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COMPONENT COMPOSITION OF POLYPHENOLIC COMPOUNDS IN INFLORESCENCES OF TAGETES ERECTA L. VAR. “ANTIGUA F 1 ORANGE”

Actuality. *Polyphenolic compounds exhibit significant antioxidant, anti-inflammatory, and immunomodulatory properties. These compounds actively participate in various biochemical processes in plants and substantially enhance the biological effects of numerous medicinal plants and the pharmaceutical products derived from them. The cultivated species of marigolds spreading (Tagetes erecta L.), along with its forms and varieties, serves as a valuable source of carotenoids for medical and economic applications. During the growing season, its inflorescences accumulate polyphenolic compounds characterized by pronounced biological activity. However, the specific composition and quantitative content of these compounds under the environmental conditions of southeastern Ukraine throughout the growing season remain unexplored.*

Aim of study is determination of the component composition and quantitative content of polyphenolic compounds in the inflorescences of Tagetes erecta L. var. “Antigua F 1 Orange”.

Material and methods. *The plant material consisted of air-dried inflorescences of Tagetes erecta L. cultivated in Ukraine, variety var. “Antigua F1 Orange”, collected during the flowering period (June – September 2023–2024). The component composition and quantitative content of polyphenolic compounds were determined using thin-layer chromatography (TLC) on glass-backed plates “Merkieselguhr F254” 20 x 20 (Merck KGaA, Germany) with the densitometer device “Biostep” CD 60 (Germany) and high-performance liquid chromatography (HPLC) “Shimadzu LC-20” with a UV detector (Japan). Standard compound samples were sourced from “Supelco Analytical, Sigma-Aldrich” (USA).*

Research results. *In the inflorescences of Tagetes erecta L. var. “Antigua F1 Orange”, the quantitative composition of 22 primary polyphenolic compounds was identified and analyzed. Among these, 13 were classified as flavonoids, while 9 were categorized as hydroxycinnamic acids. The quantitative flavonoid contents were 20,40 ± 2,14 and hydroxycinnamic acids 5,60 ± 0,64% respectively.*

Conclusion. *The substantial quantitative content of flavonoids and hydroxycinnamic acids in the inflorescences of Tagetes erecta L. var. “Antigua F1 Orange” is of significant importance for the standardization of plant raw materials, preparations derived from them, and their subsequent incorporation into medical practice.*

Key words: *marigold, Tagetes erecta L. var. “Antigua F1 Orange”, thin layer chromatography, High-Performance Liquid chromatography, qualitative and quantitative analysis, flavonoids, hydroxycinnamic acids.*

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КОМПОНЕНТНИЙ СКЛАД ПОЛІФЕНОЛЬНИХ СПОЛУК СУЦВІТЬ *TAGETES ERECTA* L. VAR. “ANTIGUA F 1 ORANGE”

Актуальність. *Поліфенольні сполуки виявляють виражену антиоксидантну, протизапальну й імуномоделюючу дію. Вони беруть активну участь у багатьох біохімічних процесах рослин, істотно доповнюють біологічну дію багатьох видів лікарських рослин, лікарських засобів на їхній основі. Культивований вид чорнобривці прямостоячі (Tagetes erecta L.), його розповсюджені форми й сорти є джерелом отримання каротиноїдів для медичного та господарського застосування. Суцвіття у вегетаційний період накопичують поліфенольні сполуки з вираженою біологічною дією. Але компонентний склад і кількісний вміст цих речовин за культивування в умовах України не досліджено.*

Мета дослідження *– визначення компонентного складу та кількісного вмісту поліфенольних сполук у суцвіттях Tagetes erecta L. var. “Antigua F 1 Orange”.*

Матеріал і методи. *Рослинною сировиною були повітряно-сухі суцвіття Tagetes erecta, культивованого в Україні сорту “Antigua F 1 Orange” під час цвітіння (червень – вересень 2023–2024 років). Компонентний склад і кількісний вміст поліфенольних сполук визначали методом ТШХ, на пластинах зі скляною підкладкою “Merkieselguhr F_{254}” 20 x 20 (Merck KGaA, Німеччина), на денситометрі “Biostep” CD 60 (Німеччина) та ВЕРХ “Shimadzu LC-20” з УФ-детектором (Японія). Використано стандартні зразки сполук фірми “Supelko Analytical, Sigma-Aldrich” (USA).*

