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Research Article

Five Edible Flowers – Valuable Source of Antioxidants in Human Nutrition

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ABSTRACT
Consumption of the edible flowers forms new trend in human nutrition, especially in Bulgaria. Therefore, the determination of bioactive compounds content in their petals presents important tasks for their evaluation as natural source of antioxidants for human diet. In this study the quantity of natural pigments, total phenolic content and total flavonoids content, as well the antioxidant capacities in 95 % (v/v) ethanol, 70 % (v/v) ethanol, 80 % (v/v) methanol and distilled water extracts obtained from five edible flowers targets (*Tagetes erecta* L.), marigold (*Calendula officinalis* L.), geranium (*Geranium macrorrhizum* L.), bougainvillea (*Bougainvillea spectabilis* Willd.), Jerusalem artichoke (*Helinathus tuberous* L.) were investigated. Total phenols and flavonoid quantification of the extracts was achieved by using Folin–Ciocalteau and $AlCl_3$ reagents, respectively. Geranium 95 % ethanol extracts were evaluated as the richest source of total phenols -19.79 mg GAE/g fw, while 80% methanol extracts of *Helinathus tuberous* L. showed the highest values of total flavonoids content - 8.89 mg QE/g fw. The highest antioxidant capacities using two methods, DPPH and FRAP assays were obtained for 95% ethanol geranium (*Geranium macrorrhizum* L.) extracts - 242.9 and 106 mM TE/g fw, respectively. There was positive linear correlation between antioxidant activity and total phenolic content for all investigated edible flower extracts. All studied flowers could be a valuable source of antioxidants for addition to dishes or fresh consumption for preventing diets. These findings showed that the tested flowers could be considered as new sources of safe natural antioxidants and colorants for food industry.

Keywords: edible fresh flowers, functional food, nutrition.

INTRODUCTION

Flowers have been consumed for years in many cultures. Ancient Greek, Roman and Chinese used flowers for their medicinal properties and nutritional value. The fresh flowers were used for treatment of certain diseases, including open wounds. In aromatherapy rose, lavender, rosemary and passionflower are widely used. In ancient times, edible fresh flowers are considered and transported as fine spices. Flowers have been used for production of various types of cooked food - syrups, jellies, sauces, and different desserts. Flowers were included in the composition of products such as liquors, vinegars, teas and other beverages, honey, oils, candied flowers, ice cubes and salads. Flower petals were eaten most often fresh in salads or as garnishes, included in food the flowers can affect the sensory characteristics of food. They contribute to improve the color, taste and aesthetic appearance of food. Rose, dandelion, viola, calendula and chamomile are the most common example of flowers, which are edible[1-4]. In recent years the interest in the consumption of fresh flowers is renewed for several reasons, not least because consumers are more demanding in their choices of food. Edible flowers are becoming more popular, flowers are used in meals as a garnish or ingredients in salads, soups, entrees, desserts and drinks[5,6]. New researches and reviews concerning the composition and nutritional value of edible flowers are also important and represents a sufficient reason for their consumption[3,5,7]. The edible flowers reveal as pharmacological resource possessing the following properties - antianxiety, anticancer, antidiabetic, antiinflammatory, anti-oxidant, diuretic, anthelmintic, immunomodulatory, antimicrobial along with its effective dosage[8-10]. It is known that antioxidants when present in the daily ration could prevent chronic diseases such as type 2 diabetes, cancer, and cardiovascular and neurodegenerative disorders. Determination of the total phenolics is one of the most important parameters used to estimate the antioxidant content[11,12]. Onanong et al. investigated the phenolic compounds and antioxidant capacities of 12 edible flowers which have been consumed as vegetable and used as ingredients in cooking. The study revealed that *Antigonon leptopus* and *Tagetes erecta* had the highest ferric reducing antioxidant power value[13]. Lee et. al., examined the anthocyanin profiles and their biological properties, including antioxidant, anticancer, and antiallergic, from the red petals of edible rose[14]. A recent study reveals that flowers with higher total phenolics content are *Antigonon leptopus*, *Bougainvillea glabra*, *Tagetes erecta*, *Cosmos sulphureus*, *Prunus mume*

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Table 1: Concentrations of total chlorophylls (ca+b) and of total carotenoids in 95 % ethanol extracts of edible flowers, μg/g fw

