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## PHYTOCHEMICAL CHARACTERIZATION OF PHENOLIC COMPOUNDS IN *ZINNIA PERUVIANA* L. HERB BY HPLC

**Actuality.** The genus *Zinnia* includes approximately twenty species of annual and perennial herbaceous plants belonging to the family Asteraceae. These plants are widely cultivated across the world due to their rapid growth, extended flowering period, and high ornamental value. Beyond their horticultural importance, species of the genus

*Zinnia* have attracted increasing scientific interest because of their potential pharmacological properties. Phytochemical investigations indicate that representatives of this genus contain a diverse range of secondary metabolites, including flavonoids, sesquiterpenes, sterols, and diterpenes.

The biological activity of *Zinnia peruviana* is believed to be associated with a complex mixture of biologically active constituents. Nevertheless, the phytochemical profile of this species remains insufficiently studied, particularly in Ukraine. For this reason, the present study aims to establish the identification of flavonoids and hydroxycinnamic acids in *Zinnia peruviana*.

**The objective of the research** is to obtain preliminary data that will support further comprehensive characterization of these compounds using high-performance liquid chromatography coupled with diode-array detection (HPLC–DAD).

**Material and methods.** The aerial parts of *Zinnia peruviana* were used as the plant material for the study. The samples were collected in the Ternopil region of Ukraine in 2024.

The identification and quantitative determination of hydroxycinnamic acids and flavonoids in the investigated plant raw material were performed using high-performance liquid chromatography coupled with diode-array detection (HPLC–DAD) on an Agilent Technologies 1200 system (USA). Identification of hydroxycinnamic acids and flavonoids was achieved by comparing the retention times and UV spectra of sample peaks with those of the corresponding reference standards. Quantitative determination of the compounds was carried out using the external standard method.

**Research results.** The HPLC–DAD method allowed the detection of 13 phenolic compounds, namely 9 hydroxycinnamic acids (hydroxyphenylacetic, chlorogenic, caffeic, syringic, *p*-coumaric, *trans*-ferulic, sinapic, *trans*-cinnamic, quinic), and 4 flavonoids (quercetin-3- $\beta$ -glycoside, naringin, fisetin, quercetin). The quantitative detection showed that the main hydroxycinnamic acids were chlorogenic ( $2781.53 \pm 0.31$   $\mu\text{g/g}$ ) and syringic ( $166.29 \pm 0.15$   $\mu\text{g/g}$ ) acids. Concerning flavonoids, the largest amounts were naringin ( $773.65 \pm 0.16$   $\mu\text{g/g}$ ) and fisetin ( $398.24 \pm 0.12$   $\mu\text{g/g}$ ).

**Conclusion.** The results of the phytochemical investigation indicate that the studied plant material represents a source of hydroxycinnamic acids and flavonoids. These findings suggest that *Zinnia peruviana* may be considered a promising plant species due to the important biological functions of phenolic compounds, which are known to participate in numerous physiological and pharmacological processes.

**Key words:** *Zinnia peruviana* L., herb, phenolic compounds, HPLC–DAD.

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## ФІТОХІМІЧНА ХАРАКТЕРИСТИКА ФЕНОЛЬНИХ СПЛУК У ТРАВІ *ZINNIA PERUVIANA* L. МЕТОДОМ ВЕРХ

**Актуальність.** Рід *Zinnia* налічує близько двадцяти видів однорічних і багаторічних трав'янистих рослин, які належать до родини *Asteraceae*. Ці рослини широко культивуються в різних регіонах світу завдяки швидкому росту, тривалому періоду цвітіння та значній декоративній цінності. Окрім значення в декоративному садівництві, види роду *Zinnia* привертають дедалі більшу наукову увагу у зв'язку з потенційними фармакологічними властивостями. Фітохімічні дослідження свідчать, що представники цього роду містять широкий спектр вторинних метаболітів, зокрема флавоноїди, сесквітерпени, стероли та дитерпени.

