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PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF THE SELECTED EDIBLE FLOWERS GROWN IN POLAND

– Research paper –

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Abstract: The purpose of this study was to determine phenolic profile and antioxidant activity of the selected edible flowers grown in Poland. A significant variation was observed in the both antioxidant activity and total phenolic content. Marigold flowers were characterized by the highest total phenolic content (89.22 mg GEA/g dry weight). In turn, begonia flowers exhibited the highest total flavonoids and phenolic acids content (21.96 mg QE/g dry weight, and 8.60 mg CAE/g dry weight, respectively). Taking into account the type of flowers, begonia and marigold flowers were the richest in phenolic acids. Caffeic and p-coumaric acids were the most frequent ones in the edible flowers grown in Poland. While gallic and p-coumaric acids were the prevalent ones in terms of their content. The begonia and marigold flowers contained quercetin and kaempferol, while hesperetin and naringenin were present in the chives flowers. The marigold flowers were characterized by a particularly high content of quercetin, and also exhibited the highest total antioxidant activity. The methanolic extracts of marigold and begonia flowers were characterized by the highest antioxidant activity, reducing activity, as well as the highest ability to neutralize free radicals.

Keywords: edible flowers; phenolic acids; flavonoids; antioxidant activity

INTRODUCTION

The culinary and medical use of flowers as foodstuffs dates thousands year back. Edible flowers are most often used as decoration, seasoning or as an ingredient of dishes. They differ in size, color, shape, structure, smell and taste. They can be eaten raw, but also may be dried, pickled, fried or cooked (Husti et al., 2013; Mlecek & Rop, 2011; Żurek et al., 2020). Due to their health-promoting properties, flowers can become one of the components of a balanced diet. Their beneficial effects on the human body are mainly used in a herbal medicine. Many types of flowers are credited with healing properties and a beneficial effect on health, condition and well-being (Araújo et al., 2019; Piresa et al., 2019; Kumari et al., 2021). Edible flowers are also widely applied in non-culinary settings (Chen et al., 2020). For instance, the essential oils that they contain are used, for example, chamazulene present in chamomile, limonene originating from chrysanthemum flowers, linalool present in lavender flowers, spinasterol in pumpkin flowers or lupeol in marigold petals. These essential oils, due to their calming, stimulating, antibacterial, appetite-enhancing and metabolism-stimulating properties, are used in an aromatherapy. The another application of edible flowers as well as their bioactive ingredients, as the factors limiting skin aging, concerns a cosmetology (Chen et al., 2020). Flowers have a low calorific value and contain no cholesterol. However, they are a rich source of macroelements, such as: magnesium, potassium, phosphorus, and calcium (Mlecek & Rop, 2011; Araújo et al., 2019; Rop et al., 2012). In addition, they are a source of fiber, polyunsaturated fatty acids, and phytosterols (Piresa et al., 2019).

The flowers with intense yellow and orange colors contain the significant amounts of provitamine A and lutein. They are also characterized by a high L-ascorbic acid content (Fernandes et al., 2017; Skrajda-Brdak et al., 2020).

Some of the flowers may also contain ingredients that are undesirable for the human body, such as: cyanogenic glucosides, erucic acid, coumarins, thujone, trypsin inhibitors, hemagglutinins or alkaloids. For this reason, the use of flowers in the human diet should be based on an appropriate knowledge (Chen et al., 2020). The consumption of edible flowers has been increasing significantly in over the recent years, which is due to their rich taste, dietary and health-promoting values. The research conducted around the world shows that edible flowers, apart from nutritional properties, have a specific healing effect (Mlecek & Rop, 2011; Kumari et al., 2021; Skrajda-Brdak et al., 2020; Nowicka & Wojdyło, 2019). The healing properties

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of edible flowers are related, among others, to their antioxidant effect, resulting from the presence of specific components, the content of which varies greatly in the individual plants (Araújo et al., 2019; Grzeszczuk et al., 2011; Skrajda-Brdak et al., 2020; Utvineantu & Vamanu, 2020).

The compounds with strong antioxidant properties include polyphenols: phenolic acids, flavonoids, anthocyanins, and catechins as well as carotenoids and vitamins C and E (Fernandes et al., 2017; Skrajda-Brdak et al., 2020; Grzeszczuk et al., 2011). The presence of these compounds in edible flowers accounts for their antioxidant, anti-inflammatory, antimutagenic, antipoliferative, vasoprotective and cardiovascular effects (Araújo et al., 2019; Fernandes et al., 2017; Skrajda-Brdak et al., 2020). Due to the presence of procyanidins that are capable of inhibiting the activity of α-glucosidase, edible flowers have also an antihyperglycaemic effect (Nowicka & Wojdyło, 2019).

Owing to the type diversity and distribution of edible flowers as well as diversity of the content of phenolic compounds present in these plants and their antioxidant potential, it is justified to conduct research on antioxidant properties and presence of polyphenols in edible flowers originating from the different geographical regions. Therefore, the purpose of this study was to assess phenolic profile and antioxidant activity of the selected edible flowers grown in Poland.

MATERIALS AND METHODS

Materials

The research materials consisted of selected edible flowers: marigold (*Tagates* L.), begonia tuberhybrida (*B. tuberhybrida Voss*.), black elderberry (*Sambucus nigra* L.), cornflower (*Centaurea cyanus* L.), chrysanthemum (*Chryzanthemum*), chives (*Allium schoneoprasum* L.), pumpkin (*Cucurbita pepo* L.), tricolor pansy (*Viola tricolor* L.), carnation (*Dianthus* L.), lavender (*Lavandula angustifolia* Mill.), daylily (*Hemerocallis hybrida hort*), pot marigold (*Calendula officinalis* L), nasturtium (*Tropaeolum majus*) and beach rose (*Rosa rugosa*). The flower samples were obtained from the suburbs of Krosno (Poland). Only flowers during the full flowering period in the spring and summer season were harvested. The flower samples collected in the amount of about 200 g and then were frozen at – 18 °C. Subsequently, the frozen flowers were subjected to a freeze-drying process in an Alpha 1-2 DL plus freeze dryer (Martin Christ GmbH, Osterode am Harz, Germany). After the freeze-drying process, the samples were ground and analyzed in terms of the water content using a Karl-Fischer method. They were stored in a tightly closed container until the further analyses were performed.

Reagents

Folin-Ciocalteu's reagent, gallic, protocatechuic, caffeic, ferulic, hydroxybenzoic, vanilic, syringic, p-coumaric and synapic acids, quercetin, kaempferol, naringenin, hesperetin, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), trolox, TPTZ (2,4,6-tris(2-pirydylo)-s-triazine), neocuproine (2,9-dimetylo-1,10-phenanthroline), EDTA, 2-thiobarbituric acid, trichloroacetic acid were purchased from Sigma-Aldrich Chemie (Germany), acetonitrile (HPLC grade) was purchased from Merck (Germany), sodium bicarbonate, sodium nitrate (III), sodium hydroxide, aluminium chloride, acetic buffer, ferric chloride(III), ammonium acetate, phosphate buffer, hydrogen peroxide, L-ascorbic acid were purchased from Avantor-POCh (Poland), Arnov's reagent, acetic and hydrochloric acid were purchased from Chempur (Poland).

Procedure of extraction

The previously prepared raw material (5g) was extracted with methanol in an ultrasonic bath Polsonic (Palczyński Sp. J., Poland) at the temperature of 25°C for 30 min. Then, the extracted sample was shaken for 60 min., and was subsequently centrifuged. The supernatants obtained from extraction were collected, and the precipitate was extracted with another portion of methanol. The extractions were carried out three times with 50 ml of methanol, the collected extracts were concentrated on a vacuum evaporator at 40°C, and the obtained residue was dissolved in methanol which was subseqently poured into a 50 ml volumetric flask and made up with methanol.