Edible plant	Chla	Chlb	Total Chlorophylls Chla+b	Total Carotenoids
Tagetes erecta L.	15.9	7.6	23.6	6.3
Calendula officinalis L.	17.9	4.6	22.5	57.2
Geranium macrorrhizum L.	41.1	0.4	41.5	-
Helianthus tuberosus L.	0.2	0.3	0.5	15.6

Table 2: Betacyanins content from *Bougainvillea spectabilis* Willd. purple flowers

Solvent	Betacyanins, %
95 % Ethanol	-
70 % Ethanol	0.026
80% methanol	0.022
water	0.039

and *Sophora viciifolia* with values >100 mg/g dw[15]. Li et al. (2014) examine the antioxidant activity of 51 edible flowers and the results shows that flowers of *Rosa rugosa*, *Limonium sinuatum*, *Pelargonium hortorum*, *Jatropha integerrima* and *Osmanthus fragrans* possessed the highest antioxidant capacities. These flowers could be used as functional food ingredients for control of diseases caused by oxidative stress[4]. In Bulgaria edible flowers represent a new direction for improving nutritional intake and healthy eating. Dishes from fresh flowers like salads offered in the restaurant still represent a challenge for most consumers. Nevertheless, the use of rose and violet are well known in culinary practice. Therefore, the aim of the current study was to evaluate the bioactive compounds in different extracts from petals of five edible flowers (*Bougainvillea spectabilis* Willd, *Calendula officinalis* L., *Geranium macrorrhizum* L., *Helinathus tuberous* L., *Tagetes erecta* L.) consumed in Bulgaria and to evaluate their *in vitro* antioxidant activities.

MATERIALS AND METHODS

The fresh edible flowers targets (*Tagetes erecta* L.), marigold (*Calendula officinalis* L.), geranium (*Geranium macrorrhizum* L.), bougainvillea (*Bougainvillea spectabilis* Willd.), Jerusalem artichoke (*Helinathus tuberous* L.) were collected from Plovdiv, Bulgaria and kept at -18 °C for two days before use. The plants samples were ground in a laboratory homogenizer with 2.5 mm particle size and prepared for further analysis.

The ultrasound-assisted extraction

The ultrasound-assisted extraction process of biologically active substances from flowers of above mentioned plants were carried out in ultrasonic bath (VWR, Malaysia) with 42 kHz frequency and 30 W power[16]. Four solvents with different polarity 95 % (v/v), 70 % (v/v) ethanol, 80 % (v/v) methanol and distilled water were used for extraction of biologically active compounds. In brief, 1.5 g frozen flowers were weighed in 50 mL centrifuge tube with screw cap and then 15 mL solvent were added to sample. The tubes were placed in ultrasonic bath at 45 °C for 25 min. The ultrasound-assisted extraction was performed in triplicate. Each extract was filtered and the combined extracts were used for further analysis.

Total Chlorophylls and Carotenoids content

Determination of chlorophyll a, chlorophyll b and the total carotenoids in the obtained flower extracts was based on measuring the absorbance at three wavelengths. Analysis was done in triplicate for each extract. The amount of these pigments was calculated according to the formulas of Lichtentaler and Wellburn[17].

Betalain conent

Betalain contents in extracts were determined by measuring the absorbance at wavelengths of 476 nm, 537 nm and 600 nm, following the method of Nilsson[18].

Total phenolic content

The total phenolic content (TPC) was determined using the Folin–Ciocalteu reagent according to Stintzing et al.[19] with some modifications. Basically, 0.2 ml test edible flowers extract was mixed with 1 ml Folin–Ciocalteu reagent diluted five times and 0.8 mL 7.5 % Na_2CO_3. The reaction was carried out 20 min at room temperature in darkness. After reaction time, the absorption of sample was recorded at 765 nm against blank sample. The results were expressed in mg equivalent of gallic acid (GAE) per g fresh weight, according to calibration curve; build in range of 0.02 - 0.10 mg[11].

The total flavonoids content

was determinated by $Al(NO_3)_3$ reagent. The absorbance was measured at 415 nm. The results were presented as mg equivalents quercetin (QE) per g fresh weight according to the calibration curve, linear in the range of 10-100 mg/mL quercetin as a standard[20].

Antioxidant activity (AOA)

The antioxidant activities of edible flowers extracts were evaluated by two methods: DPPH (1,1 - diphenyl-2-picrylhydrazyl) radical based on mixed hydrogen atom transfer (HAT) and FRAP (ferric reducing antioxidant power) based only on single electron transfer mechanism.

