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RESEARCH ARTICLE

Antioxidant capacity of *Cosmos sulphureus* plants grown in the temperate climate

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Abstract

Cosmos sulphureus is an adventitious species for Europe and Ukraine in particular. It originates from Central and South America, where it grows in tropical and subtropical climates. The climatic conditions of Kyiv are characterized as temperate, with an absolute temperature minimum of -32.2°C , an absolute maximum of $+39.9^{\circ}\text{C}$, and average annual rainfall of 649 mm.

Plants were analyzed in the flowering phase, divided into inflorescences, leaves, stems, and roots, dried at $+35^{\circ}\text{C}$ and then extracted with methanol and water. The determination of the antiradical activity was carried out according to a modified method using a DPPH (2,2-diphenyl-1-picrylhydrazyl) radical inhibition reaction. The highest antiradical activity was detected in inflorescences (59.60–81.81 % inhibition) and leaves (79.81–89.12 % inhibition). Stem extracts had an average level of inhibition (19.63–65.93 %), and root extracts showed only 2.54–39.46 % inhibition. Correlation analysis showed a strong relationship between leaves and stems ($r = 0.84$), leaves and roots ($r = 0.81$), and stems and roots ($r = 0.91$).

Extracts of *C. sulphureus* plants grown in temperate climate were found having a high antioxidant potential but lower than that reported for tropical and subtropical regions. It was found that higher intensity of coloration of marginal florets of the capitulum does not correlate with a higher antiradical activity. Methanolic and water extracts of inflorescences of the genotype CSCO-368812 with intensily colored perianth inhibited only 59.60 % and 71.50 % of radicals, while similar extracts of the genotype CS-361294 with lighter florets inhibited 71.17 % and 81.81 % of radicals, respectively. Instead, there was a difference in antiradical activity depending on applied extractant. Methanolic extracts of vegetative organs (leaves, stems, roots) prevail over water extracts in terms of their antiradical activity. However, water extracts of inflorescences of both genotypes demonstrated higher level of antiradical activity.

Keywords: *Cosmos sulphureus*, antiradical activity, DPPH, methanolic extract, water extract

Authors' contributions: O. Andrushchenko developed the concept of research, analyzed literary sources, and interpreted the results, statistical processing of the experimental data and wrote the manuscript. O. Vergun was engaged in the preparation and conduct of the biochemical analyzes, writing methodological part of the research. D. Rakhmetov was coordinated research and editorial support in writing the article.

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Introduction

Cosmos sulphureus Cav. (Asteraceae Bercht. & J. Presl) originated from Central and South America (Vargas-Amado et al., 2013). This is an annual herbaceous plant with a strongly branched stem up to 2 m high (Nash & Williams, 1976). Currently, it is a widespread plant in Europe and Ukraine, which is mainly used for ornamental purposes (Kuzemko, 2013). It is also applied in ethnomedicine as a hepatoprotector, tonic, and a support meaning in malaria (Botsaris, 2007; Saleem et al., 2019). *Cosmos sulphureus*, like other species of the genus *Cosmos* Cav., is also widespread in South Africa (Ram et al., 2013) and Northern Thailand (Kaisoon et al., 2012), where it is used as a food plant. Researchers from India and Europe have identified several biologically active substances (i.e., butin, apigenin, kaempferol, myricetin, rutin and essential oils), which determine the antioxidant and bactericidal properties of *C. sulphureus* (Schlangen et al., 2010; Ram et al., 2013). Hepatoprotective properties are associated with quercetin and phenolic compounds such as gallic, caffeic, and chlorogenic acids (Saleem et al., 2019). The essential oil from the florets has an anthelmintic effect; at a concentration of 100 µg/ml, it causes the death of *Schistosoma mansoni* (Aguilar et al., 2013).

