

ФАРМАЦІЯ

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INVESTIGATION OF THE CONTENT OF HYDROXYCINNAMIC ACIDS IN PHYTOSUBSTANCES FROM *ZINNIA ELEGANS* JACQ. AND *ZINNIA ANGUSTIFOLIA* KUNTH. HERB OBTAINED BY MACERATION WITH STIRRING

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The genus Zinnia belongs to the Asteraceae family and includes numerous species originating from North America. Plants of this genus contain various biologically active compounds, such as flavonoids, tannins, glycosides, phenols, anthocyanins, and saponins. Species such as Zinnia elegans Jacq. and Zinnia angustifolia Kunth. exhibit a wide range of biological activities, including antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, antimalarial, cytotoxic, and insecticidal properties. Zinnia angustifolia is less common in gardens but is gradually gaining popularity due to its decorative qualities. Zinnia elegans is widely cultivated for its diversity of flower shapes and colors, as well as its low maintenance requirements. Numerous scientific studies highlight the potential of Zinnia species as sources of biologically active compounds with therapeutic potential.

The article presents the results of studies on the quantitative determination of the total content of hydroxycinnamic acids in phytosubstances obtained from the herbs of Zinnia elegans Jacq. and Zinnia angustifolia Kunth. by maceration with stirring, using 10 % ethanol as an extractant, at a raw material to extractant ratio of 1:10. The quantitative content of hydroxycinnamic acids, recalculated as chlorogenic acid, was determined by spectrophotometric method using a Lambda 25 Perkin Elmer spectrophotometer (USA).

The herbs of Zinnia elegans Jacq. and Zinnia angustifolia Kunth. were collected at the beginning of the flowering period in 2024 in the Lviv region.

It was established that the total content of hydroxycinnamic acids in the phytosubstance derived from Zinnia angustifolia Kunth. amounted to $(3.98 \pm 0.15) \%$, while in the phytosubstance derived from Zinnia elegans Jacq. it was $(3.90 \pm 0.06) \%$. The obtained results indicate the prospects for further phytochemical, microbiological, and pharmacological studies of the phytosubstances from the herbs of Zinnia angustifolia Kunth. and Zinnia elegans Jacq. with the aim of developing plant-based medicinal products and dietary supplements.

Key words: *Zinnia elegans Jacq., Zinnia angustifolia Kunth., трава, гідроксикоричні кислоти, спектрометричний метод.*

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Дослідження вмісту гідроксикоричних кислот у фітосубстанціях із трави *Zinnia elegans* Jacq. і трави *Zinnia angustifolia* Kunth., одержаних методом мацерації з перемішуванням

Рід *Zinnia* належить до родини *Asteraceae* та містить багато видів, що походять із Північної Америки. Рослини цього роду містять різні біологічно активні сполуки, як-от флавоноїди, таніни, глікозиди, феноли, антоціани та сапоніни. Види, зокрема *Zinnia elegans* Jacq. та *Zinnia angustifolia* Kunth., проявляють широкий спектр біологічної активності, включно з антиоксидантними, антибактеріальними, протигрибковими, противірусними, гепатопротекторними, протималарійними, цитотоксичними та інсектицидними властивостями. *Zinnia angustifolia* є менш поширеною в садах, проте поступово набуває популярності завдяки своїм декоративним властивостям. *Zinnia elegans* широко культивується завдяки різноманітності форм і кольорів квіток та невибагливості в догляді. У дослідженнях багатьох науковців акцентується увага на перспективності використання видів роду *Zinnia* як джерел біологічно активних сполук із терапевтичним потенціалом.

У статті представлено результати досліджень визначення кількісного вмісту суми кислот гідроксикоричних у фітосубстанціях із трави *Zinnia elegans* Jacq. і трави *Zinnia angustifolia* Kunth., одержаних методом мацерації з перемішуванням, при використанні як екстрагента 10 % етанолу, у співвідношенні сировина : екстрагент – 1:10. Спектрофотометричним методом визначали кількісний вміст суми кислот гідроксикоричних у перерахунок на хлорогенову кислоту на спектрофотометрі Lambda 25 Perkin Elmer (USA).