Біологічна активність *Zinnia peruviana* пов'язана з наявністю комплексу біологічно активних сполук. Однак фітохімічний склад цього виду залишається недостатньо вивченим, особливо в Україні. У зв'язку із цим це дослідження спрямоване на встановлення наявності та ідентифікації флавоноїдів та гідроксикоричних кислот у рослинній сировині *Zinnia peruviana*.

**Метою дослідження** було отримання попередніх даних, необхідних для подальшої комплексної характеристики цих сполук, із застосуванням методу високоефективної рідинної хроматографії з діодно-матричним детектуванням (ВЕРХ – ДМД).

**Матеріали та методи.** Матеріалом для дослідження була надземна частина *Zinnia peruviana*. Рослинну сировину було зібрано на території Тернопільської області (Україна) у 2024 році.

Ідентифікацію та кількісне визначення гідроксикоричних кислот і флавоноїдів у досліджуваній рослинній сировині проводили методом високоефективної рідинної хроматографії з діодно-матричним детектуванням (ВЕРХ – ДМД) з використанням системи *Agilent Technologies 1200* (США). Ідентифікацію гідроксикоричних кислот та флавоноїдів здійснювали шляхом порівняння часу утримування досліджуваних зразків із відповідними стандартними зразками. Кількісне визначення фенольних сполук проводили методом зовнішнього стандарту.

**Результати дослідження.** Методом ВЕРХ у досліджуваній рослинній сировині було виявлено 13 фенольних сполук, зокрема 9 гідроксикоричних кислот (гідроксифенілоцтову, хлорогенову, кавову, сирінгову, *p*-кумарову, транс-ферулову, синапову, транс-коричну, хінну) та 4 флавоноїди (кверцетин-3- $\beta$ -глікозид, нарингін, фізетин, кверцетин).

Кількісний аналіз показав, що серед гідроксикоричних кислот домінували хлорогенова ( $2781,53 \pm 0,31$  мкг/г) та сирінгова ( $166,29 \pm 0,15$  мкг/г) кислоти. Серед флавоноїдів найбільший вміст було встановлено для нарингину ( $773,65 \pm 0,16$  мкг/г) та фізетину ( $398,24 \pm 0,12$  мкг/г).

**Висновки.** Результати проведеного фітохімічного дослідження свідчать, що досліджувана рослинна сировина є джерелом гідроксикоричних кислот і флавоноїдів. Отримані дані дають змогу розглядати *Zinnia peruviana* як перспективний рослинний вид, зважаючи на важливу роль фенольних сполук у численних фізіологічних і фармакологічних процесах.

**Ключові слова:** *Zinnia peruviana* L., трава, фенольні сполуки, ВЕРХ – ДМД.

**Introduction.** The family *Asteraceae* represents one of the largest and most taxonomically diverse groups of angiosperms. It comprises approximately 1,600 genera and more than 24,000 species, including annual, biennial, and perennial herbaceous plants, as well as

shrubs and, less frequently, trees. Members of this family exhibit remarkable morphological and ecological diversity, which has contributed to their successful adaptation to a wide range of environmental conditions (Shi et al., 2011).

Species of *Asteraceae* are distributed worldwide, with particularly high diversity observed in regions such as South Asia, South Africa, and South America (Rahman et al., 2008). Many representatives of this family possess considerable economic importance and are widely cultivated as food, medicinal, and ornamental plants. Notable edible species include *Lactuca sativa*, *Cynara scolymus*, and *Helianthus annuus*, which are extensively used in agriculture and food production. In addition, numerous ornamental species, such as *Cosmos sulphureus* and *Zinnia elegans*, are widely cultivated for their decorative value and diverse floral characteristics (Alshymaa et al., 2019).

