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RESEARCH ARTICLE

The content of flavonoids in *Cosmos sulphureus*

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Abstract

Flavonols, anthocyanins, and chalcones were determined during the flowering phase in two genotypes of *Cosmos sulphureus* (regular species and its cultivar 'Cosmic Orang') grown in the M.M. Gryshko National Botanical Garden in Kyiv. Inflorescences, leaves, stems, roots, and separated ray and disc florets were dried and crushed to prepare extracts following three different techniques. In particular, with 80 % (v/v) ethanol following Andreeva & Kalinkina (2000), 3.5 % HCl following Kriventsov (1982), and 0.1 N HCl following Udovenko (1988). The absorbance of flavonoids was measured at 390 nm wavelength for flavonols, 530 nm – for anthocyanins, and 364 nm – for chalcones. The highest content of flavonols was found in inflorescences of both genotypes (87.79 ± 1.64 and 87.99 ± 1.75 mg / 100 g of dry weight (DW), respectively). The content of anthocyanins was found to be ranked by overground organs: inflorescences > leaves > stems. In particular, the content of anthocyanins in the inflorescences of these two genotypes was 188.95 ± 5.20 and 177.14 ± 6.81 mg / 100 g DW, respectively. In the leaves, the content of anthocyanins was 61.32 ± 1.97 and 41.33 ± 2.27 mg / 100 g DW, respectively. In the stems, the content of anthocyanins was 31.63 ± 1.16 and 25.31 ± 0.95 mg / 100 g DW, respectively. In the roots, the anthocyanins were not detected. Among the flavonoids, the highest content, in general, was found for anthocyanins. Similarly, chalcones were also localized in overground organs only and mostly in the inflorescences (39.65 ± 1.25 and 37.93 ± 0.88 mg / 100 g DW, respectively). The content of chalcones in the leaves and stems was much lower than the content of the anthocyanins and flavonols; it significantly varied for two investigated genotypes. During the detailed investigation of the flavonoids content in different parts of the inflorescence, it was found that disc florets in both genotypes had fewer flavonoids than the ray florets.

Keywords: *Cosmos sulphureus*, flavonoids, flavonols, anthocyanins, chalcones

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Introduction

Cosmos sulphureus Cav. (Asteraceae) originates from Central and South America. It is used as an edible plant in its original range of distribution and in Asia, where it was first introduced as ornamental (Fernald & Kinsey, 2012; Kaisoon et al., 2012; de Morais et al., 2020). The inflorescences of *C. sulphureus* are the best investigated and also are most often used in