Результати дослідження. *У суцвіттях чорнобривців прямостоячих Tagetes erecta L. сорту “Antigua F 1 Orange” ідентифіковано та встановлено кількісний вміст до 22 основних поліфенольних сполук. З них 13 віднесені до флавоноїдів і 9 до гідроксикоричних кислот. Кількісний вміст суми флавоноїдів – до 20,40 ± 2,14, гідроксикоричних кислот – 5,60 ± 0,64%.*

Висновок. *Значний кількісний вміст флавоноїдів і гідроксикоричних кислот у суцвіттях чорнобривців прямостоячих Tagetes erecta L. сорту “Antigua F 1 Orange” має велике значення для стандартизації рослинної сировини, препаратів із неї, подальшого їх упровадження в медичну практику.*

Ключові слова: *чорнобривці, Tagetes erecta L. сорту “Antigua F 1 Orange”, ТШХ, ВЕРХ, якісний і кількісний аналіз, флавоноїди, гідроксикоричні кислоти.*

Actuality. Polyphenolic compounds are a class of organic substances commonly found in plants, characterized by the presence of more than one phenolic group in their molecular structure. They are secondary metabolites that plants utilize to protect themselves from UV radiation and other adverse environmental factors. These compounds play a role in controlling insect and pest populations during plant growth. Additionally, they exhibit antiseptic properties against pathogenic fungi and microorganisms responsible for infectious diseases (Vivek, 2024).

Polyphenolic compounds most often accumulate in flowers, fruits, leaves, and seeds during the flowering and fruiting stages of medicinal and edible plants, particularly among medicinal plants, fruits, vegetables, cereals, and etc.

As components of herbal raw materials, medicinal or prophylactic agents, polyphenolic compounds demonstrate significant antioxidant, anti-inflammatory, immunomodulatory, and wound-healing properties, contribute to overall health and improve quality of life. These compounds are utilized in the prevention and treatment of diabetes, obesity, cardiovascular diseases, immune stimulation, and neuroprotective effects (Devika, 2013).

Non-toxic polyphenolic compounds are used in food products, plant raw materials, dietary supplements, and phytopharmaceuticals. They have a positive effect on the flavor properties and shelf life of active compounds in medicinal products when used for medical and prophylactic purposes (Tiwari, 2023).

Proper level of intake of polyphenolic compounds lowers the risk of numerous chronic diseases, including cancer and cardiovascular conditions. These compounds inhibit the formation of free radicals, thereby preventing cellular damage caused by oxidation and aging. Furthermore, they help reduce the inflammatory processes associated with infectious diseases (Singh, 2020).

In addition, as usual, they contribute to improving digestion, normalizing brain function, regulating blood sugar levels, and offering protection against blood clots, heart disease, and cancer. Currently, it has been established that plants contain up to 8,000 polyphenolic compounds, with the most common being flavonoids (up to 60%) and hydroxycinnamic acids (30%) (Stromsnes 2021).

Flavonoids are structural derivatives of benzo-γ-pyrone, containing reactive phenolic radicals and a carboxyl group. They include derivatives of: anthocyanins, flavan-3-ols, flavones, isoflavones, flavonols, flavononols, and chalcones. While most flavonoids are aglycones, glycosides are more prevalent in plants. The biological activity of flavonoids depends on the structural characteristics of the compounds, exhibiting effects such as capillary strengthening, anti-inflammatory, antioxidant, immunomodulatory, and wound-healing properties (Chandhare, 2024).

Other polyphenolic compounds include hydroxycinnamic acids, tannins, anthocyanidins, anthraquinones, coumarins, and stilbenoids. These compounds possess anti-inflammatory, antimicrobial, and immunostimulatory activities (Janigashvili, 2024).

All known polyphenolic compounds are not considered as nutrients and do not have established recommended daily intake levels in the European Union, the United Kingdom, or the United States (Patel, 2018).