The genus *Zinnia* comprises approximately 20 species of annual and perennial herbaceous plants belonging to the family *Asteraceae*. These species are primarily native to regions of Mexico and South America, where they occur in diverse ecological habitats (Nimra et al., 2024). Members of the genus are widely cultivated due to their attractive, brightly colored solitary inflorescences, which contribute to their high ornamental value. Owing to their rapid growth, prolonged flowering period, and suitability as cut flowers, *Zinnia* species are considered among the most popular ornamental plants cultivated worldwide (Wahba et al., 2014).

In addition to their decorative significance, *Zinnia* species have attracted considerable scientific attention because of their diverse biological activities. Previous pharmacological studies have demonstrated that extracts from several species exhibit antibacterial, antifungal, antioxidant, and hepatoprotective properties (Greer et al., 2010; Mohamed et al., 2015). Phytochemical and biological investigations have been conducted for a number of species within the genus, including *Zinnia tenuiflora*, *Zinnia citrea*, *Zinnia pauciflora*, *Zinnia elegans*, *Zinnia linearis*, *Zinnia multiflora*, *Zinnia peruviana*, *Zinnia angustifolia*, *Zinnia verticillata*, *Zinnia haageana*, and *Zinnia acerosa*. The results of these studies indicate that representatives of this genus possess significant phytochemical diversity and promising medicinal potential (Toscano & Romano, 2021).

Review of the available literature on the genus *Zinnia* revealed the presence of a wide diversity of secondary metabolites belonging to different classes, including flavonoids, sesquiterpenes, sterols, and diterpenes (Tulub, & Burda, 2022; Tulub, & Burda, 2023). The analysis showed that numerous compounds have been isolated from species of the genus *Zinnia*. Among them, sesquiterpene lactones represent the major constituents and are considered responsible for various pharmacological properties of the genus. Furthermore, sesquiterpene lactones isolated from the aerial parts of *Z. flavicoma* and

*Z. grandiflora* have been reported to exhibit cytotoxic activity (Bashyal et al., 2006; Tallez et al., 1995).

There are also reports concerning the investigation of other plant species belonging to the genus *Zinnia*, particularly *Zinnia pauciflora*. Phytochemical investigations of this plant material have revealed the presence of essential oil and flavonoid compounds, including anthocyanins. Furthermore, the fatty acid composition of this species has also been studied (Hend et al., 2014; Hemaia et al., 2015; Tulub, & Burda, 2023).

The pharmacological activity of *Zinnia peruviana* is associated with a complex mixture of biologically active compounds; however, the phytochemical composition of this species has not yet been sufficiently investigated in Ukraine. Therefore, the present study focuses on establishing an analytical basis for the identification of flavonoids and hydroxycinnamic acids in *Zinnia peruviana*.

**The aim of the research is** to obtain preliminary data for further comprehensive analysis of these compounds using high-performance liquid chromatography with diode-array detection (HPLC–DAD). The results of this study will contribute to the scientific foundation for the rational utilization and further development of plant resources of cultivated species of the genus *Zinnia*.

**Materials and methods.** The aerial parts of *Zinnia peruviana* L. were collected in the Ternopil region of Ukraine in 2024. The plant material was taxonomically authenticated by Prof. Svitlana Marchyshyn from the Department of Pharmacognosy and Medical Botany, I. Horbachevsky Ternopil National Medical University (Ternopil, Ukraine) (Budniak et al., 2022). The voucher specimen of herbal raw material has been deposited in the departmental herbarium for future records (Feshchenko et al., 2021).

HPLC-grade acetonitrile, trichloroacetic acid, and methanol (E. Merck, Darmstadt, Germany) were used for chromatographic analysis. Reference standards of rutin, quercetin-3-*b*-glycoside, naringin, neohesperidin, quercetin, naringenin, kaempferol, luteolin, apigenin, kaempferol-3-*b*-glycoside, fisetin, silibinin, baicalein, rhamnetin, casticin, hydroxyphenylacetic acid, chlorogenic acid, caffeic acid, syringic acid, *p*-coumaric acid, *trans*-ferulic acid, sinapic acid, *trans*-cinnamic acid, and quinic acid ( $\geq 96\%$  HPLC purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA) (Budniak et al., 2024).