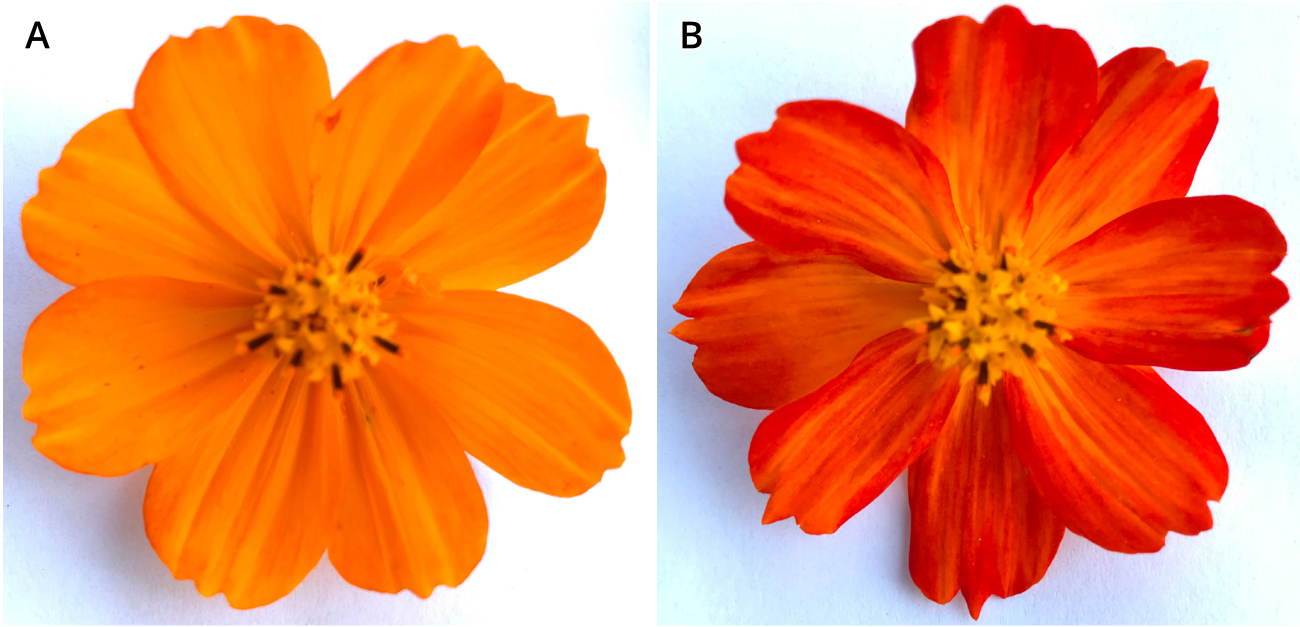

Figure 1. Inflorescence of regular *Cosmos sulphureus* (**A**) and *C. sulphureus* 'Cosmic Orange' (**B**).

food (Fernald & Kinsey, 2012), for treatment (Botsaris, 2007; Saleem et al., 2019), and dyeing (Jansen, 2005; Kale et al., 2006; Sabatini et al., 2020). *Cosmos sulphureus* inflorescences were used in traditional Native American culture as a bright dye preserved on 2000-year-old wool products (Sabatini et al., 2020). Application of these plants in the traditional household was possible primarily due to the high content of flavonoids. Inflorescences of *C. sulphureus* contain quercetin, which Saleem et al. (2019) considered the cause of hepatoprotective properties of this plant. Myricetin, kaempferol, rutin, and apigenin, having an immuno-protective function, were reported for *C. sulphureus* (Cavaiuolo et al., 2013). Different subgroups of other flavonoids, including cosmonidin (antocianidin), sulfuretin (auron), quercetin (flavonol), luteolin (flavon), butein (chalcone) and eriodictyol (flavanon) are represented in these plants too (Iwashina et al., 2019). However, the distribution of flavonoids in different overground organs and parts of the inflorescence of *C. sulphureus* is not sufficiently elucidated.

Material and methods

Material sampling

The plants of regular species of *C. sulphureus* and its cultivar 'Cosmic Orang' were chosen for the investigation due to the different coloration of the ray florets and their number per inflorescence (Fig. 1). The color of ray florets was determined by the Royal Horticultural Society's Colour Chart (2007). In the regular *C. sulphureus*, it corresponded to orange group N25B; in the cultivar 'Cosmic Orang', it has an orange leading tone (N25B) with orange-red stripes (N30A).

The analyzed plants were grown in the open soil of the M.M. Gryshko National Botanical Garden of the NAS of Ukraine. Plant material was collected in the flowering phase (the first ten days of August) in sunny weather. After that, it was segregated onto the inflorescences, leaves, stems, and roots. The vegetative parts were ground. The inflorescences were divided into two fractions (ray and disc florets) to explore and compare the composition of flavonoids in the ray and disc florets. All material was dried at +35 °C using an electric dryer Ezidri Ultra FD1000.

Determination of flavonols

Quantitative determination of flavonols was carried out according to a technique based on their ability to form a colored complex with an alcoholic solution of aluminum chloride, which causes a bathochromic shift in the long-wave absorption band and, at the same time, gives the main maximum absorption at a wavelength of 390 nm. The mixture of solutions of aluminum chloride and acetic acid served as a control (Andreeva & Kalinkina, 2000). The absorbance of the studied solutions was measured using Zalimp KF 77 (Poland) spectrophotometer. The number of parallel measurements was three. The obtained data were presented in mg / 100 g of dry weight (DW) in terms of rutin.

To determine the content of flavonols, an analytical sample (0.2–0.3 g) was ground, transferred to a flask with 3 ml of 80 % (v/v) ethanol, and heated under a reflux condenser for 45 min in a water bath. After that, the flask was cooled to room temperature, and the suspension was filtered through a paper filter into a 100 ml volumetric flask. The resulting solution was brought to the mark with 80 % (v/v) ethanol (solution A). Next, 2 ml of solution A was placed in a 25 ml volumetric flask, 1 ml of a 2 % (w/v) solution of aluminum chloride in 95 % (v/v) ethanol was added, and the volume of the solution was brought to the mark with 95 % (v/v) ethanol. The absorbance of the solution was measured after 20 min at 390 nm in a cuvette with a layer thickness of 10 mm.

Determination of anthocyanins

The content of anthocyanins was determined spectrophotometrically at 530 nm wavelength, using ethanol extraction from a homogenate of plant raw material acidified with 3.5 % HCl (Kriventsov, 1982). The number of parallel determinations was three. The data obtained were presented as mg / 100 g of DW in terms of cyanidin glycosides.