The antioxidant activity of *C. sulphureus* is determined mainly by secondary metabolites: phenols and flavonoids (Phuse & Khan, 2018). According to FRAP assay performed by Lim (2014), antioxidant activity of *C. sulphureus* is caused by the accumulation of phenols (102.5 mg GAE/g of dry weight, DW) in its inflorescences. For *C. sulphureus* capitula containing 13.08 GAE/g (fresh weight, FW) of phenols, the total antioxidant capacity of 320.36 µM TE/g FW was recorded (Chensom et al., 2019). Similarly, Cavaiuolo et al. (2013) performed DPPH assay and detected in *C. sulphureus* florets the total phenol content of 86.8–102.5 mg/g DW (according to the FRAP assay – 99.9–538.6 µmol Fe²⁺/g DW) resulting in 87.0% inhibition.

Despite numerous phytochemical studies, in Ukraine *C. sulphureus* was analyzed only as an ornamental plant (Prysedskyi, 2014). Therefore, we aimed to investigate the antioxidant activity of *C. sulphureus* plants grown in the temperate climate and to identify

potential differences depending on the genotype, selected plant parts, and extract preparation technique.

Material and methods

Biological material

Two genotypes of *C. sulphureus* (CS-361294) and *C. sulphureus* ‘Cosmic Orang’ (CSCO-368812) different in the color of the ray florets and their number in the inflorescence were chosen for the investigation (Fig. 1). The analyzed plants were grown in the open soil of the M.M. Gryshko National Botanical Garden. Samples were taken in sunny weather in beginning of August 2021.

Plants were analyzed in the flowering phase. The aboveground biomass of the plants was divided into parts: inflorescences, leaves, stems, and roots. The vegetative parts were crushed. All material was dried at +35°C using an electrodryer Ezidri Ultra FD1000 (Czech Republic).

Climatic characteristics of the region of growing

Experimental plots of the M.M. Gryshko National Botanical Garden are characterized by the forest Atlantic-continental climate. The average monthly temperature of January is –3.5°C, of July – +20.5°C; absolute minimum is –32.2°C; absolute maximum – +39.9°C. The average annual rainfall is 649 mm; the minimum – in October (35 mm); the maximum rainfall is in July (88 mm) (Vrublevska & Katerusha, 2012).

Determination of antiradical activity

The investigation of antioxidant capacity of experimental plants and procedure of determination of DPPH scavenging activity was conducted according to Brand-Williams et al. (1995). This method is based on the inhibition reaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with plant extracts. Visually it is confirmed by the reaction of discoloration. 1 g of plant powder was mixed with 25 ml of solvent (99.95% methanol or distilled water) and extracted for 12 hours at 8000 rpm in the shaker LT2 (Czech Republic). From the obtained filtrate, we took 0.1 ml and added it to 3.9 ml



Figure 1. Flowering *Cosmos sulphureus* plants: **A** – CS-361294 genotype; **B** – CSCO-368812 genotype.

of a radical solution. The radical solution was prepared by the following procedure: 0.025 g of 2,2-diphenyl-1-picrylhydrazyl was mixed with methanol in volumetric flask to get 100 ml. Obtained solution was diluted (1:10) and used for the next step. The optical density of the working radical solution ranged from 0.700 to 0.800 units. The antiradical activity was measured using spectrophotometer UV/VIS Unico 2800 (USA) at 515 nm wavelength. Measuring each repetition was done before adding sample extract and after 10 min with sample extract. These results are used in the inhibition equation showing the antioxidant potential of analyzed extracts. Each sample was measured in triplicate.

Statistical analysis

The data obtained were expressed as mean \pm standard deviation and were calculated using IBM SPSS Statistics Base 22.0 (2013, USA). ANOVA by Fisher's test was used to determine the significant effects ($p < 0.05$). The variation coefficient was calculated for each variant. To investigate the relationship between the values of the antiradical activity of different

plant parts, a bilateral Pearson correlation analysis was performed at $p < 0.001$, $p < 0.05$, and $p < 0.10$ significance levels.