Chromatographic analysis was carried out using a high-performance liquid chromatography system equipped with diode-array detection (HPLC–DAD) (Agilent Technologies 1200, USA), including a G1311A quaternary pump, G1315B diode array detector, G1313A autosampler, and G1316A column compartment. Data acquisition and processing were performed using Agi-

lent Technologies software (Budniak et al., 2021; Demydiak et al., 2023).

Separation was achieved on a Zorbax SB-C18 column (150 mm × 4.6 mm, 5 μm; Agilent, USA). The column temperature was maintained at 30 °C, and the mobile phase flow rate was set at 1.0 mL/min with an injection volume of 20 μL. The mobile phase consisted of solvent A (0.1% trichloroacetic acid in water) and solvent B (acetonitrile), applied according to the gradient program presented in Table 1.

Table 1

**HPLC-DAD gradient solvent system for hydroxycinnamic acids and flavonoids separation**

| Time/min | Solvent A (%) | Solvent B (%) |
|----------|---------------|---------------|
| 0–2      | 98            | 2             |
| 2–25     | 90            | 10            |
| 25–40    | 85            | 15            |
| 40–48    | 80            | 20            |
| 48–68    | 75            | 25            |

Detection was performed using a diode-array UV–Vis detector within a wavelength range of 210–700 nm (with signal registration for flavonoids at 280 and 365 nm, at 250 and 275 nm for hydroxycinnamic acids). Identification of hydroxycinnamic acids and flavonoids was achieved by comparing the retention times and UV spectra of sample peaks with those of the corresponding reference standards. Quantitative determination of the compounds was carried out using the external standard method.

**Research results and discussion.** The HPLC–DAD analysis of *Zinnia peruviana* L. herb revealed the presence of several phenolic compounds (Table 2). The chromatographic profile of hydroxycinnamic acids detected in the plant material is presented in fig. 1, while the chromatogram of flavonoids is shown in fig. 2. In total, thirteen phenolic constituents were identified in the studied raw material. These included hydroxycinnamic acids – hydroxyphenylacetic, chlorogenic, caffeic, syringic, *p*-coumaric, *trans*-ferulic, sinapic, *trans*-cinnamic, and quinic acids – as well as the flavonoids quercetin-3-β-glycoside, naringin, fisetin, and quercetin (Table 2).

The quantitative levels of hydroxycinnamic acids and flavonoids in *Zinnia peruviana* raw material determined by the HPLC method are presented in Table 2. Among the identified hydroxycinnamic acids, chlorogenic acid was found to be the predominant compound, with a concentration of  $2781.53 \pm 0.31$  μg/mg. Chlorogenic acid, also known as 3-(3,4-dihydroxycinnamoyl) quinic acid, is one of the most widespread phenolic acids occurring in numerous plant-derived foods (Contardi et al., 2021). This compound is recognized as an important biologi-

cally active dietary polyphenol exhibiting a broad spectrum of pharmacological activities. According to previous studies, chlorogenic acid demonstrates antioxidant, hepatoprotective, antibacterial, anti-inflammatory, cardioprotective, antipyretic, anti-obesity, neuroprotective, antiviral, and antihypertensive properties, as well as free radical scavenging activity (Naveedab et al., 2018; Mazulin et al., 2025). In addition, it has been reported to play a significant role in the regulation of lipid and glucose metabolism, which may contribute to the prevention or management of various metabolic disorders, including cardiovascular diseases, hepatic steatosis, diabetes, and obesity (Santana-Gálvez et al., 2017).