Траву *Zinnia elegans* Jacq. і траву *Zinnia angustifolia* Kunth. заготовляли на початку цвітіння рослин у 2024 році на території Львівської області.

Установлено, що вміст суми гідроксикоричних кислот у фітосубстанції з трави *Zinnia angustifolia* Kunth. становить $(3.98 \pm 0.15) \%$, у фітосубстанції із трави *Zinnia elegans* Jacq. – $(3.90 \pm 0.06) \%$.

Отримані результати свідчать про перспективність подальших фітохімічних, мікробіологічних та фармакологічних досліджень фітосубстанцій із трави *Zinnia angustifolia* Kunth. і трави *Zinnia elegans* Jacq. з метою розробки лікарських засобів та дієтичних добавок на рослинній основі.

Ключові слова: *Zinnia elegans* Jacq., *Zinnia angustifolia* Kunth., herb, hydroxycinnamic acids, spectrophotometric method.

Introduction. The genus *Zinnia* belongs to the *Asteraceae* family. It includes numerous species that are particularly popular, especially in their region of origin – North America. Mexico is considered the center of diversity for the genus *Zinnia* [1–3].

The genus *Zinnia* was named in honor of Johann Gottfried Zinn, a professor of botany at the University of Göttingen.

The primary biologically active compounds in the flowers and aerial parts of *Zinnia* include flavonoids, tannins, glycosides, phenols, anthocyanins, and saponins [3; 4].

Species of the genus *Zinnia* exhibit a range of biological activities, including antioxidant, antibacterial, antifungal, antiviral, hepatoprotective, antimalarial, cytotoxic, and insecticidal properties [3].

Zinnia angustifolia Kunth. (also known as *Zinnia linearis*) is less commonly found in gardens compared to *Zinnia elegans* Jacq., but it is gradually gaining popularity. Additionally, this plant has smaller solitary flowers and narrower leaves [4; 5].

In 1750, double-flowered forms of *Zinnia angustifolia* were bred in France. To date, *Zinnia angustifolia* (varieties such as *Gloriensa*, *Sombrero*, *Classic*) are considered among the most promising plants of the *Asteraceae* family for flower bed decoration, landscaping of areas, forest parks, and parks.

Using the HPLC method, hydroxyphenylacetic, chlorogenic, caffeic, syringic, *p*-coumaric, *trans*-ferulic, sinapic, *trans*-cinnamic, and quinic acids were identified in the herb of *Zinnia angustifolia* Kunth [6].

Zinnia elegans Jacq. (synonym *Zinnia violacea*) is one of the most well-known and widespread species of the genus *Zinnia*. The introduction of this plant into cultivation in Europe dates back to approximately 1790 when *Zinnia elegans* Jacq. began to gain popularity as a garden crop. This species is represented by a large number of varieties [1; 7]. The flowers of *Zinnia elegans* Jacq. are characterized by various shapes and a diverse color palette, including shades of yellow, red, pink, lavender, green, crimson, orange, and white [7].

Zinnia elegans Jacq. thrives in fertile, evenly moist, well-drained soils with good sunlight exposure.

Zinnia elegans Jacq. is widely used for ornamental landscaping due to its long flowering period, low maintenance requirements, and bright colors [7].

Scientists have identified various classes of biologically active compounds in certain parts of the plant. Studies of alcoholic extracts obtained from the herb or leaves have confirmed the presence of flavonoids, saponins, polyphenols, glycosides, and steroids [4; 8].

In the herb and leaves of *Zinnia elegans* Jacq., the GC/MS method identified the presence of 13 fatty acids, including 7 saturated, 5 unsaturated, and 1 unidentified. In the flowers of *Zinnia elegans* Jacq., 14 fatty acids were determined, among which 5 were saturated, 7 were unsaturated, and 2 were unidentified. The stems of the studied plant contained 13 fatty acids, of which 6 were saturated, 6 were unsaturated,

and 1 was unidentified. In the roots of *Zinnia elegans* Jacq., 14 fatty acids were detected, including 5 saturated, 7 unsaturated, and 2 unidentified.