Hydrolysis of the extracts of edible flower

In order to determine the total content of phenolics chromatographically, namely, the sum of bound and free forms of phenolic compounds in the extracts of edible flowers, an alkaline hydrolysis was performed according to the procedure reported by Nardini and Ghiselli (2004). Briefly, 10 ml of the studied extract was subjected to an alkaline hydrolysis by mixing with 90 ml 2 M NaOH solution containing 10 mM EDTA and 1% L-ascorbic acid following by incubation for 30 min. at 30°C in argon atmosphere. After end of this process, the mixture was cooled, adjusted to pH = 3 with using 4 M HCl solution following by saturation with NaCl in the amount of 300 mg per ml. The obtained

mixture was extracted three times using ethyl acetate as an extractant in the volume of 25 ml for each extraction. The obtained fraction of ethyl acetate were subsequently collected and evaporated to a dryness at a temp. of 40°C using a vacuum evaporator. The obtained dry residue was subsequently dissolved in 5 ml of methanol. For the each edible flower extract, an alkaline hydrolysis followed by the extraction process was conducted in two independent replications.

Spectrophotometric analysis of color

Determination of color parameters of the edible flowers under study (in a form of powder) were established using a Color i5 (X-Rite, USA) spectrophotometer. The following measurement conditions were used: illuminant D65 at the measuring geometry d/8°, and measuring gap 10°.The values of color parameters i.e., L* , a* , b* were determined using program X-Rite Color Master. The lightness value (L*) defines black at 0 and white at 100. The a* parameter is relative to the green–red opponent colors, with negative values toward green and positive values toward red. The b* parameter represents the blue–yellow opponents, with negative numbers toward blue and positive toward yellow.

Total phenolic content

The total phenolic content was evaluated according to the method using Folin-Ciocalteu's reagent described by Singelton and Rossi (1965). Briefly, 0.5 ml of the methanolic extract was mixed with 2.5 ml of Folin-Ciocalteu's reagent (0.2 N). After 5 min, 2 mL of sodium carbonate solution (7.5 g/100 ml) was added. After incubation for 2 hours at room temperature, the measurements of absorbance were made at λ = 760 nm against the blank sample. The calibration curve was made in the concentration range of 0–20 μg/ml, using gallic acid as a standard. Total phenolic content was calculated as mg of gallic acid equivalent (GAE)/g d.w. (dry weight) of the plant material.

Total flavonoids content

The total content of flavonoids was determined in reaction of the sample with aluminium chloride according to the procedure described by Barnum (1977). The 1 ml of extract was mixed with 4 ml of deionized water and 0.3 ml of 15% (w/v) sodium nitrate (III) solution. Then, 0.3 ml of 10% (w/v) aluminum chloride solution was added and the obtained mixture was made up to 10 ml with deionized water. After incubation for 15 min. at room temperature, the measurements of absorbance were conducted at λ = 510 nm against the blank sample. The calibration curve was made in the concentration range of 0 - 50 μg/ml using quercetin as a standard. The total content of flavonoids was calcuated as mg of quercetin equivalent (QE)/g d.w. of the plant material.

Total content of phenolic acids

Determination of the total content of phenolic acids was performed in the reaction of the sample with Arnov's reagent according to the method described by Nalewajko-Sieliwoniuk et al. (2019). The 1 ml of the extracts was mixed with 5 ml of deionized water, 1 ml of hydrochloric acid solution (18 g/l), 1 ml of Arnov's reagent and 1 ml 4M of sodium hydroxide solution and made up to 10 ml with deionized water. The measurements of absorbance were made at λ = 490 nm against the blank sample. The total content of phenolic acids was calculated as mg of caffeic acid equivalent (CAE)/per 1 g of d.w. of the plant material using the following expression: $CAE=A\times1.7544/m$; where *A* is the absorbance of the sample at 490 nm and *m* is the mass of the plant material.

Determination of phenolic profile using chromatographic (HPLC) method

Phenolic profile of the studied samples was conducted using a high performance liquid chromatography (HPLC). The determination of both flavonoids and phenolic acids contents in the extracts under study after an alkaline hydrolysis was conducted using a liquid chromatograph (HPLC, LC-Net II/ADC, Jasco, Japan) equipped with a diode array detector (DAD, MD 2018 plus, Jasco, Tokyo, Japan). The chromatographic analysis was conducted on an RP-18 column (Purospher, 250 mm × 4 mm, 5 μm particle size, Merck, Germany) at a temp. of 30°C, and the flow rate of 1 ml per min. The injection volume was 20 μl. The eluent system was a linear gradient with using two solvents: solvent A: solution of acetic acid in water (2.5 g/100 ml) and solvent B: acetonitrile. The analyzes were carried out as follows: 0 – 10 min – a linear gradient was applied with solvent B contribution increasing from 3 to 8%. The solvent B contribution was subsequently increased to 15, 20, 30 and 40% at 20, 30, 40 and 50 min of chromatographic analysis. The qualitative analysis of analyzed phenolics was performed by comparison the UV spectra of analyzed extracts with those for the standards of phenolics (i.e., flavonoids and phenolic acids) using a DAD detector and by the comparison of the standards' retention times with that of the peaks present in the chromatograms. The hydroxybenzoic acids, including gallic, *p*-hydroxybenzoic and protocatechuic acids were detected at the

wavelength of 280 nm, whereas, the hydroxycinnamic acids (i.e.,caffeic, *p*-coumaric and ferulic acids) were detected at 320 nm, respectively. Among the flavonoids, quercetin and kaempferol were identified at the wavelength of 360 nm, while hesperetin and naringenin were identified at 280 nm, respectively. The quantitative analysis of phenolic compounds was performed basing on the calibration curves which were plotted separately for the each phenolic standard in the concentration range from 0.02 to 0.2 mg per ml.

Determination of the total antioxidant activity using an ammonium molybdate assay

Determination of the total antioxidant activity was performed in reaction of the sample with ammonium molybdate according to the method described by Prieto et al. (1999). The 0.3 ml of extract was mixed with 3 ml of reaction mixture ($H_2SO_4/Na_3PO_4/(NH_4)_6Mo_7O_{24}$). After incubation process for 60 min. at 95ºC, the measurements of absorbance were made at λ = 695 nm. The calibration curve was made in the concentration range of 0 - 0,3 μM, using L-ascorbic acid. The total antioxidant activity was calculated as μM of *L*-ascorbic acid (AAE)/g d.w. of the plant material.

Determination of reducing capacity using a FRAP assay

Determination of reducing capacity using a FRAP assay was performed according to the procedure described by Benzie et al. (1999). Briefly, 3.3 ml of acetate buffer (pH = 3.6) was mixed with 0.33 ml of 20 mM $FeCl_3$ and 0.33 ml of 10 mM TPTZ solution. After incubation process for 5 min. at 37ºC, 0.33 ml of the studied extract was added. After another incubation process for 15 min. at 37ºC, the measurements of absorbance were made at λ = 450 nm. Subsequently, the calibration curve was made in the concentration range of 200 - 1200 μM/l with using aqueous solution of ferrous sulphate as a standard. The reducing capacity was calculated as μmol of Fe(II) ions/1g of d.w. of the plant material.

Determination of reducing capacity using a CUPRAC assay

Determination of the reducing activity using a CUPRAC assay was made according to the method described by Apak et al. (2004). The 0.5 ml of extract was mixed with 1 ml of 10 mM $CuCl_2$, 1 ml of 7.5 mM neocuproine, 1 ml of ammonium acetate (pH = 7.0) solutions and 0.5 ml of deionized water. The measurements of absorbance were made at λ = 450 nm. The calibration curve was made in concentration range 0 - 0.009 μM using the standard solutions of trolox. The reducing capacity was calculated as μmol of trolox equivalent (TE)/1 g d.w. of the plant material.

