonoid content in these phytosubstances was determined spectrophotometrically at a wavelength of 408 nm and expressed in terms of rutin equivalent.

The results of the total flavonoid content determination in the obtained phytosubstances are shown in fig. 1.

The study found that the ethanol concentration during extraction significantly affects the total flavonoid content in the obtained phytosubstances. As the ethanol concentration increased from 10 to 30%, a gradual rise in flavonoid content was observed – from 1% in the first series to 1,8% in the third. The use of 40% ethanol resulted in a sharp increase to 4,9%, and at 50% – to 6,3%. Further elevation to 60% yielded 8,1%, while the maximum value of 9,2% was achieved with 70% ethanol, indicating that this concentration is optimal for flavonoid extraction.

In contrast, the use of 80% ethanol led to a decrease in the amount of extracted compounds, likely due to reduced solubility of polar components in such a concentrated solvent. Thus, the most efficient extraction of total flavonoids from *Zinnia elegans* Jacq. herb is achieved using 70% ethanol as the extractant, while higher concentrations reduce the extraction efficiency.

In Series 7, the qualitative composition and quantitative content of individual flavonoid compounds were determined using the HPLC method.

The results of the conducted study are presented in fig. 2 and table 2.

In the phytosubstance (Series 7) obtained from *Zinnia elegans* Jacq. herb, the qualitative composition and quantitative content of individual flavonoid compounds – rutin, isoquercetin, astragalol, silybin, and rhamnetin – were determined using HPLC. The total flavonoid content was found to be 7,87 mg/g, with rhamnetin showing the highest concentration (4,57 mg/g).

Rhamnetin is an aglycone distinguished from quercetin by the presence of a methyl group at the 7th position. Owing to its structural features, this compound demonstrates pronounced antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and anticancer properties.

As an antioxidant, rhamnetin efficiently neutralizes free radicals and suppresses the formation of reactive oxygen species, thereby protecting cells from oxidative stress. Its

Table 1

Gradient elution parameters

Time, min	Eluent A, %	Eluent B, %
0	5	95
20	30	70
50	100	0
60	100	0

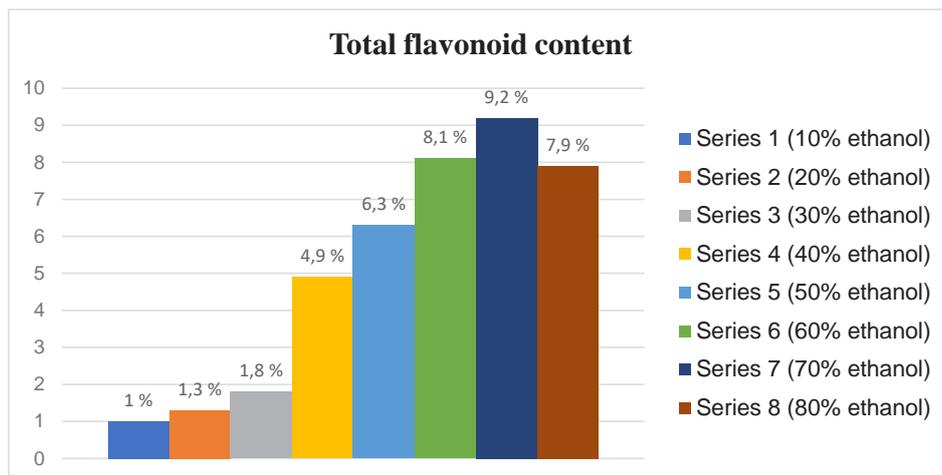


Fig. 1. Diagram of the total flavonoid content in phytosubstances from the herb of *Zinnia elegans* Jacq.

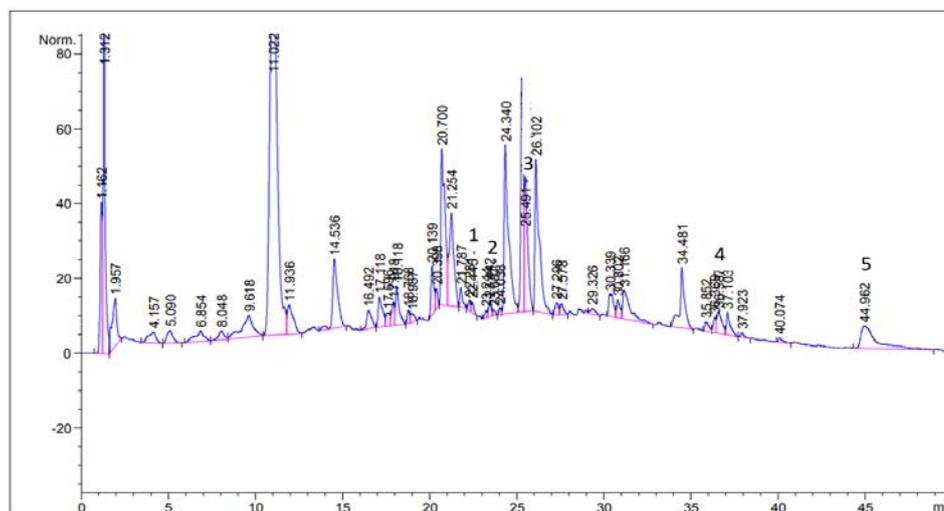


Fig. 2. HPLC chromatogram of individual flavonoid compounds in the phytosubstance (Series 7) from *Zinnia elegans* Jacq. herb: 1 – rutin, 2 – isoquercitrin, 3 – astragaline, 4 – silybin, 5 – rhamnetin

Qualitative composition and quantitative content of individual flavonoid compounds in the phytosubstance (Series 7) from *Zinnia elegans* Jacq. herb

Compound name	Quantitative content, mg/g
Rutin	0,18
Isoquercetin	0,13
Astragaline	2,40
Silybin	0,59
Rhamnetin	4,57
Total	7,87

Table 2

anti-inflammatory potential is linked to the inhibition of pro-inflammatory cytokines, including TNF- α , IL-6, IL-1 β , and IL-18, through modulation of caspase-1 activity, which leads to a reduction in the inflammatory response.

Rhamnetin also exhibits antibacterial activity, particularly against carbapenem-resistant *Acinetobacter baumannii*, by reducing bacterial colonization in organs and improving survival outcomes in sepsis models. Additionally, the compound demonstrates hepatoprotective effects by lowering hepatic enzyme levels and restoring normal liver histology in cases of toxic injury.

Recent studies emphasize the potential of rhamnetin as an anticancer agent, particularly in hepatocellular carcinoma, namely through its dual mechanism of inhibiting tumor cell proliferation and inducing apoptosis, which effectively suppresses cancer cell growth and survival (Lee, 2022; Kim, 2023; Abdel-Rasol, 2025).

Conclusions. 1. The study confirmed that ethanol concentration significantly influences the extraction efficiency of flavonoids from *Zinnia elegans* Jacq. herb. The highest total flavonoid yield (9,16%) was obtained using 70% ethanol, while higher concentrations reduced extraction efficiency.

2. HPLC analysis of the phytosubstance obtained with 70% ethanol revealed five individual flavonoids, including rutin, isoquercitrin, astragalol, silybin, and rhamnetin, with a total content of 7,87 mg/g. Rhamnetin predominated (4,57 mg/g), indicating its potential pharmacological importance.

3. The findings highlight *Zinnia elegans* Jacq. as a promising source of biologically active flavonoids with antioxidant and hepatoprotective potential. Further research should focus on evaluating their biological activity and standardizing extraction processes for phytopharmaceutical development.

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