Thus, unsaturated fatty acids predominate in all types of *Zinnia elegans* Jacq. raw material. The highest content of fatty acids was observed in the flowers of *Zinnia elegans* Jacq. (71.94 %), while the lowest content was found in the leaves (56.50 %). Among the unsaturated fatty acids, linoleic acid was dominant in all raw materials. Additionally, linolenic acid was present in large amounts in the stems, herb, and leaves, whereas oleic acid was predominant in the flowers. The content of arachidic acid was highest in the leaves of *Zinnia elegans* Jacq. (11.05 %) compared to other raw materials. Among the saturated acids, palmitic acid was dominant in all types of raw materials [9].

In the herb, leaves, roots, and flowers of *Zinnia elegans* Jacq., tartaric, malic, and citric acids were identified. In the herb and leaves, ascorbic and salicylic acids were determined, while oxalic acid was found in the roots. The highest content of organic acids was observed in the leaves – (7.28 ± 0.34) %. The quantitative content of organic acids in flowers was (4.90 ± 0.24) %, in the herb – (5.46 ± 0.26) %. The lowest accumulation of organic acids was found in the roots of *Zinnia elegans* Jacq. – (2.93 ± 0.14) % [10].

Several classes of natural metabolites were identified in *Zinnia elegans* Jacq. Among the flavonoids were apigenin 7-O-glucoside, apigenin 4'-O-glucoside, kaempferol 3-O-glucoside, kaempferol 3-O-xyloside-7-O-glucoside, luteolin 7-O-glucoside, and quercetin 3-O-glucoside [11].

The scientific literature presents data on the antioxidant, antifungal, hepatoprotective, and antimalarial activities of this species [3; 4; 8].

Given the ongoing search for new plants that could serve as sources of biologically active substances with therapeutic potential, this study focused on ornamental plants that are widely cultivated and in which certain classes of compounds have been identified, potentially indicating therapeutic potential.

The aim of our study was to determine the quantitative content of total hydroxycinnamic acids in phytosubstances derived from the herb of *Zinnia elegans* Jacq. and *Zinnia angustifolia* Kunth., obtained by the maceration method with stirring.

Methods of Research. The objects of the study were phytosubstances obtained from the herb of *Zinnia elegans* Jacq. and *Zinnia angustifolia* Kunth. using 10 % ethanol as an extractant by the maceration method with stirring, at a raw material-to-extractant ratio of 1:10.

The dried herb was ground using a manual mill into particles of 3–5 mm in size. The raw material was placed in glass containers and covered with ethanol “to the mirror level”.

Maceration was carried out for 7 days at a temperature of 15–20°C, with periodic stirring. After infusion, the extract was filtered through a paper filter and concentrated at a temperature of 40°C.

The aerial parts of two *Zinnia* species were harvested at the beginning of their flowering stage in 2024 in the Lviv region.

The plants were authenticated by Prof. Svitlana Marchyshyn, Department of Pharmacognosy and Medical Botany (TNMU, Ternopil, Ukraine). The voucher specimens No. 371 and No. 372 are kept at the Department of Pharmacognosy and Medical Botany, TNMU.

The qualitative composition of hydroxycinnamic acids in the studied plant raw material was previously investigated [6]. Chlorogenic acid was the predominant hydroxycinnamic acid. Therefore, the quantitative content of hydroxycinnamic acids in the phytosubstances obtained from *Zinnia elegans* Jacq. and *Zinnia angustifolia* Kunth. was determined using a spectrophotometric method, recalculated as chlorogenic acid [12].

A precisely weighed sample of 0.1 g of the phytosubstance was placed in a 25 mL volumetric flask and diluted to the mark with 20 % ethanol. The resulting solution was filtered through a dry paper filter into a dry flask (solution B).