Results and discussion

To evaluate the quality of *C. sulphureus* raw material serving a food and for prophylactic purposes, its antiradical activity should be determined. This study aimed to detect the combined effect of biologically active compounds depending on their location in the plant parts. The inflorescences of *C. sulphureus* are edible (Kaisoon et al., 2012; Lim, 2014; Chensom et al., 2019). The extract from *C. sulphureus* inflorescences showed over 87% inhibition following DPPH (Cavaiuolo et al., 2013). In our experiment, the antiradical activity of methanolic (M) and water (W) extracts of the inflorescences of both samples was in ranges of 59.60–71.17% and 71.50–81.81% inhibition, accordingly. The highest inhibition level was observed in plants of CS-361294 genotype (Table 1). The mean values of antioxidant capacity of CS-361294 inflorescences were higher by 11.57% and

Table 1. Antiradical activity of water and methanol extracts of different parts of *Cosmos sulphureus* (DPPH radical reaction), % inhibition.

Genotype	Plant parts	Methanol solvent		Water solvent	
		M±SD	V, %	M±SD	V, %
CS-361294	Inflorescences	71.17±0.65	0.92	81.81±0.57	0.69
	Leaves	89.12±0.70	0.79	79.81±0.54	0.68
	Stems	57.32±0.57	0.99	28.84±0.79	2.74
	Roots	39.46±2.75	6.97	10.95±1.21	11.02
CSCO-368812	Inflorescences	59.60±0.43	0.73	71.50±1.24	1.73
	Leaves	89.01±0.72	0.81	83.81±0.74	0.88
	Stems	65.93±0.77	1.16	19.63±0.38	1.91
	Roots	28.78±1.19	4.14	2.54±0.25	9.87

Note. M – arithmetic mean; SD – standard deviation; V, % – variation coefficient; $p < 0.05$.

10.34% than in CSCO-368812 genotype in the water and methanol extracts, respectively.

Analyzing the properties of individual plant parts, it was found that leaves extracts had the highest antiradical activity. In both *C. sulphureus* genotypes, the inhibition rate of methanol extracts of the leaves was the highest. The water extract of CSCO-368812 leaves also demonstrated the highest inhibition rate. However, the water extract of CS-361294 leaves showed relatively lower antiradical activity (Table 1). The high biological value of *Cosmos* leaves as a raw material is also evidenced by Phuse & Khan (2018).

Smaller number of biologically active compounds is accumulated in the stems, but the methanolic extracts of CS-361294 and CSCO-368812 showed the free radical scavenging activity at medium level – 57.32% and 65.93%, respectively. Water extracts of *C. sulphureus* stems had much lower values in both samples (Table 1). Root extracts were characterized by low inhibition, which ranged from 2.54% to 39.46%. Small values of variation coefficient ($V < 10\%$) of the antiradical activity of the extracts of inflorescences, leaves, stems, and roots indicate the homogeneity of the experimental results (Lakin, 1990).

Correlation analysis was used to establish the relationships between the values of the antiradical activity of different parts of the *C. sulphureus* plants (Table 2). A weak correlation was found when comparing the antiradical activity of inflorescences with other parts of the plant. Instead, the antioxidant

potential of leaves – stems, leaves – roots, and stems – roots were strongly interrelated.

Assessing the level of antioxidant potential of plant raw materials is usually quite a difficult task. This is due to the wide variety of test systems that use different free radical inducers and different mechanisms of action. In the cells of living organisms, many biochemical processes result in the formation of free radicals. Excessive accumulation of the latter is balanced by the action of antioxidants of various biochemical nature. The great variety of these processes explains different methods for assessing antioxidant activity. In world practice, we see a set of such studies performed by several methods: DPPH, FRAP, ORAC, CAA, and NOSA (Nitric oxide scavenging activity) (Table 3). Our task was to compare the antioxidant properties of *C. sulphureus* by several factors, so we chose the most available method – DPPH radical scavenging activity.

Inflorescences of *C. sulphureus* are the most often consumed, so they attract more researchers' attention than other plant organs. The antioxidant activity of different flowers was studied by Kaisoon et al. (2012), Chensom et al. (2019), and Jadav & Gowda (2017). At the same time, only Phuse & Khan (2018) studies were performed regarding *C. sulphureus* leaves. Thus, there was no complete idea of the properties of all parts of *C. sulphureus*.