Table 2

**Results of HPLC-DAD analysis of phenolic compounds in *Zinnia peruviana* L.**

| Rt, min               | Common name of identified compound | Quantitative content of phenols, μg/g |
|-----------------------|------------------------------------|---------------------------------------|
| hydroxycinnamic acids |                                    |                                       |
| 9.37                  | hydroxyphenylacetic acid           | $81.20 \pm 0.12$                      |
| 11.05                 | chlorogenic acid                   | $2781.53 \pm 0.31$                    |
| 12.20                 | caffeic acid                       | $42.32 \pm 0.12$                      |
| 14.33                 | syringic acid                      | $166.29 \pm 0.15$                     |
| 16.25                 | <i>p</i> -coumaric acid            | $85.15 \pm 0.08$                      |
| 17.88                 | <i>trans</i> -ferulic acid         | $22.93 \pm 0.09$                      |
| 19.66                 | sinapic acid                       | $11.51 \pm 0.07$                      |
| 22.18                 | <i>trans</i> -cinnamic acid        | $37.74 \pm 0.11$                      |
| 22.92                 | quinic acid                        | $62.59 \pm 0.17$                      |
| Flavonoids            |                                    |                                       |
| 23.47                 | quercetin-3-β-glycoside            | $153.40 \pm 0.23$                     |
| 26.09                 | Naringin                           | $773.65 \pm 0.16$                     |
| 27.27                 | Fisetin                            | $398.24 \pm 0.12$                     |
| 33.72                 | Quercetin                          | $258.31 \pm 0.06$                     |

Syringic acid was also present in a considerable concentration among the detected hydroxycinnamic acids, with a content of  $166.29 \pm 0.15$  μg/mg. According to previous studies, syringic acid exhibits a range of beneficial biological activities, particularly in the context of metabolic syndrome. It has been reported to exert protective effects against conditions such as diabetes, cardiovascular diseases, dyslipidemia, and obesity. Experimental studies in animal models have demonstrated that syringic acid may produce antidiabetic effects by reducing blood glucose levels and improving insulin regulation. Furthermore, this phenolic compound has been shown to attenuate cardiac injury biomarkers, decrease oxidative stress, and improve lipid metabolism parameters (Mashayekhi et al., 2025).

Flavonoids represent an important group of plant secondary metabolites and serve as a significant source of biologically active compounds. The quantitative content of flavonoids identified in *Zinnia peruviana* L. is pre-

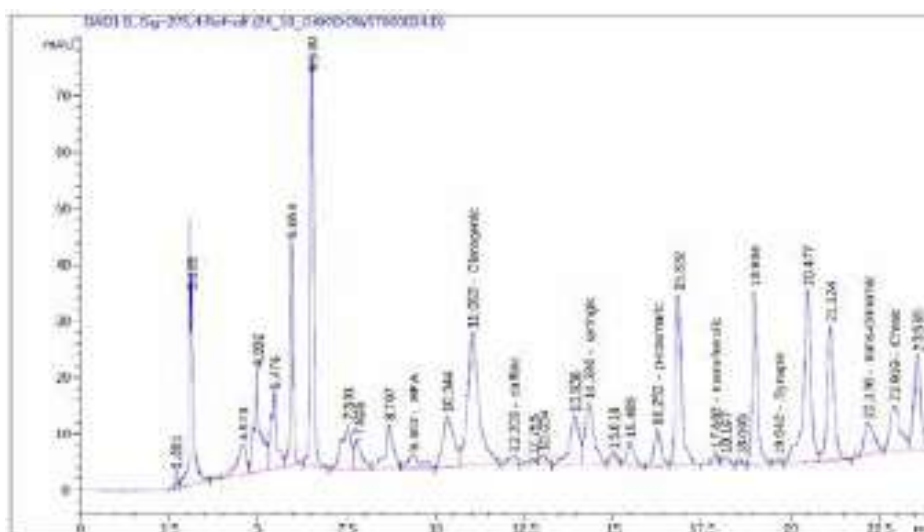


Fig. 1. HPLC-DAD chromatogram of hydroxycinnamic acids identified in *Zinnia peruviana* L.