Determination of antiradical activity using an DPPH assay

Determination of antiradical activity using a DPPH assay was performed in reaction of the sample with a DPPH radical accordingly to the procedure described by Blois (1958). Briefly, 1 ml of the methanolic extract was mixed with 3 ml DPPH radical methanolic solution (0.1 mM). After incubation process for 60 min. at room temperature, the measurements of absorbance were conducted at λ = 515 nm. The calibration curve was made in the concentration range of 0 - 0.9 μM, using trolox solution as a standard. The antiradical activity was calculated as μmol of trolox equivalent (TE)/g d.w. of the plant material.

Determination of hydroxyl radical scavenging ability using a deoxyribose degradation assay

Determination of hydroxyl radical scavenging activity was made according to the method described by Halliwell et al. (1987). The 0.4 ml of the extract was mixed with 0.2 ml of 28 mM deoxyribose solution. Then, 1 ml of phosphate buffer solution (pH = 7.4), 0.2 ml of $FeCl_3$ water solution, 0.2 ml of 1 mM EDTA water solution, 0.2 ml of 1 mM H_2O_2 solution, and 0.2 ml of 1% ascorbic acid solution were added and the mixture was incubated for 60 min. at 37°C. Subsequently, 1 ml of 1% 2-thiobarbituric acid and 1 ml of 0.05 M trichloroacetic acid solutions were added. After incubation for 15 min. in a boiling water bath the measurements of absorbance were conducted at λ = 530 nm. The calibration curve was made in the concentration range of 0 - 400 μM using quercetin solution as a standard. The results were calculated as μM of quercetin equivalent (QE)/1 g d.w. of the plant material. All spectrophotometric analyses were carried out on an UV/Vis V-630 spectrophotometer (Jasco, Tokyo, Japan).

Statistical analysis

In order to estimate the statistical differences between the mean values, the data were treated by an one-way analysis of variance (ANOVA) then the last significant difference (LSD) values at a level of significance of 0.05 were calculated using a Fisher test. The Pearson's linear correlation coefficients between the investigated parameters were also calculated. Additionally, a Principal Component Analysis (PCA) was applied in order to differentiate the chosen parameters using Statistica v. 12.0 software (StatSoft, Inc., Tulsa, OK, USA)

RESULTS AND DISCUSSION

Color parameters, total content of phenolics, flavonoids and phenolic acids in the edible flowers.

Phenolic compounds are commonly present in all the anatomical parts of plants, including flowers. They perform a number of functions, such as protecting against the harmful effect of an UV radiation, they are also the hormones and regulators of plant growth, and provide them the characteristic color (Chen et al., 2020; Skrajda-Brdak et al., 2020). Anthocyanins and carotenoids are the most important dyes in edible flowers. They are responsible for the color of flowers, but also they are very valuable substances showing biological activity (Piresa et al., 2019; Chen et al., 2020). Anthocyanins are the natural dyes which may be of different colors which depend on the pH of environment. A particular species of plant may contain even a dozen of different types of anthocyanins, which means that it can take on various colors and shades. Among the anthocyanins present in flowers, the most important are: cyanidin, pelargonidin and delphinidin (Kumari et al., 2021). Due to their strong antioxidant properties, anthocyanins effectively reduce the harmful influence of free radicals on the human body, have a positive effect on the cardiovascular system, stimulate the secretion of nitric oxide, inhibit platelet aggregation, support liver function, and also have antimicrobial properties (Piresa et al., 2019; Kumari et al., 2021; Fernandes et al., 2017). At the same time, carotenoids, which are responsible for the yellow, orange and red color of flowers, are also the precursors of vitamin A and exhibit antioxidant properties. They have also a protective function against photooxidation processes. The most important carotenoids occurring in flowers, are α and β-carotene, cryptoxanthin, lycopene, lutein and zeaxanthin (Chen et al., 2020; Fernades et al., 2017; Kumari et al. 2021). Color parameters of the studied edible flowers, are provided in Table 1. The values of parameter L*, indicating that brightness of the sample ranged from 45.20 for cornflower to 68.56, 68.96 and 69.09 for pot marigold, pumpkin and carnation flowers, respectively. The obtained results of the brightness of color corresponded to the visual observations. The cornflower flowers were characterized by an intense sapphire-blue color; in turn, pumpkin and carnation ones were light yellow. The tricolor pansy flowers were also characterized by a low value of parameter L*, as well as these flowers have also yellow and/or white areas, apart from a purple color.

Among the studied edible flowers, only carnation had a negative value of parameter a*, which indicates a lack of share of red color in the total overall color of these flowers. With respect to the other studied flowers tested, the values of parameter a* ranged from 1.27 for lavender flowers having a characteristic purple color to 23.71 for begonia tuberhybrida flowers (Table 1) characterized by an intensely red color. In turn, the values of parameter b* varied from – 15.62 for the intense bluish-sapphire-blue cornflower flowers to 42.44 for pot marigold flowers having an intense yellow color.

Table 1. Parameters characterizing the color of the analyzed varieties of edible flowers

Name (Common Name)	L*	a*	b*
Marigold (*Tagates* L.)	54.44 ± 0.03^{d}	22.49 ± 0.05^{j}	28.45 ± 0.53^{j}
Begonia tuberhybrida (*B. tuberhybrida Voss.)*	60.00 ± 0.01^{g}	23.71 ± 0.20^{k}	10.36 ± 0.07^{d}
Black elderberry (*Sambucus nigra* L.)	65.52 ± 0.05^{i}	6.01 ± 0.01^{e}	26.02 ± 0.05^{i}
Cornflower (*Centaurea cyanus* L.)	45.20 ± 0.19^{a}	2.51 ± 0.03^{c}	-15.62 ± 0.07^{a}
Chrysanthemum *(Chryzanthemum*)	66.10 ± 0.13^{j}	3.73 ± 0.04^{d}	23.57 ± 0.04^{h}
Chives (*Allium schoneoprasum* L.)	58.29 ± 0.10^{f}	2.30 ± 0.01^{c}	14.11 ± 0.08^{f}
Pumpkin (*Cucurbita pepo* L.)	68.96 ± 0.03^{l}	10.58 ± 0.01^{f}	34.39 ± 0.05^{k}
Tricolor pansy (*Viola tricolor* L.)	49.59 ± 0.09^{b}	2.86 ± 0.03^{c}	3.98 ± 0.05^{c}
Carnation (*Dianthus* L.)	69.09 ± 0.07^{l}	-0.62 ± 0.00^{a}	23.16 ± 0.02^{g}
Lavender (*Lavandula angustifolia* Mill.)	56.10 ± 0.07^{e}	1.27 ± 0.05^{b}	13.24 ± 0.05^{e}
Daylily (*Hemerocallis hybrida hort*)	64.95 ± 0.04^{h}	12.76 ± 0.06^{g}	34.14 ± 0.02^{k}
Pot marigold (*Calendula officinalis* L)	68.56 ± 0.02^{k}	14.22 ± 1.71^{h}	42.44 ± 0.05^{l}
Nasturtium (*Tropaeolum majus*)	54.40 ± 0.06^{d}	14.59 ± 0.04^{h}	23.48 ± 0.04^{h}
Beach rose (*Rosa rugosa*)	53.12 ± 0.19^{c}	17.32 ± 0.06^{i}	-4.85 ± 0.04^{b}

Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level α = 0.05

Total content of phenolic compounds, flavonoids and phenolic acids

The edible flowers under study differed in the values of total phenolic content (TPC) (Table 2), which varied in the range from 7.15 to 89.22 mg GAE/g d.w. The chives, cornflower and lavender flowers were characterized by the lowest values of TPC, while the marigolds flowers were the richest in this respect. Although the total phenolic content varied significantly and dependent on the type of flowers, the values of this parameter were within the broad range of value reported in the literature concerning phenolic content in the edible flowers originating from Portugal (Araújo et al., 2019), the Czech Republic (Kucekova et al., 2011; 2013), Spain (Navarro-González et al., 2015), Thailand (Kaisoon et al., 2011) and China (Xiong et al., 2011; Chen et al., 2015). However, a somewhat narrower but similar range of total phenolic content in the edible flowers (2.84 - 71.07 mg/g d.w.) was reported by Nowicka & Wojdyło (2019).