Our investigations demonstrate the importance of each *C. sulphureus* plant organ in the distribution of compounds with antioxidant properties. We found the high

Table 2. Correlation coefficients of a linear relationship between the values of the antiradical activity of different plant parts in two genotypes of *Cosmos sulphureus*.

Plant parts	Inflorescences	Leaves	Stems	Roots
Inflorescences	1	-0.28	0.27	0.26 *
Leaves		1	0.84 **	0.81 ***
Stems			1	0.91
Roots				1

Note. Significance according to the *t*-test; * – $p < 0.10$; ** – $p < 0.05$; *** – $p < 0.001$.

antiradical activity of both inflorescences and leaves (Table 1). However, investigated antiradical activity of *C. sulphureus* was lower than the data presented in known publications. Probably, the difference could be explained by the climatic peculiarities of the regions where the analyzed plants grew. Most of published studies were realized in the regions with tropical climates: Bangalore, India (Jadav & Gowda, 2017); Shegaon, Buldhana district, India (Phuse & Khan, 2018); Maha Sarakham, Thailand (Kaisoon et al., 2012). There are also published data on *C. sulphureus* from subtropical climates, i.e., Aichi, Japan (Chensom et al., 2019). The plants we tested were cultivated in the temperate climate (Kyiv, Ukraine).

Inflorescences and leaves have high values of antioxidant activity. For example, inflorescences of *C. sulphureus* demonstrated 87.0–89.9% inhibition, and its leaves – up to 80.2% inhibition (Table 3). However, it is impossible to compare the data correctly because they are obtained by different methods (DPPH and NOSA). In our experiment, the antiradical activity of methanolic extracts from the leaves was higher (89.01% and 89.12%) than antiradical activity of extracts from the inflorescences (59.60% and 71.17%) in both genotypes (Table 1). Water extracts did not show such apparent regularity.

Marginal florets of *C. sulphureus* have different color intensities. We did not find data among the published results on antioxidant activity depending on this factor. Jang et al. (2008) studied the inflorescences of *C. bipinnatus* Cav. with different pigmentation intensities, from purple to white, and reported higher radical scavenging activity in the purple florets (Table 3). Surprisingly, Jang et al. (2008) mentioned *C. bipinnatus* with orange florets, although this species does not have such

inflorescence coloration (Paniagua-Ibáñez et al., 2015). We suppose that the authors misidentified *C. bipinnatus* with *C. sulphureus*. The extract from the florets of this genotype had one of the highest levels of radical scavenging activity, close to the *C. bipinnatus* florets of purple color (Table 3).

In our experiment, we compared the antiradical activity of inflorescences with orange (CS-361294) and red-orange (CSCO-368812) coloration, but did not find any positive dependence on the pigmentation intensity. Both methanolic (71.17% inhibition) and water (81.81% inhibition) extracts of orange florets dominated over the corresponding extracts of the red-orange samples (59.60% and 71.50% inhibition, respectively). We observed a similar trend when studying the content of flavonoids in the same samples of *C. sulphureus* (Andrushchenko & Levon, 2021). The amount of anthocyanins and chalcones (188.95 and 39.65 mg/100 g DW, respectively) in inflorescences of CS-361294 with orange florets was higher than in CSCO-368812 with more intense coloration (177.14 and 37.93 mg/100 g DW, respectively). The content of flavonols in both samples was at the same level – 87.79 (orange) and 87.99 (red-orange) mg/100 g DW. We can assume that pigments giving brighter color to *C. sulphureus* petals do not have a significant antioxidant effect, unlike the pigments of *C. bipinnatus*.

The choice of solvent for extraction is essential in identifying the antioxidant properties of plant raw materials. We chose methanol and water for comparison, as both solvents are widely applied in consumer practice. Among alcoholic solvents, methanol is preferred because it is more commonly used for the DPPH method. Testing water extracts was especially important because the most common use of *C. sulphureus* is tea

Table 3. Antioxidant and radical scavenging properties of *Cosmos* species.