Currently, modern methods such as microwave, ultrasonic, supercritical extraction, fermentation, and liquid chromatography are used for the extraction and purification of polyphenolic compounds (Zardeto, 2024). For determining their composition and quantitative content, the most suitable methods of physicochemical analysis include high-performance thin-layer and liquid chromatography, modifications of UV- and IR-spectroscopy, chromatography-mass spectrometry, X-ray structural analysis, and others (Lin, 2020).

Species of the genus *Tagetes* L. grow wild in South America and Mexico (Patel, 2018). Species, forms, and varieties of *Tagetes* erecta L. are cultivated in Europe, Asia, Africa, Australia, and Madagascar. The inflorescences of these plants are used as food and serve as a source of the carotenoid lutein for industrial and medical purposes (Ordogh, 2021).

Globally, the most widely distributed species of the genus *Tagetes* L. include *T.* patula L. (spreading marigold), *T.* erecta L. (upright marigold), *T.* signata Bartl. (marked marigold), *T.* minuta L. (small marigold), and *T.* tenuifolia Cav. (fine-leaved marigold). During their vegetative growth, these plants accumulate a wide range of bioactive compounds, including carotenoids, xanthophylls, flavonoids, hydroxycinnamic acids, essential oils, fatty acids, organic acids, amino acids, and others (Maliuhina, 2013; Moghaddam, 2021; Burlec, 2021; Novianti, 2022).

In contemporary medicine, extracts and bioactive compounds derived from *Tagetes* L. species are utilized as therapeutic and prophylactic agents, exhibiting anti-inflammatory and wound-healing properties, providing photoprotection, and enhancing visual acuity. The pronounced biological activity of these plants is largely attributed to their polyphenolic compounds. However, no comprehensive studies have been conducted to date on the accumulation of these compounds in the inflorescences of promising low-growing varieties of *Tagetes* erecta L. (Youssef, 2020).

Thus, it is both relevant and scientifically justified to investigate the component composition and quantitative content of polyphenolic compounds in the inflorescences of the high-yielding dwarf variety *Tagetes* erecta L. var. "Antigua F1 Orange" to standardize plant raw materials and facilitate the development of pharmaceutical products based on this species.

The purpose of the work. Determination of the component composition and quantitative content of polyphenolic compounds in the inflorescences of *Tagetes* erecta *L. var.* "Antigua F1 Orange".

Materials and methods. The plant material consisted of air-dried inflorescences of the cultivated low-growing marigold species Tagetes *erecta L. var.* "Antigua F1 Orange" collected during the flowering period (June–October 2023–2024). This species, along with its forms and varieties, is widely distributed in Ukraine and globally. Its extended vegetative period and relatively small above-ground biomass facilitate active biochemical processes in the plants, leading to a high accumulation of polyphenolic compounds. The plant material was harvested from central, southern, and eastern regions of Ukraine (Kharkiv, Zaporizhzhia, Dnipropetrovsk, Vinnytsia, and Kyiv regions).

The *Tagetes* erecta L. var. "Antigua F1 Orange" variety is a genetically developed compact hybrid with a height of 25–30 cm and a spread of 25–45 cm. It features optimal flowering timing, reduced sensitivity to photoperiod, fast growth, and adaptability to various soil and moisture conditions. The flowers are large, abundant, densely double, and orange-colored, with a diameter of 8–10 cm. They are borne on long, hollow, thickened stems, either solitary or arranged in dense, tufted inflorescences. The plant has an upright, hollow, and glabrous stem. The leaves are opposite or alternate, pinnately dissected or divided, rarely entire. Leaflets are lanceolate, linear-lanceolate, or serrated, and green to dark green in color. The fruit is an achene, flattened and elongated. Flowering occurs from June to October. For early blooming, seeds are sown in early April for seedlings, with germination occurring within 5–6 days. Direct sowing in open ground is carried out at a depth of 1,5–2 cm.

The plant material consisted of individual flowers and impurities of leaves with twig fragments not exceeding 2%. The collection and drying of the plant material were carried out in accordance with the recommendations of the State Pharmacopoeia of Ukraine (SPhU).

The drying was carried out for 48 hours in a drying cabinet "Termolab СНОЛ 24/350" at a temperature of 35 °C and a layer thickness of 10 mm, until the moisture content reached no more than 10%.