Taking into account the flowers color, the highest content of TPC was found in marigold flowers which had a relatively low value of parameter L*, and by a high value of parameter a* indicating a significant share of red color. On the contrary, the lowest content of total phenolics was found in chives, cornflower and lavender flowers which exhibited the various values of parameter L*, the low values of parameter a*, and the very diverse values of parameter b*. In the present study, there was no statistically significant ($p > 0.05$) linear correlation between the total phenolic content, and color parameter L* which characterize the brightness of the sample and parameter b* indicating the balance between blue and green yellow color. Whereas, the values of parameter a* indicating the share of red or green color correlated significantly with total phenolic content (r = 0.60). As revealed by the total phenolic content, the edible flowers under study showed different values of total flavonoid content (Table 2). The begonia flowers contained the highest amount of flavonoids (21.96 mg QE/g d.w.), whereas, the nasturtium flowers were the poorest in this respect (0.79 mg QE/g d.w.). A slightly wider range of flavonoid content in the edible flowers originating from Portugal and Thailand was reported by Araújo et al. (2019), and Kaisoon et al. (2011), respectively. Whereas, Chen et al. (2015) found presence of flavonoids in the chosen Chinese flowers at the level very close to our results. Flavonoids, as a one of the most diversified and widespread natural substances in nature, are among the most important polyphenols. The numerous epidemiological studies show that an increased consumption of food rich in flavonoids results in reduction of risk of developing the cardiovascular diseases as well as certain types of cancer (Piresa et al., 2019; Fernandes et al., 2017). Flavonoid content does not always correlate with total phenolic content. The highest content of flavonoids which was found in begonia flowers did not correlate with the highest total phenolic content which was observed in the marigold flowers. Analogously, the lowest content of flavonoids observed in nasturtium flowers did not correspond to the total phenolic content in these flowers for which the value of the latter parameter was not the lowest among the edible flowers tested. The value of linear correlation coefficient (r) between total phenolic content and total flavonoids content was equal to 0.52, however, such a value of degree of freedom resulted in a lack of statistically significant correlation. These observations confirm the data reported by Kaisoon et al. (2011).

Table 2. Total phenolic (TPC), total flavonoids (TFC) and total phenolic acids (TPAC) content in the edible flowers

Sample	TPC (mg GAE/g d.w.)	TFC (mg QE/g d.w.)	TPAC (mg CAE/g d.w.)
Marigold	89.22±4.09^{i}	14.66±0.13^{j}	6.63±0.03^{l}
Begonia tuberhybrida	33.00±0.86^{f}	21.96±0.21^{k}	8.60±0.00^{n}
Black elderberry	14.36±1.29^{cd}	2.06±0.08^{c}	4.98±0.01^{j}
Cornflower	7.34±0.02^{a}	4.80±0.13^{e}	1.66±0.00^{c}
Chrysanthemum	8.71±0.08^{a}	5.71±0.04^{f}	2.56±0.00^{g}
Chives	7.15±0.15^{a}	4.56±0.15^{e}	0.78±0.00^{a}
Pumpkin	13.01±0.13^{bc}	3.61±0.12^{d}	2.01±0.00^{f}
Tricolor pansy	46.78±0.33^{g}	1.53±0.03^{b}	7.41±0.03^{m}
Carnation	15.88±0.04^{de}	11.88±0.31^{h}	1.86±0.00^{e}
Lavender	7.41±0.07^{a}	2.10±0.00^{c}	1.72±0.00^{d}
Daylily	15.73±0.07^{de}	9.55±0.22^{g}	2.62±0.00^{h}
Pot marigold	17.16±0.30^{e}	7.81±0.12^{f}	1.36±0.00^{b}
Nasturtium	11.78±0.30^{b}	0.79±0.02^{a}	3.41±0.00^{i}
Beach rose	56.18±0.49^{h}	14.36±0.16^{i}	6.03±0.02^{k}

Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level α = 0.05. GAE – gallic acid equivalents, QE – quercetin equivalents, CAE – caffeic acid equivalents, d.w. – dry weight.

Phenolic acids are the essential antioxidant ingredients of edible flowers. Their content in the edible flowers under study was varied and ranged from 0.78 mg CAE/g d.w. for chives to 8.60 mg CAE/g d.w. for begonia flowers (Table 2). A slightly lower content, as the sum of phenolic acids content determined chromatographically in the edible flowers was reported by Nowicka & Wojdyło (2019). In turn, a similar range of phenolic acids content in the edible flowers originating from China was reported by Zheng et al. (2019). In plants, phenolic acids are occuring mainly in bound forms as esters and glycosides being a part of lignins and hydrolyzable tannins. They account for a sour and bitter taste of some plant food products, they also give them the astringent properties. Their antioxidant, reducing and chelating activities are closely related to the chemical structure of individual phenolic acids, and in particular to the number of functional groups, including hydroxyl groups. The content of this group of compounds correlated significantly and linearly (r = 0.73) with the total phenolic content. No correlation was observed between the content of phenolic compounds, flavonoids and phenolic acids in the tested edible flowers, and color parameter L*, which characterize brightness of the sample as well as parameter b* indicating a balance between blue and yellow color. Whereas, the values of parameter a* showing the presence of red and green color correlated with total phenolic content (r = 0.60), total flavonoids content (r = 0.66), and total content of phenolic acids (r = 0.68).

Content of phenolic acids and flavonoids – HPLC-DAD analysis

Phenolic acids and flavonoids are the important ingredients responsible for stimulating of plant tissues. First of all, they exhibit antioxidant activity. In addition, they regulate cholesterol level, possess diuretic, anti-inflammatory activity, and seals blood vessels (Araújo et al., 2019; Fernandes et al., 2017). The action of individual phenolics is strongly correlated with their chemical structure, hence an analysis of only their total content does not provide complete information about their activity. For this reason, a number analytical techniques, especially liquid chromatography, are used for the both quantitative and qualitative analysis of the particular phenolic compound. The exemplary chromatograms for lavender flowers extract were shown in Figure 1. Whereas, the content of the determined phenolic acids was presented in Table 3. Ferulic acid was identified and quantified in the ten samples, and it was present in the highest amount in nasturtium flowers. This acid was previously found in *Allium schoenoprasum* flowers by Kucekova et al. (2011; 2013). Also Kaisoon et al. (2011) and Xiong et al. (2014) confirmed the diversified presence of this acid in edible flowers. Ferulic acid positively influences the improvement of microcirculation, neutralizes reactive oxygen species (e.g., free radicals), inhibits carcinogenesis and has anti-inflammatory properties (Kucekova et al. 2011; 2013).