Species	Analyzed plant parts	Received values	Applied assay *	Applied solvent	References
<i>C. sulphureus</i>	Florets	87.0 % inhibition	DPPH	Ethyl acetate	Kaisoon et al., 2012
		99.9–538.6 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	Ethyl acetate	Kaisoon et al., 2012
		214.8 $\mu\text{mol T Eg}$ /g DW	ORAC	Ethyl acetate	Kaisoon et al., 2012
		966.1 $\mu\text{M QE}$ /g DW	CAA	Ethyl acetate	Kaisoon et al., 2012
		320.36 $\mu\text{mol T Eg}$ /g DW	ORAC	Ethyl acetate	Chensom et al., 2019
		89.87% inhibition	DPPH	Methanol	Jadav & Gowda, 2017
	Leaves	18.1–80.2% inhibition	NOSA	Ethyl acetate	Phuse & Khan 2018
<i>C. bipinnatus</i>	Florets white	1.65 mg /ml	DPPH RSA (IC_{50})	Methanol	Jang et al., 2008
	Florets pink	1.45 mg /ml	DPPH RSA (IC_{50})	Methanol	Jang et al., 2008
	Florets violet	0.61 mg /ml	DPPH RSA (IC_{50})	Methanol	Jang et al., 2008
	Florets orange **	0.84 mg /ml	DPPH RSA (IC_{50})	Methanol	Jang et al., 2008
<i>C. caudatus</i>	Herb	0.047 mg /ml	DPPH RSA (IC_{50})	Methanol	Mediani et al., 2013
		0.054 mg /ml	DPPH RSA (IC_{50})	Ethanol	Mediani et al., 2013
	Leaves	87.52% inhibition	DPPH	100% methanol	Cheng et al., 2016
		63.70% inhibition	DPPH	100% ethanol	Cheng et al., 2016
		52.64% inhibition	DPPH	95% ethanol	Cheng et al., 2016
		70.85% inhibition	DPPH	50% ethanol	Cheng et al., 2016
		30.76% inhibition	DPPH	Distilled water	Cheng et al., 2016
		28.98% inhibition	DPPH	Juice	Cheng et al., 2016
		1197 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	100% methanol	Cheng et al., 2016
		1113.50 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	100% ethanol	Cheng et al., 2016
		840.73 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	95% ethanol	Cheng et al., 2016
		1820.70 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	50% ethanol	Cheng et al., 2016
		392.94 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	Distilled water	Cheng et al., 2016
		229.85 $\mu\text{mol Fe}^{2+}$ /g DW	FRAP	Juice	Cheng et al., 2016
Young leaves	502.21 μM /TE /ml	FRAP	Distilled water	Dian-Nashiela et al., 2015	
Mature leaves	332.00 μM /TE /ml	FRAP	Distilled water	Dian-Nashiela et al., 2015	
Old leaves	239.18 μM /TE /ml	FRAP	Distilled water	Dian-Nashiela et al., 2015	

Note. * – methods are used to evaluate antioxidant activity: **FRAP** (Ferric reducing ability of plasma), **DPPH** (diphenylpicrylhydrazyl), **ABTS** (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), **ORAC** (oxygen radical absorption capacity) (Kaisoon et al., 2012; Fernandez et al., 2017), **CAA** (cellular antioxidant activity), **NOSA** (Nitric oxide scavenging activity), **DPPH RSA** (diphenylpicrylhydrazyl radical scavenging activity); ** – probably it is *C. sulphureus*.