Subsequently, 1 mL of solution B was transferred into a 25 mL volumetric flask and brought to volume with 20 % ethanol.

The optical density of the resulting solution was measured using a Lambda 25 Perkin Elmer spectrophotometer (USA) at a wavelength of 327 nm. The reference solution was 20 % ethanol.

The content of hydroxycinnamic acids, recalculated as chlorogenic acid, was determined using the following formula:

$$X = \frac{A_1 \cdot 25 \cdot 25 \cdot 100}{E_{1\text{cm}}^{1\%} \cdot a_1 \cdot 1 \cdot (100 - W)},$$

where: A_1 – optical density of the test solution;

a_1 – weight of the phytosubstance, g;

$E_{1\text{cm}}^{1\%}$ – specific absorbance of chlorogenic acid;

W – moisture content of the phytosubstance, %.

The research results were processed using methods of mathematical statistics with the help of Excel (Microsoft Office). The obtained data were statistically analyzed according to the methodology of the State Pharmacopoeia of Ukraine (SPhU) [13].

Table 1

Results of the determination of the quantitative content of the total hydroxycinnamic acids in the phytosubstance from the herb of *Zinnia angustifolia* Kunth., obtained using 10 % ethanol

Number of measurements	Number of degrees of freedom	X_i	X_{cp}	S^2	S_{cp}	P	t (P, n)	Confidence interval	ε_{-} , %
5	4	3.8999	3.98	0.0137	0.0523	0.95	2.78	3.98 ± 0.1453	3.65
		3.8361							
		3.9729							
		4.0371							
		4.1357							

Table 2

Results of the determination of the quantitative content of the total hydroxycinnamic acids in the phytosubstance from the herb of *Zinnia elegans* Jacq., obtained using 10 % ethanol

Number of measurements	Number of degrees of freedom	X_i	X_{cp}	S^2	S_{cp}	P	t (P, n)	Confidence interval	ε_{-} , %
5	4	3.8783	3.90	0.0022	0.0212	0.95	2.78	3.90 ± 0.0589	1.51
		3.8694							
		3.8483							
		3.9646							
		3.9275							

Discussion of Results. The results of determining the quantitative content of the total hydroxycinnamic acids in phytosubstances obtained from the aerial parts of *Zinnia elegans* Jacq. and *Zinnia angustifolia* Kunth. are presented in Tables 1 and 2.

In the phytosubstance from the herb of *Zinnia angustifolia*, obtained by maceration with stirring using 10 % ethanol as the extractant, the content of the total of hydroxycinnamic acids was determined. The results of the determination are presented in Table 1.

The content of the total hydroxycinnamic acids in the phytosubstance from the aerial parts of *Zinnia elegans* Jacq., obtained using 10 % ethanol as an extractant and the maceration with stirring method, is presented in Table 2.

The results of the conducted research demonstrate that the total content of hydroxycinnamic acids in the phytosubstances derived from two *Zinnia* species is nearly identical, amounting to (3.98 ± 0.15) % in the herb of *Zinnia angustifolia*

Kunth. and (3.90 ± 0.06) % in the herb of *Zinnia elegans* Jacq.

Conclusions. 1. Phytosubstances from the herb of *Zinnia angustifolia* Kunth. and *Zinnia elegans* Jacq. were obtained using 10 % ethanol as the extractant, with maceration under stirring as the extraction method, and a raw material to extractant ratio of 1:10.

2. The quantitative content of the total hydroxycinnamic acids in the phytosubstances derived from the herbs of the studied plant species was determined by the spectrophotometric method, recalculated as chlorogenic acid. The content of hydroxycinnamic acids was found to be (3.98 ± 0.15) % in the phytosubstance from the herb of *Zinnia angustifolia* Kunth. and (3.90 ± 0.06) % in the phytosubstance from the herb of *Zinnia elegans* Jacq.

3. The obtained results substantiate the feasibility of further phytochemical and pharmacological investigations of phytosubstances derived from the herbs of *Zinnia angustifolia* Kunth. and *Zinnia elegans* Jacq.

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