In turn, gallic acid was determined and quantified in the eight samples. Beach rose flowers were characterized by the highest amount of this acid (Table 3). The presence of this acid was previously confirmed in *Tragopogon pratensis* flowers by Kucekov et al. (2011; 2013). Also Li et al. (2009), Kaisoon et al. (2011), Xiong et al. (2014) and Stanciu et al. (2019) confirmed the diversified presence of this acid in the edible flowers. Significant amounts of this acid in the flowers of various rose varieties were determined by Chen et al. (2015) and Li et al. (2009). Gallic acid, besides its antioxidant properties, exhibits also neuroprotective and anti-carcinogenic properties, inducing apoptosis and demonstrating cytotoxicity against cancer cells (Kucekova et al., 2013; Xiong et al., 2014).

p-Hydroxybenzoic acid was present at a low level in the only five samples among all the edible flowers under study. The presence of this acid in edible flowers was previously confirmed by Kaisoon et al. (2011) and Xiong (2014). Especially large amounts of this phenolic have been observed in *Hibiscus sabdariffa* flowers (Xiong et al., 2014).

The presence of caffeic acid was observed in all the studied samples (Table 3), and its highest amount was established in black elderberry flowers. The significant amounts of this acid have also been found in lavender, nasturtium and chrysanthemum flowers. The presence of this acid in chrysanthemum flowers and various rose varieties was observed by Chen et al. (2015). Also, Xiong et al. (2014) found a high content of caffeic acid in the flowers of *Chrysanthemum morifolium*. The significant amounts of this acid in the flowers of *Cichorium intybus* were previously found by Kucekova et al. (2011; 2013). Caffeic acid is commonly present in plants because it is a key intermediate in lignin biosynthesis (Xiong et al., 2014). It possesses strong antioxidant properties. It also has the ability to block carcinogenic compounds formed by metabolic changes and, like other cinnamic acid derivatives, protects the LDL cholesterol fraction against oxidation and thus inhibits atherogenesis.

The analyzed edible flowers were also rich in *p*-coumaric acid (Table 3), the presence of which was

not found only in chrysanthemum flowers. Its highest levels were established in tricolor pansy, daylily, and lavender flowers. The absence of this acid in chrysanthemum flowers was previously confirmed by Xiong et al. (2014) and Chen et al. (2015). Whereas, Kaisoon et al. (2011) confirmed the presence of *p*-coumaric acid in the various edible flowers, and its significant amounts were determined in the flowers of rose Xiong et al. (2014) and Chen et al. (2015). This acid is one of the main building blocks of lignocellulose and limits the formation of nitrosamines.

Protocatechuic acid was identified and determined in the nine samples (Table 3). Lavender flowers exhibited a particularly high amount of this acid. Kaisoon et al. (2011) observed the presence of significant amounts of this acid in the edible flowers grown in Thailand. What is more, the presence of this acid in lavender flowers was reported earlier by Xiong et al. (2014). In turn, synapic acid was identified only in begonia and carnation flowers, while syringic acid was found in marigold, tricolor pansy and pot marigold flowers. The presence of sinapic acid in the flowers of Rumex actosa was previously reported by Kucekova et al. (2013) and in different varieties of Chinese flowers by Xiong et al. (2014). In turn, a large amount of this acid in rose flowers was found by Chen et al. (2015). Whereas, Frum (2017) identified and determined the significant amounts of syringic acid in calendula flowers. Vanillic acid was identified only in pumpkin flowers (Table 3).

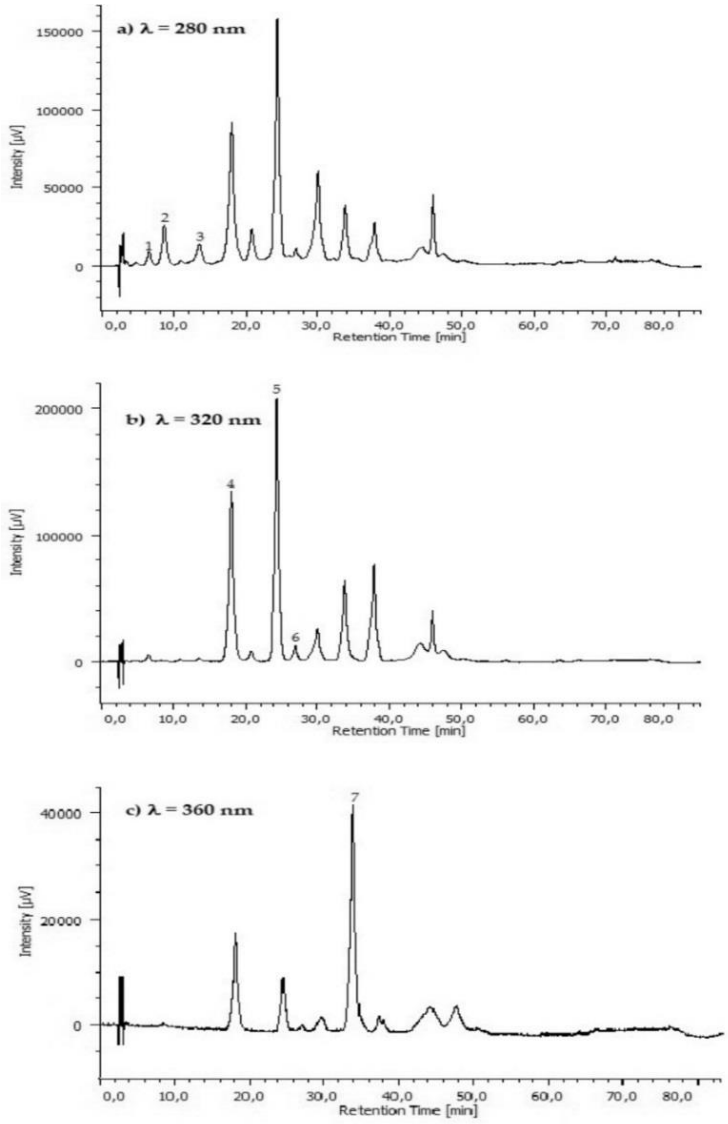

Figure 1. HPLC-DAD chromatograms obtained for lavender flower extract at the different wavelengths: a) 280 nm; b) 320 nm; c) 360 nm; 1 – gallic acid; 2 – protocatechuic acid; 3 – *p*-hydroxybenzoic acid; 4 – caffeic acid; 5 – *p*-coumaric acid; 6 – ferulic acid; 7 -kaempferol.

Table 3. Phenolic acids content (μg/g d.w.) in the edible flowers

Sample	Ferulic acid	Gallic acid	*p*-Hydroxybenzoic acid	Caffeic acid	*p*-Coumaric acid	Protocatechuic acid	Synapic acid	Syringic acid	Vanillic acid
Marigold	16.3 ± 0.8^{bc}	3344.9±29.6^{c}	nd	60.1±1.4^{c}	33.3±3.3^{a}	421.3±3.1^{d}	nd	282.1±4.7^{c}	nd
Begonia tuberhybrida	19.7 ± 0.6^{c}	531.0±37.3^{b}	45.0±1.1^{c}	328.4±2.5^{f}	557.1±0.8^{c}	106.6±5.1^{b}	36.3±5.6^{a}	nd	nd
Black elderberry	135.5 ± 3.4^{e}	nd	nd	2426.3±141.0^{k}	661.7±47.8^{cd}	nd	nd	nd	nd
Cornflower	4.4 ± 0.5^{a}	nd	nd	238.2±3.2^{e}	164.5±19.7^{b}	92.7±1.4^{b}	nd	nd	nd
Chrysanthemum	nd	nd	nd	605.8±2.5^{h}	nd	nd	nd	nd	nd
Chives	158.3 ± 4.3^{f}	nd	nd	7.4±0.3^{a}	730.5±29.6^{d}	24.1±0.7^{a}	nd	nd	nd
Pumpkin	19.0 ± 0.6^{c}	19.9±0.7^{a}	15.7±0.5^{b}	28.3±3.2^{b}	128.6±1.9^{b}	15.0±0.9^{a}	nd	nd	12.5±1.7
Tricolor pansy	nd	39.8±1.0^{a}	nd	62.0±3.4^{c}	1543.4±40.8^{f}	179.1±1.2^{c}	nd	17.4±0.7^{a}	nd
Carnation	266.7 ± 8.0^{h}	nd	8.9±0.1^{a}	21.0±1.2b	633.532.5^{cd}	22.7±1.3^{a}	81.2±4.9^{b}	nd	nd
Lavender	81.6 ± 5.8^{d}	29.8±0.4^{a}	92.1±1.3^{e}	1182.5±69.8^{j}	1337.1±76.5^{e}	831.2±35.4^{e}	nd	nd	nd
Daylily	10.2 ± 0.5 ^{ab}	9.1±0.2^{a}	nd	468.3±1.7^{g}	1436.7±5.3^{ef}	nd	nd	nd	nd
Pot marigold	nd	nd	nd	104.1±3.0^{d}	12.0±1.2^{a}	nd	nd	44.8±0.6^{b}	nd
Nasturtium	237.5 ± 4.1^{g}	43.1±1.8^{a}	56.1±0.2^{d}	790.8±24.8^{i}	695.1±12.5^{d}	14.6±1.4^{a}	nd	nd	nd
Beach rose	nd	10736.4±32.5^{d}	nd	60.9±3.3^{c}	179.0±3.0^{b}	nd	nd	nd	nd

Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level α = 0.05; nd - not detected; d.w. – dry weight.

The presence of identified and quantified phenolic acids (Table 3) confirms the previous data concerning the occurrence of phenolic acids in Polish edible flowers (Wojciak-Kosior et al., 2003; Dudek et al., 2006). Taking into account the type of edible flowers, begonia and marigold flowers exhibited the highest number quantity of phenolic acids, namely, the six out of nine different acids determined chromatographically. According to Nowicka & Wojdyło (2019), daisy and hawthorn flowers are particularly rich in phenolic acids.

By contrast, chrysanthemum flowers exhibited the lowest level of phenolic acids, where only caffeic acid was identified. The presence of this acid in chrysanthemum flowers was earlier confirmed by Chen et al. (2015). In turn, Li et al., (2009) identified gallic and protocatechuic acids in chrysanthemum flowers. In addition, the cited authors established the presence of protocatechuic acid in beach rose flowers. In the present study, the obtained results indicate that caffeic and *p*-coumaric acids were predominant ones in terms of the frequency of their occurrence in the edible flowers grown in Poland, while ferulic and gallic acids were prevalent ones in terms of their content. Beach rose flowers should be paid attention due to a very large amount of gallic acid, despite the fact that only three out of nine phenolic acids were identified chromatographically in these flowers. The presence of significant amounts of this acid in beach rose flowers was stated earlier by Xiong et al. (2014) and Chen et al. (2015).

The diversity of flower types themselves and the areas of their occurrence results in a significant variation in the content of individual phenolic compounds, and thus antioxidant activity. Zheng et al., (2019) reported that gallic, protocatechuic and *p*-hydroxybenzoic acids were predominant in the Chinese edible flowers. In turn, Kaisoon et al., (2011) stated that gallic, ferulic and sinapic acids are prevalent in the edible flowers originating from Thailand. Besides phenolic acids, flavonoids are an important group of compounds possessing antioxidant activity. The levels of four flavonoids determined chromatographically, were provided in Table 4. Among the samples under study, only cornflower flowers had all the identified flavonoids. The begonia and marigold flowers contained quercetin and kaempferol, while hesperetin and naringenin were present in the chives flowers. Naringenin was also present in black elderberry flowers, while kaempferol was found to be characteristic for the lavender flowers. The presence of the latter flavonoid in lavender flower was confirmed by Xiong et al., (2014). A special attention should be paid to the high level of quercetin in marigold flowers which were characterized by the highest content of phenolic compounds, and a high content of flavonoids (Table 2). For this reason, quercetin can be considered as the predominant flavonoid in the studied samples. This observation is reflected by the literature data. Zheng et al. (2019) reported that quercetin and hesperetin were predominant flavonoids in the edible flowers originating from China. Whereas, Kaisoon et al. (2011) informs about the dominant importance of both rutin and quercetin in the edible flowers originating from Thailand. In the remaining edible flowers under study, the determined flavonoids were not found. A low content of this group of compounds in the edible flowers was partially confirmed by the previous studies. Xiong et al., (2014) found no presence of any flavonoids among the four ones identified in the present study, when investigating beach rose, chrysanthemum and daylily flowers from Thailand.

Table 4. Flavonoid (μg/g d.w.) content in the edible flowers

Sample	Quercetin	Kaempferol	Naringenin	Hesperetin
Marigold	13762.6 ± 55.0^{c}	61.0 ± 6.9^{a}	nd	nd
Begonia tuberhybrida	1251.0 ± 6.4^{b}	1092.0 ± 28.5^{c}	nd	nd
Black elderberry	nd	nd	429.4 ± 27.5^{b}	nd
Cornflower	304.7 ± 7.9^{a}	140.1 ± 5.1^{b}	79.6 ± 0.8^{a}	64.8 ± 0.7^{b}
Chrysanthemum	nd	nd	nd	nd
Chives	nd	nd	35.7 ± 1.6^{a}	5.0 ± 0.4^{a}
Pumpkin	nd	nd	nd	nd
Tricolor pansy	nd	nd	nd	nd
Carnation	nd	nd	nd	nd
Lavender	nd	81.3 ± 0.0^{ab}	nd	nd
Daylily	nd	nd	nd	nd
Pot marigold	nd	nd	nd	nd
Nasturtium	nd	nd	nd	nd
Beach rose	nd	nd	nd	nd

nd - not detected; Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level α = 0.05; d.w. – dry weight.

Antioxidant activity

The consequence of the presence of polyphenolic compounds in flowers, are antioxidant properties of their extracts (Fernandes et al., 2017; Żurek et al., 2020). Due to the variety of components responsible for antioxidant properties, the analysis of antioxidant activity should include the variety of tests based on the different mechanisms, including among the most known, such as: hydrogen atom transfer (HAT), single electron transfer (SET), reducing capacity, and metal chelating capacity. On the other hand, the analysis of antioxidant properties estimated in a variety of ways, makes the results obtained by individual authors not always directly comparable between each other. The values of total antioxidant activity of extracts of the studied edible flowers were shown in Figure 2. Marigold flowers were characterized by the highest antioxidant activity, and they also exhibited the highest total phenolic content, and the significant content of total flavonoids and phenolic acids (Table 2). Moreover, marigold flowers had the highest level of syringic acids, and quercetin, as well as the significant amounts of gallic and protocatechuic acids (Tables 3 and 4). Beach rose flowers also exhibited a high antiradical activity, and were characterized by a high total content of phenolics, flavonoids, and phenolic acids (Table 3), as well as by the highest content of gallic acid (Table 4). Similarly, tricolor pansy flowers exhibited a high antioxidant activity, having at the same time the highest level of *p*-coumaric acid (Table 3). In turn, the extracts of cornflower and lavender flowers having the lowest total antioxidant activity, also exhibited the lowest total polyphenols content and a low level of phenolic acids (Table 2). In the present study, the significant ($p < 0.05$) linear correlations between total antioxidant activity, and total phenolic ($r = 0.97$), and phenolic acids content ($r = 0.58$) were observed.