For the identification of polyphenolic compounds, reactions with solutions of heavy metal salts were used, along with the TLC method after extraction with 96% ethanol, using the densitometer "Biostep" CD 60 (Germany).

Approximately 1,0 g (accurately weighed) of plant material was finely ground to a particle size of d = 0,3 mm, placed in a 100 ml flask, and 30 ml of ethanol was added. The mixture was heated in a boiling water bath "ВБ-4 micromed" (t = 100 °C) for 30 minutes with constant stirring. This extraction procedure was repeated twice more with fresh portions of the solvent. The extracts were combined, cooled for 30 minutes, centrifuged using the "CM-3.01. micromed" centrifuge, and filtered into a 100 ml flask, bringing the volume to the mark. The resulting filtrates were passed through a filter (d = 0,45 μm) into vials for further analysis. Chromatographic separation and quantification of individual components were performed using selected chromatographic systems. For flavonoids, the following system was used: ethyl acetate – acetic acid – purified water (10 : 2 : 3), and for hydroxycinnamic acids, n-butanol – acetic acid – purified water (4 : 1 : 5).

The confirmation of compound accumulation and determination of their quantitative content were performed using HPLC on a Shimadzu LC-20 system with a UV detector (Japan). The chromatographic column used was Phenomenex Luna C18 (2), 250 mm × 4,6 mm × 5 μm. The column temperature was maintained at 35 °C, the detection wavelength was 330 nm, the mobile phase flow rate was 1 ml/min, and the sample volume was 5 μl. The mobile phase consisted of: eluent A – 0,1% trifluoroacetic acid solution in purified water, and eluent B – 0,1% trifluoroacetic acid solution in acetonitrile.

The identification of compounds was carried out based on the retention time of standard samples and spectral characteristics, comparing the UV spectra of the studied compounds to those of the standards. Standard samples were obtained from Supelco Analytical, Sigma-Aldrich (USA) (Demydiak, 2023).

The applied methods enable simultaneous separation, identification, and quantitative determination of the studied components. Key advantages include the use of small sample quantities, rapid analysis, reliable reproducibility of results, and low relative measurement error.

The obtained results were processed using mathematical statistics with the licensed software "Statistica 6.0 for Windows" (Stat. Soft. Inc., License № AXXR712D-833214FANS). The reliability of the observed differences was evaluated according to the requirements of the State Pharmacopoeia of Ukraine (SPhU) using Student's t-test (p > 95%).

Research results

The results are presented in fig. and table.

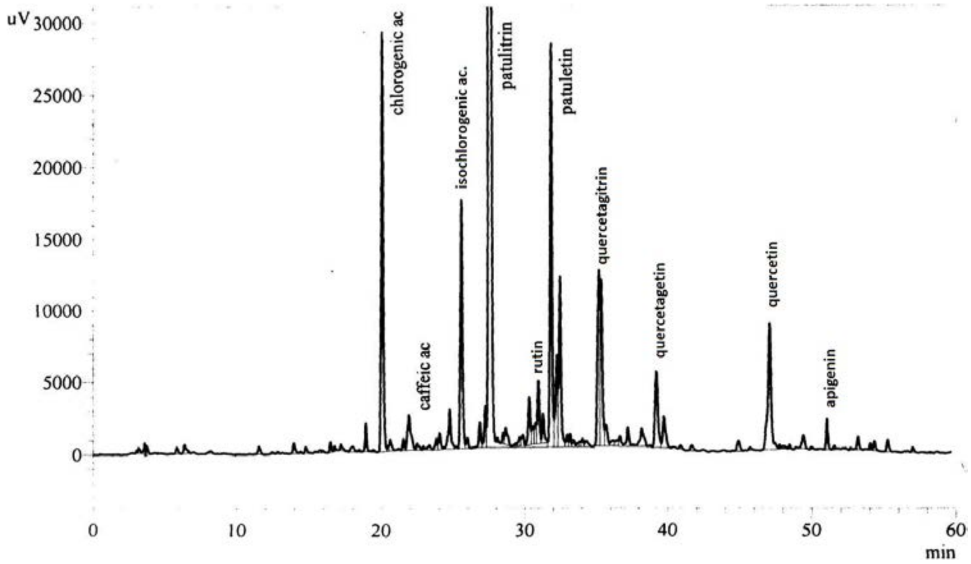

Fig. Results of HPLC analysis of polyphenolic compounds from the inflorescences of Tagetes erecta L. var. "Antigua F1 Orange" during the flowering period (June – October 2023–2024), Solene village, Dnipropetrovsk region ($x \pm \Delta\bar{x}$) %, μ = 6