Determination of chalcones

Determination of chalcones was performed spectrophotometrically following the modified protocol of Udovenko (1988). For analysis, a sample of 0.2 g was taken, ground, filled with 0.1 N HCl solution, and infused for two hours with periodic shaking. After that, the solution was drained into a dry test tube and centrifuged at 2 K rpm for 2–3 min. The absorbance of the solution was measured at 364 nm wavelength. Due to the high absorbance, the initial solution was diluted five times with 0.1 N HCl. The number of parallel determinations was three.

Statistical analysis

Statistically processed data are presented as arithmetic means and their standard deviation. The significance level was set at $\alpha = 0.05$. The statistical analysis was performed with IBM SPSS Statistics, release 26.0.0.1.

Results and discussion

Flavonoids is a large group of biologically active compounds, mainly dissolved in plant cell sap and chloroplasts (Kovaliov, 2019). The most common and diverse group of flavonoids are flavonols, exhibiting strong dyeing properties (Jansen, 2005). Quercetin is one of the well-known representatives of flavonols (Ivashkiv, 2010). This group also includes kaempferol and myricetin, present in *C. sulphureus* plants (Cavaiuolo et al., 2013).

The inflorescences of investigated plants had the highest content of flavonols (Table 1). Moreover, it was similar for both genotypes despite the coloration difference. Other plants' parts contained a significantly lower amount of flavonols. Their content in the roots was the lowest, almost twice lower than in the leaves.

Compounds from the anthocyanin group usually give plants a blue, red, or pink color (Kovaliov, 2019). Their availability in the plant parts can be determined visually. For example, Saito (1979) showed that cosmocyanin could be isolated only from the colored corollas of *C. bipinnatus* Cav. In colorless floral parts, cosmocyanin was not detected. The availability of anthocyanins in the varieties of *C. atrosanguineus* (Hook.) Voss was investigated by Amamiya & Iwashina (2016). Inflorescences of *C. bipinnatus* and *C. atrosanguineus* are pinkish-purple, while the inflorescence of *C. sulphureus* has orange pigmentation, not characteristic for anthocyanins. Hence, it is not possible to determine these compounds directly by pigmentation, although in the vegetative phase of development on the leaves' petioles, purple color is noticeable. Moreover, recently, Iwashina et al. (2019) isolated a new compound of this group, cosmonidin, from the inflorescences of *C. sulphureus*.

Anthocyanins were localized only in the overground parts of the studied plants and

Table 1. The content of flavonoids in different organs of *Cosmos sulphureus*, mg / 100 g DW.

Samples	Color of ray florets	Inflorescences	Leaves	Stems	Roots
Flavonols					
C. sulphureus	Orange	87.79 ± 3.41	26.14 ± 2.02	9.10 ± 0.76	12.90 ± 0.76
C. sulphureus 'Cosmic Orange'	Red-orange	87.99 ± 3.41	12.74 ± 0.76	10.62 ± 0.76	6.54 ± 0.82
Anthocyanins					
C. sulphureus	Orange	188.95 ± 11.80	61.32 ± 3.41	31.63 ± 1.52	n.d.
C. sulphureus 'Cosmic Orange'	Red-orange	177.14 ± 11.80	41.33 ± 3.93	25.31 ± 2.02	n.d.
Chalcones					
C. sulphureus	Orange	39.65 ± 2.16	16.34 ± 0.92	4.55 ± 0.44	n.d.
C. sulphureus 'Cosmic Orange'	Red-orange	37.93 ± 1.52	8.50 ± 0.71	2.28 ± 0.44	n.d.

Note. n.d. – not detected in the determination conditions.

were not found in the roots. Their most considerable content was also found in the inflorescences and was much lower in the stems and leaves (Table 1).

Butein is known from the group of chalcones in *C. sulphureus* (Shimokoriyama & Hattori, 1953; Iwashina et al., 2019). In our investigation, chalcones were found in all overground organs (Table 1). Chalcones predominated in the inflorescences and differed slightly in the two investigated genotypes. In the leaves and stems of both samples, their amount gradually lowered, and in the roots they were absent at all. In contrast, Saito (1979) found no butein in the leaves and colorless floral parts of *C. bipinnatus*.

It was shown that all studied types of compounds have the highest concentration in the inflorescences. Therefore, they are the most suitable for use as raw material. The cultivar 'Cosmic Orange' has florets with distinctly more intense color. However, none of the groups of flavonoids we examined affected the color intensity. The content of flavonols was close in both genotypes, while anthocyanins and even chalcones predominated in the genotype with lighter florets. Therefore, it was necessary to determine how flavonols, anthocyanins, and chalcones are distributed in different types of florets within a single genotype. Ray florets were found to have the highest content of flavonoids (Table 2). At the same time, disc florets contained relatively high of flavonoids too.