The antioxidant activity of extracts of edible flowers under study was also determined using reducing properties of a sample against iron and copper ions (Table 5). The reducing activity determined using a FRAP assay varied in the range from 53.59 to 1328.24 µmol Fe(II)/g d.w. (Table 5). The obtained values were within a broad range of value reported by the other authors (Dudek et al., 2006; Chen et al., 2015; Kaisoon et al., 2011; Wojciak-Kosior et al., 2003; Xiong et al., 2014). The extracts of chives and pumpkin flowers exhibited the lowest reducing activity (Table 5). By contrast, marigold flowers had the highest values of this parameter, and also were characterized by the highest total phenolic content, and a high level of flavonoids and phenolic acids (Table 2), including ferulic acid (Table 3), and quercetin (Table 4). Beach rose had also a high reducing activity and exhibited a high total content of phenolics (Table 2), and the highest level of gallic acid (Table 3). In this study, the significant linear correlations between the reducing activity measured using a FRAP assay, and total phenolic content ($r = 0.96$), total phenolic acids content ($r = 0.64$), total antioxidant activity ($r = 0.92$), and color parameter a* ($r = 0.56$) were observed.

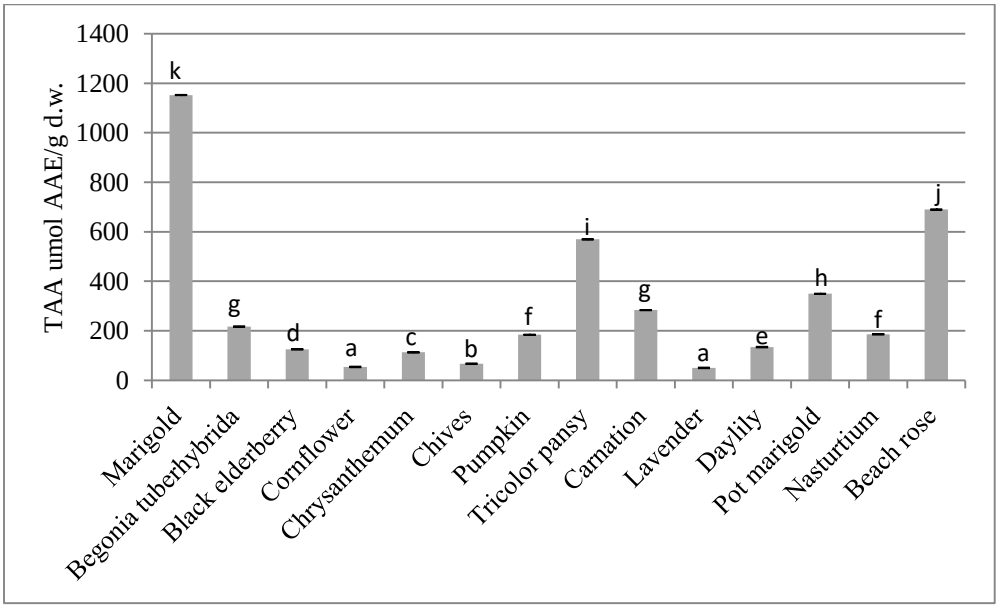

Figure 2. Total antioxidant activity of the edible flowers extracts determined using an ammonium molybdate assay. Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level $\alpha = 0.05$; d.w. – dry weight.

The values of reducing activity as regards copper ions determined using a CUPRAC assay do not confirm the trend observed in the case of FRAP method. The highest reducing capacity determined using a CUPRAC method was observed for begonia flowers (Table 5), which exhibited an average content of total phenolic, and the highest total flavonoids and phenolic acids content (Table 2). Furthermore, the latter ones contained seven out of nine phenolic acids evaluated chromatographically (Table 3), and were characterized by the highest content of kaempferol (Table 4). The values of reducing activity of extracts in the reaction with copper ions correlated significantly ($p < 0.05$) with the total flavonoids content ($r = 0.66$).

The antioxidant activity of edible flowers extracts was also evaluated using the ability to neutralize free radicals (Table 4). The ability of studied extracts to neutralize DPPH radicals varied in the range from 5.39 to 519.88 μmol TE/g d.w. (Table 5). The obtained data fell within the range of values reported by the other authors (Xiong et al., 2014; Chen et al., 2015; Butnariu & Coradini, 2009). The extracts of chives flowers exhibited the lowest antioxidant activity against DPPH radicals (Table 5). However, the marigold flowers were found to be the most active against DPPH radicals, and they also were characterized by the highest total phenolic (Table 2), ferulic acid (Table 3) and quercetin content (Table 4). The beach rose flowers also exhibited a high ability to neutralize DPPH radicals, accompanied by a high total phenolic content (Table 2), and the highest content of gallic acid. Significant ($p < 0.05$) linear correlations were observed between the ability to neutralize DPPH radicals and total phenolic content ($r = 0.94$), total content of phenolic acids ($r = 0.68$), total antioxidant activity ($r = 0.91$), and reducing activity determined using a FRAP assay ($r = 0.98$). The obtained results concerning the ability to neutralize hydroxyl radical do not confirm the trend observed for a DPPH method in this study. The highest ability to neutralize hydroxyl radicals was observed in the case of begonia flowers extract which exhibited the highest total flavonoids and phenolic acids content (Table 2), as well as the highest level of kaempferol (Table 4). Values of ability of the extracts to neutralize hydroxyl radicals correlated significantly ($p < 0.05$) with the total phenolic content ($r = 0.67$), and with reducing activity determined using a CUPRAC method ($r = 0.71$).

The results of principal component analysis were presented in Figure 3. The first component explains 59.37% of variation, while the second one 28.55%, which gives a satisfactory total results 87.92% of the explained variability. The projection of variables is shown in Figure 3a, and it confirms the earlier information on the correlation between them. A strong positive correlation can be observed for the variables, including total phenolic content, total antioxidant activity, reducing activity determined using a FRAP method, and antiradical activity measured against DPPH radicals. These variables did not correlate with the reducing activity, determined using a CUPRAC method, and with antiradical activity measured against OH radicals, that both create the different groups. Two last variables are present among these two groups: total phenolic acids and total flavonoids content, the both showing a weak correlation with the other variables. The localization of analyzed samples on the surface of two first variables that explaining 87.92% of the variation, is shown in Figure 3b.

Table 5. Reducing power and antiradical activity of the edible flowers extracts

Sample	FRAP μmol Fe(II)/g d.w.	CUPRAC μmol TE/g d.w.	DPPH• μmol TE/g d.w.	OH• μmol QE/g d.w.
Marigold	1328.42 ± 46.25^{i}	78.39 ± 0.04^{a}	519.85 ± 0.18^{m}	54.47 ± 0.18^{d}
Begonia tuberhybrida	317.44 ± 22.44^{f}	761.60 ± 15.96^{j}	99.27 ± 1.20^{j}	227.44 ± 6.83^{i}
Black elderberry	175.48 ± 0.66^{e}	80.37 ± 0.04^{a}	84.97 ± 0.82^{h}	52.14 ± 0.37^{d}
Cornflower	64.24 ± 0.62^{ab}	246.28 ± 1.83^{g}	33.05 ± 0.00^{ef}	19.52 ± 1.60^{a}
Chrysanthemum	70.53 ± 1.67^{ab}	259.88 ± 0.17^{h}	33.69 ± 0.22^{f}	73.89 ± 0.07^{g}
Chives	55.15 ± 1.74^{a}	131.58 ± 0.47^{c}	5.39 ± 0.28^{a}	18.80 ± 0.15^{a}
Pumpkin	53.59 ± 6.93^{a}	114.70 ± 1.08^{b}	10.77 ± 0.18^{b}	95.55 ± 1.39^{h}
Tricolor pansy	465.14 ± 14.25^{g}	226.38 ± 5.39^{f}	294.29 ± 1.37^{k}	95.72 ± 0.93^{h}
Carnation	101.04 ± 0.36^{cd}	245.90 ± 0.81^{g}	27.60 ± 0.10^{d}	21.23 ± 0.54^{a}
Lavender	184.93 ± 2.89^{e}	156.42 ± 0.71^{d}	94.21 ± 0.28^{i}	61.70 ± 0.23^{e}
Daylily	67.17 ± 1.97^{ab}	372.53 ± 9.34^{i}	32.20 ± 0.09^{e}	30.05 ± 2.17^{b}
Pot marigold	84.47 ± 2.14^{bc}	124.02 ± 1.07^{bc}	16.14 ± 0.10^{c}	18.42 ± 0.27^{a}
Nasturtium	117.37 ± 2.04^{d}	71.68 ± 0.34^{a}	41.94 ± 0.09^{g}	66.01 ± 0.59^{f}
Beach rose	1042.8 ± 84.29^{h}	189.01 ± 13.66^{e}	468.82 ± 1.94^{l}	47.93 ± 0.96^{c}

Mean values ± standard deviation marked with the same letters in each column do not differ significantly at the significance level $\alpha = 0.05$; TE – Trolox equivalents, QE – quercetin equivalents; d.w. – dry weight.