Table

Quantitative content of polyphenolic compounds in the inflorescences of marigolds Tagetes erecta L. var. "Antigua F1 Orange" during the flowering period (June – October 2023–2024), Solene village, Dnipropetrovsk region ($x \pm \Delta\bar{x}$) %, μ = 6

№	Name of the compound	Retention time (min)	Quantitative content (%)	$\lambda_{max.}$ (nm)
1	Protocatechuic acid	12,143	0,17 ± 0,02	208; 218; 260; 294
2	Caftaric acid	14,081	0,18 ± 0,02	290
3	Rosmarinic acid	16,520	0,19 ± 0,02	215; 275; 325
4	Chlorogenic acid	20,042	2,20 ± 0,21	208; 218; 260; 294
5	Ferulic acid	21,740	0,08 ± 0,01	240; 280
6	Caffeic acid	21,924	0,23 ± 0,03	290
7	Isochlorogenic acid	25,623	0,87 ± 0,09	219; 235; 245; 300; 329
8	Patulitrin	27,581	9,32 ± 1,28	259; 273; 338
9	Luteolin-7-glucoside	29,228	0,81 ± 0,02	255; 267; 348
10	Tannic acid	29,405	0,83 ± 0,08	220; 275
11	Trans-cinnamic acid	30,321	0,85 ± 0,09	204; 216; 278
12	Rutine	30,708	0,90 ± 0,09	259; 362,5
13	Patuletin	32,151	2,08 ± 0,21	259; 371
14	Hyperoside	32,210	1,10 ± 0,20	257; 289; 299; 362
15	Dihydroquercetin	32,331	0,21 ± 0,12	289; 331
16	Quecetagitrin	36,921	2,18 ± 0,20	249; 347; 388
17	Apigenin-7-O-glucoside	37,052	0,09 ± 0,10	268; 339
18	Isoquercitrin	38,057	0,66 ± 0,07	258; 360
19	Quercetagetin	40,113	0,03 ± 0,03	260; 274; 365
20	Quercetin	48,057	1,12 ± 0,19	255; 374
21	Luteolin	49,077	0,58 ± 0,06	254; 359
22	Apigenin	53,982	1,32 ± 0,14	218; 240; 324; 298
	Total content of flavonoids, %		20,40 ± 2,14	
	Total content of hydroxycinnamic acids, %		5,60 ± 0,64	

Conclusions. 1. Using the methods of TLC and HPLC, 22 compounds were identified, of which 13 were classified as flavonoids and 9 as hydroxycinnamic acids.

2. Among the polyphenolic compounds, flavonoids constituted the highest quantative content (up to 20,40 ± 2,14%), while the accumulation of phenolic.

3. Among the polyphenolic compounds, flavonoids constituted the highest quantitative content (up to 20,40 ± 2,14%), while the accumulation of hydrocynnamic acids was significantly lower (up to 5,60 ± 0,64%).

4. The raw material predominantly contained compounds with pronounced antioxidant and anti-inflammatory activities, including patulitrin (9,32 ± 1,28%), quercetagitrin (2,18 ± 0,20%), patuletin (2,08 ± 0,21%), quercetin (1,12 ± 0,19%), quercetagetin (0,03 ± 0,03%), chlorogenic acid (2,20 ± 0,21%), and isochlorogenic acid (0,87 ± 0,09%).

5. The substantial quantitative content of polyphenolic compounds in the inflorescences of Tagetes erecta L. var. "Antigua F1 Orange" during flowering highlights its potential for the development of complex phytopreparations with pronounced anti-inflammatory, antioxidant, and wound-healing activities.

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