making. We found that methanolic extracts are more effective for vegetative organs (leaves, stems, roots), unlike generative organs (inflorescences). Water extracts of inflorescences of both samples had higher antiradical activity (Table 1). There is no

confirmation of our findings for *C. sulphureus* in published sources. However, Cheng et al. (2016) confirmed the same tendency for another *Cosmos* species, *C. caudatus* Kunth. In particular, Cheng et al. (2016) showed the dependence of antioxidant activity on the type

of solvent, using DPPH and FRAP methods and leaves extracts (Table 3). According to their data, methanol extract has the highest antioxidant activity (87.52%) compared to different concentrations of ethanol and water extracts when tested by the DPPH method. When using ethanol as a solvent, its concentration is important. In particular, 50% solution had a significant advantage over higher concentrations, which was especially shown with the use of the FRAP test system (Cheng et al., 2016). Similarly, Mediani et al. (2013) confirmed the greater efficiency of methanol extracts for *C. caudatus* (Table 3).

Dian-Nashiela et al. (2015) demonstrated surprising radical scavenging activity of *C. caudatus* plants depending on the maturity of the leaves. This aspect is not investigated for *C. sulphureus* and requires further explorations.

Conclusions

We found that inflorescences and leaves of *C. sulphureus* have the highest antioxidant activity. Their extracts can effectively neutralize oxidation products. Stem extracts, in particular methanol ones, also revealed a moderate level of antioxidant activity. This allow the use of all aboveground plant parts when harvesting raw material. However, leaves are especially valuable.

As a solvent, methanol was more effective than water for vegetative organs and vice versa for inflorescences. This should be stressed in repetitive tests and taken into account when developing innovative products.

A significant difference was found when comparing the antioxidant activity of two *C. sulphureus* genotypes, which argue the need of deeper study of *Cosmos* plants from different populations and cultivars.

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Антиоксидантна здатність рослин *Cosmos sulphureus* за вирощування в умовах помірного клімату

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Cosmos sulphureus є адвентивним видом для Європи загалом та України зокрема. Він походить із Центральної та Південної Америки, де зростає в тропічному та субтропічному кліматі. Умови Києва характеризуються помірним кліматом з абсолютним мінімумом температури $-32,2^{\circ}\text{C}$, абсолютним максимумом $+39,9^{\circ}\text{C}$ та середньорічною кількістю опадів – 649 мм.

Рослини аналізували у фазі квітіння, розділяли на суцвіття, листки, стебла та корені. Сушили при $+35^{\circ}\text{C}$ та екстрагували метанолом і водою. Визначення антирадикальної активності проводили

за модифікованою методикою за допомогою реакції інгібування радикалів DPPH (2,2-дифеніл-1-пікрилгідразил). Найвища антирадикальна активність була виявлена у екстрактів суцвіть (59,60–81,81 % інгібування) та листків (79,81–89,12 % інгібування). Екстракти стебел мали середній рівень інгібування (19,63–65,93 %), а екстракти коренів – лише 2,54–39,46 % інгібування. Кореляційний аналіз показав сильну залежність між листками і стеблами ($r = 0,84$), листками і коренями ($r = 0,81$), а також стеблами і коренями ($r = 0,91$).

Виявлено, що екстракти рослин *C. sulphureus* вирощених в умовах помірного клімату, мають високий антиоксидантний потенціал, але нижчий, ніж дослідники повідомляють для тропічних і субтропічних регіонів. З'ясовано, що більша інтенсивність забарвлення крайових квіток суцвіття не свідчить про більш високу антирадикальну активність як метанольного, так і водного екстрактів. Метанольний і водний екстракти суцвіть яскраво забарвленого генотипу CSCO-368812 інгібували лише 59,60 % і 71,50 % радикалів, в той час як аналогічні екстракти генотипу CS-361294 зі світлішими суцвіттями – 71,17 % і 81,81 % радикалів, відповідно. При цьому, існує відмінність у ефективності інгібування залежно від використаного екстрагенту. Метанольні екстракти вегетативних органів (листки, стебла, корені) мали вищу антирадикальну активність ніж водні. Натомість для суцвіть обох генотипів водні екстракти продемонстрували вищий рівень антирадикальної активності.

Ключові слова: *Cosmos sulphureus*, антирадикальна активність, DPPH, метанольний екстракт, водний екстракт