The conducted analysis made it possible to distinguish three groups of variables. The first group includes extracts of flowers with a high total phenolic content – marigold, beach rose, and tricolor pansy flowers. In this group, the marigold flowers exhibited the highest level of syringic acid and quercetin, the highest reducing activity (FRAP) and the highest ability to neutralize DPPH radicals. In turn, beach rose flowers were characterized by a high total phenolic content, as well as the highest content of gallic acid, while pansy flowers by a high total content of phenolic compounds and the highest content of *p*-coumaric acid. The extracts originated from the latter flowers exhibited also a high reducing activity (FRAP), and a high ability to neutralize of OH radicals. The separate group, clearly different from the others constitute begonia flowers having the highest total content of flavonoids and phenolic acids, as well as the highest amount of kaempferol. Begonia flowers were also characterized by the highest reducing activity (CUPRAC), and the highest ability to neutralize OH radicals. The last, third group includes the remaining flower extracts with similar antioxidant properties.

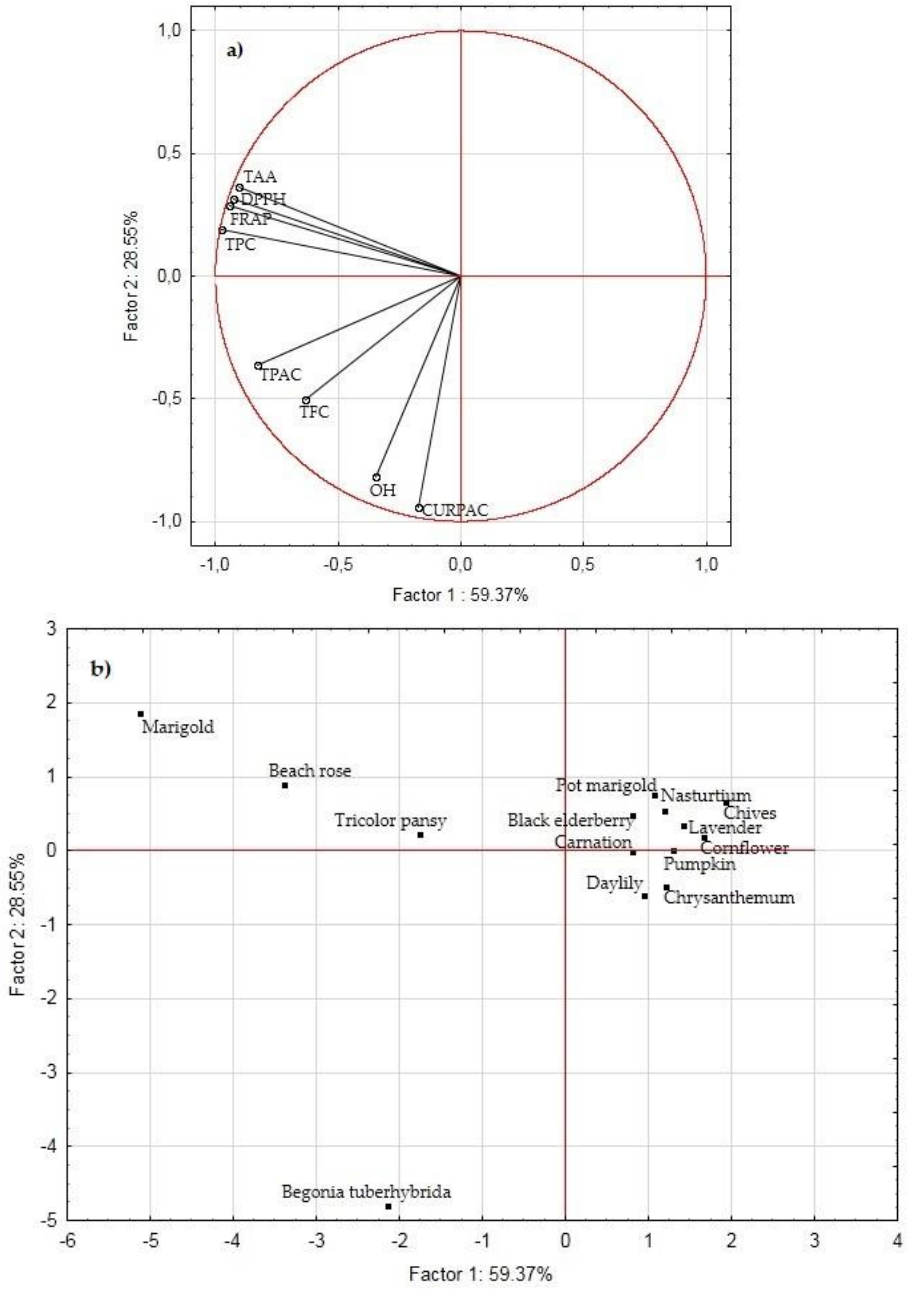

Figure 3. Results of Principal Component Analysis (PCA); a) projection of variables on the plane of reduced factors, b) projection of cases on the plane of reduced factors.

CONCLUSION

The analyzed extracts of edible flowers were characterized by a varied content of phenolics, flavonoids, and phenolic acids, as well as varied in antioxidant and antiradical properties, as well as reducing activity. Marigold flowers were characterized by the highest content of phenolic compounds. The significant amounts of this type of compounds were also present in beach rose and tricolor pansy flowers. While begonia flowers exhibited the highest total content of both phenolic acids and flavonoids. Large amounts of these compounds were also found in the beach rose, marigold and tricolor pansy flowers. Among the phenolic acids determined chromatographically, rose flowers was the richest in this respect. The obtained results indicate that caffeic and *p*-coumaric acids are the predominant phenolic acids in terms of the frequency of their occurrence, while gallic and *p*-coumaric acids were prevalent in terms of their content. Among the samples under study, only cornflower flowers contained all the four flavonoids determined chromatographically. The marigold flowers exhibited a particularly high content of quercetin, what suggests that this flavonoid can be considered as the dominant one. The content of both total phenolic and individual groups of compounds influenced the observed antioxidant properties of the studied extracts related to their reducing ability, as well as ability to neutralize the free radicals. The extract of marigold flowers was characterized by the highest total antioxidant activity, reducing activity measured using a FRAP assay, and the highest ability to scavenge DPPH radicals. While begonia flowers exhibited the highest reducing capacity determined using a CUPRAC assay and the highest ability to neutralize OH radicals. The performed PCA analysis enabled the illustration of the differences and similarities between the analyzed edible flowers in terms of their antioxidant properties. Due to the high content of polyphenols with proven health-promoting properties and significant antioxidant potential, marigold (*Tagates* L.), beach rose (*Rosa rugosa*) and tricolor pansy (*Viola tricolor* L.) should be considered particularly interesting edible flowers grown in Poland.

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Conflicts of Interest: The authors declare no conflict of interest.

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