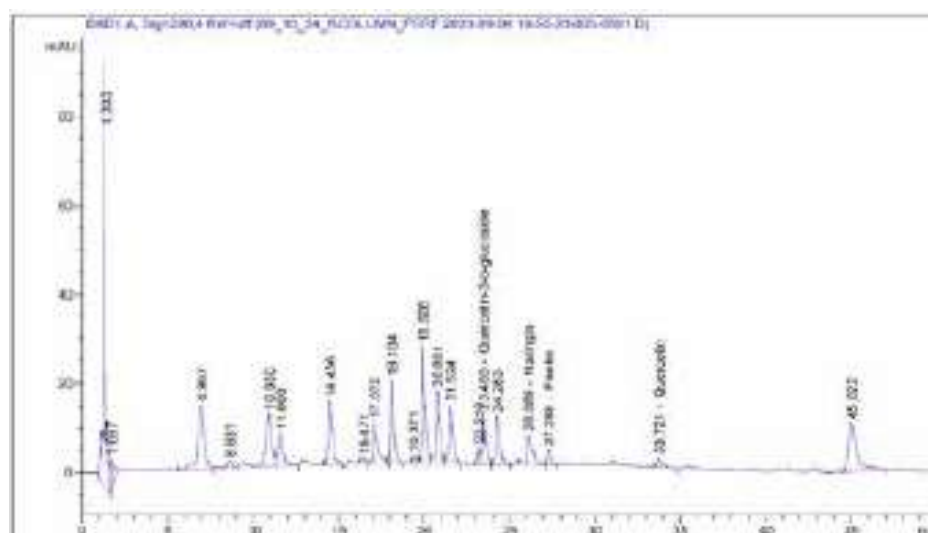


Fig. 2. HPLC-DAD chromatogram of flavonoids identified in *Zinnia peruviana* L.

sented in Table 2. Among the detected compounds, naringin was found in the highest concentration ( $773.65 \pm 0.16 \mu\text{g}/\text{mg}$ ), followed by fisetin ( $398.24 \pm 0.12 \mu\text{g}/\text{mg}$ ). According to previous studies, naringin exhibits a wide range of biological activities, including antioxidant, anti-apoptotic, anti-inflammatory, anti-osteoporotic, anti-ulcer, and anticancer effects (Wang et al., 2013). Similarly, fisetin has attracted considerable scientific interest due to its diverse pharmacological properties. Preclinical studies have demonstrated that this flavonoid possesses antioxidant, anti-inflammatory, anticancer, antimicrobial, and antidiabetic activities (Antika & Dewi, 2021).

Conclusions. Polyphenolic compounds are widely distributed in medicinal plants and have been exten-

sively studied due to their diverse biological activities, including antioxidant, cardioprotective, and anticancer effects. The present study focused on the identification and quantification of two major classes of polyphenols (flavonoids and hydroxycinnamic acids) in the herb of *Zinnia peruviana* L.

HPLC analysis demonstrated that *Zinnia peruviana* is a valuable source of phenolic compounds with potential pharmacological significance. Quantitative analysis revealed that among hydroxycinnamic acids, chlorogenic and syringic acids were the predominant constituents. In the flavonoid fraction, the highest concentrations were observed for naringin and fisetin.

The obtained findings expand the current knowledge on the phytochemical composition of *Zinnia peruviana* and may contribute to the development of quality control approaches for this plant material, as well as support its potential application in the formulation of herbal medicinal products.

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**Authors' contribution:**

**Slobodianiuk L.V.** – research concept and design, material collection, writing the article, final approval of the article;

**Marchyshyn S.M.** – research design, editing the article, final approval of the article;

**Danyliv S.I.** – material collection, analyzing the data obtained and their statistical processing, writing an article;

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