Moreover, according to our observations, each inflorescence has about nine ray and 49 disc florets. The average dry weight of one floret is 4.1 mg and 1.7 mg, respectively. Hence, the total weight of ray florets per inflorescence is about 38 mg, and disk florets – about 84 mg (DW). As a result, disc florets predominate more than two times. Therefore, disc florets are a significant component of plant raw materials of this species despite the lower content of flavonoids. At the same time, leaves containing all investigated flavonoids can be preferred as raw material for food, particularly to enrich the diet with biologically valuable compounds.

The study of the content of flavonoids in certain parts of *C. sulphureus* allows developing different ways of plant biomass use. Depending on the area of consumption (pharmaceutical, food, dyeing, etc.), it can be preferred to harvest the separated parts of the plant or its completely aboveground mass. If the production conditions allow harvesting by hand, it is possible to expect the maximum yield of all inflorescences produced by plants during the floreting period (60–80 days). Under machine harvesting, raw materials will include inflorescences, leaves, and stems. Their number and ratio will depend on several factors, such as the phase of development, the height of the cut biomass, etc.

Knowledge of the redistribution of flavonoid compounds in different parts of the plant can be essential because of human consumption needs and provide helpful information for understanding the physiological processes of *C. sulphureus*.

Conclusions

Flavonols are present in all organs of *Cosmos sulphureus*, while anthocyanins and chalcones available only from the overground parts of these plants. The highest concentration of flavonoids was observed in inflorescences, in particular in the ray florets. Anthocyanins are the most distributed component of flavonoids in the leaves. The presence of flavonoids determines the value of leaves' mass as a raw material for food and enrichment of the diet with biologically valuable compounds. Flavonols, chalcones, and especially anthocyanins are more available in ray florets than in disc florets.

Table 2. The content of flavonoids in the florets of *Cosmos sulphureus*, depending on their type, mg / 100 g DW.

Flavonoids	Ray florets	Disk florets
Flavonols	95.58 ± 2.66	88.23 ± 3.27
Anthocyanins	210.35 ± 2.66	149.89 ± 6.81
Chalcones	41.15 ± 1.33	34.31 ± 1.63

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Вміст флавоноїдів у *Cosmos sulphureus*

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Флавоноли, антоціани та халкони визначали протягом фази квітування у рослин *Cosmos sulphureus* двох генотипів (*C. sulphureus* та *C. sulphureus* 'Cosmic Orang'), культивованих в Національному ботанічному саду імені М.М. Гришка НАН України. Суцвіття, листки, стебла, корені, а також окремо крайові та дискові квітки сушили та подрібнювали для приготування витягів згідно трьох різних методик. Зокрема, витяги готували з 80 % етиловим спиртом згідно Andreeva & Kalinkina (2000), 3,5 % соляною кислотою згідно Kriventsov (1982) та 0,1 N соляною кислотою згідно Udovenko (1988). Визначення флавонолів проводили спектрофотометричним методом, вимірюючи поглинання при довжині хвилі 390 нм для флавонолів, 530 нм – для антоцианів і 364 нм – для халконів. Найвищий вміст флавонолів було виявлено у суцвіттях обидвох генотипів (87,79 ± 1,64 і 87,99 ± 1,75 мг / 100 г сухої ваги, відповідно). Вміст антоцианів ранжується за локалізацією: суцвіття > листки > стебла. Зокрема, найвищий вміст антоцианів було виявлено у суцвіттях (188,95 ± 5,20 та 177,14 ± 6,81 мг / 100 г сухої ваги, відповідно для двох досліджених генотипів *C. sulphureus*). У листках вміст антоцианів становив 61,32 ± 1,97 та 41,33 ± 2,27 мг / 100 г сухої ваги, відповідно. У стеблах, вміст антоцианів становив 31,63 ± 1,16 та 25,31 ± 0,95 мг / 100 г сухої ваги, відповідно. У коренях антоциани не виявлено. При цьому, серед проаналізованих флавоноїдів, найбільшим вмістом відрізнялися саме антоциани. Подібним чином, халкони було виявлено лише у надземних органах. Найбільша їх кількість зосереджена у суцвіттях (39,65 ± 1,25 та 37,93 ± 0,88 мг / 100 г сухої ваги, відповідно). Сполук із групи халконів значно менше у листках та стеблах, а їх вміст також сильно різниться для двох досліджених генотипів. При детальному вивчені вмісту флавоноїдів у різних частинах суцвіття, було виявлено, що дискові квітки мають менший, у порівняні з крайовими квітками, вміст флавоноїдів.

Ключові слова: *Cosmos sulphureus*, флавоноїди, флавоноли, антоциани, халкони