The DPPH radical-scavenging ability

Each extract of flowers (0.15 ml) was mixed with 2.85 ml freshly prepared 0.1mM solution of DPPH in methanol. The sample was incubated for 15 min at 37 °C in darkness. The reduction of absorbance at 517 nm was measured by spectrophotometer in comparison to the blank containing methanol[21]. A standard curve was built with 6-hydroxy-2,5,7,8-tetramethylchroman- 2- carboxylic acid (Trolox) in concentration between 0.005 and 1.0 mM. The results

Table 3: Total phenolic content (mg GAE/g fw) and total flavonoids content (in 95 % ethanol[A], 70% ethanol[B], 80% methanol[C], and water[D] extracts and extracts from five edible flowers

Edible flower extracts	TFC, mg GAE/g fw	Flavonoids, mg QE/g fw
	Tagetes (*Tagetes erecta* L.)	
Tagetes [A]	0.79±0.02	6.05±0.02
Tagetes [B]	3.27±0.02	2.62±0.03
Tagetes [C]	5.24±0.03	6.15±0.02
Tagetes[D]	0.35±0.03	0.45±0.01
	Marigold (*Calendula officinalis* L.)	
Marigold[A]	1.18±0.02	2.60±0.01
Marigold[B]	3.30±0.02	1.40±0.02
Marigold[C]	5.1±0.03	3.30±0.05
Marigold[D]	0.7±0.02	0.70±0.03
	Geranium (*Geranium macrorrhizum* L.)	
Geranium[A]	19.79±0.11	1.52±0.05
Geranium[B]	10.48±0.03	0.82±0.06
Geranium[C]	9.89±0.05	0.80±0.02
Geranium[D]	12.35±0.07	0.74±0.03
	Bougainvillea (*Bougainvillea spectabilis* Willd.)	
Bougainvillea[A]	1.80±0.13	1.26±0.05
Bougainvillea[B]	1.65±0.02	1.03±0.03
Bougainvillea[C]	1.96±0.11	1.24±0.01
Bougainvillea[D]	2.25±0.01	0.86±0.02
	Jerusalem artichoke (*Helianthus tuberosus* L.)	
Jerusalem artichoke[A]	3.03±0.03	3.79±0.04
Jerusalem artichoke[B]	17.36±0.12	4.66±0.02
Jerusalem artichoke[C]	15.20±0.04	8.89±0.05
Jerusalem artichoke[D]	6.35±0.02	3.32±0.09

were expressed in mM Trolox® equivalents (TE) per g fresh weight (fw)[22].

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain with slight modification[23]. The FRAP reagent was freshly by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3.6H_2O$ in d. H_2O. The reaction was started by mixing 3.0 ml FRAP reagent with 0.1 ml of investigated extract. The reaction time was 10 min at 37 °C in darkness and the absorbance was measured at 593 nm against blank prepared with methanol[20]. Antioxidant activity was expressed as mM Trolox® equivalents (TE) per g fresh weight (fw).

Statistical analysis

All the experiments were performed in triplicate, and the results were expressed as mean ± SD (standard deviation). Statistical analysis was performed using and Excel 2010.

RESULTS AND DISCUSSION

Natural pigment content in edible flowers

Natural dyes and pigments gain more importance in food industries because of their non toxic and eco friendly characteristics[24-27], as well the abundant and renewable source from which they are extracted. Therefore, the knowledge for the presence of safety coloring compounds in some edible flowers suitable for food purposes is new trend for preparation of functional foods and innovative recipes for nutritionist. The natural pigments responsible for green and yellow color of edible flowers extracts were presented in Table 1. *Calendula officinalis* L. flower extracts showed the highest content of carotenoids among the other investigated plants -57 μg/g fw, followed by *Helianthus tuberosus* L. and *Tagetes erecta* L. flowers, where its content was 15.6 and 6.3 μg/g fw, respectively. The obtained results for total carotenoids in marigold petals were in agreement with Muley et al.[24] - 7.7 % for dry weight. Traditionally, golden-orange marigold (*Calendula officinalis*) flowers were used in the treatment of skin inflammation and have been found to have antioxidant, antifungal and wound healing properties[28]. It was reported that *C.officinalis*-derived carotenoid pigments and polyunsaturated fatty acids (calendic acid), possessed inflammatory properties *in vivo* and inhibitory properties *in vitro*[29]. Therefore, due to their biological activity, in this study marigold flowers were shown as potential flavoring and coloring agents in food products as ice-creams, sauces and cheese with potential healthy effect for consumers. Among the investigated flowers samples 95 % ethanol *Geranium macrorrhizum* L. were evaluated as the richest source of total chlorophylls - 41.5 μg/g fw (Table 1). The gentle floral bracts of *Bougainvillea* flowers gave purple extracts color for most of the extracts except 95% ethanolic one. It is well-known that betalain pigments are mainly responsible for the purple color, particularly betacyanins[25]. Recent reports deal with importance of these pigments as powerful antioxidants with anti-inflammatory and anticarcinogenic activity[18,26]. The results for betacyanins content in different extracts were presented in Table 2. From the obtained results for Bougainvillea flowers were found that water was the best solvent for their extractions. Our results were in agreement

Table 4: The antioxidant activities of five edible flowers (mM TE/g fw) in 95 % ethanol[A], 70% ethanol[B], 80% methanol[C], and water [D] extracts

Edible flower extracts	*Tagetes erecta* L.	*Calendula officinalis* L.	*Geranium macrorrhizum* L.	*Bougainvillea spectabilis* Willd.	*Helianthus tuberosus* L.
DPPH[A]	73.2±0.1	1.8±0.2	242.9±0.1	3.9±0.1	68.6±0.3
DPPH[B]	43.6±0.2	0.6±0.3	162.1±0.4	10.4±0.2	125.5±0.1
DPPH[C]	62.8±0.5	0.7±0.5	156.8±0.3	8.3±0.4	151.9±0.5
DPPH[D]	48.2±0.2	0.3±0.1	192.4±0.1	8.0±0.6	87.6±0.1
FRAP[A]	76.6±0.2	5.6±0.6	106.3±0.4	8.2±0.4	16.4±0.6
FRAP[B]	22.7±0.8	3.0±0.7	97.7±0.2	7.63±0.1	75.6±0.1
FRAP[C]	24.3±0.2	2.8±0.5	67.7±0.2	8.14±0.5	107.5±0.8
FRAP[D]	9.2±0.1	2.2±0.6	97.7±0.5	12.1±0.7	29.5±0.5

with Mai et al.[25] who received the highest betacyanin extraction yield (0.032%). The results showed that, water could extract betacyanin from *Bougainvillea* flower better than other solvent. According to these authors the ratio solvent and raw materials is 5:1 giving the highest extraction efficiency (0.036%), which coincide with our results obtained in the same ratio (0.039 %). The highest yield in our case could be explained with the efficiency of ultrasound-assisted extraction process. In accordance with previous report for water soluble red purple pigment from floral bracts of *Bougainvillea glabra*[27], the current study evaluated *Bougainvillea spectabilis* flower as alternative sources of betacyanins as a natural colorant for foods with potential application in some foods such as dairy products, frozen desserts or event meat.

Total phenolic content in extracts from edible flowers

The total phenolic contents of 5 edible flowers were estimated using the Folin–Ciocalteu method. The results for the determination of the total phenols and flavonoids contents of these bioactive compounds in all five studied edible flowers were shown in the Table 3**.** The total phenolic content in five edible flowers extracts varied in the wide range from 0.7 ± 0.02 to 19.79 ± 0.56 mg GAE/g fw, as their content were higher in 95 % ethanol extracts or water-containing solvents (Table 3). Two edible flowers, geranium and Jerusalem artichoke showed remarkably high total phenolic content in all investigated extracts, while water extracts of tagetes and marigold showed the lowest content – 0.3 and 0.7 mg GAE/g fw, respectively. Geranium (*Geranium macrorrhizum* L.) 95 % ethanol extract was evaluated as the highest source of total phenolic compounds - 19.79 mg GAE/g fw. It was reported that hydro-ethanol, methanol and water extracts obtained from leaves or roots of *Geraniaceae* species contained phenolic acids (mainly gallic acid), flavonoids and tannins[30-32]. It was demonstrated that these extracts found application in traditional medicine for curing stomach-ache, duodenal ulcers as well as in diarrhoea and hemorrhoids and also possessed significant antiviral, antibacterial, antiallergic, anti-inflammatory, antihepatotoxic, antidiarrhoeic, antinociceptive, antioxidant and radical scavenging activities[30-32]. The total phenolic content in water and ethanol geranium flower extracts were higher than *Geranium sanguineum* roots extracts[31]. To best of our knowledge, for the first time the water and ethanol flower extracts of the medicinal plant *Geranium macrorrhizum* L. were evaluated as edible plant for culinary purpose. For all investigated *Bougainvillea spectabilis* extracts, the aqueous one showed the highest total phenolic contents of 2.25 mg GAE/g fw. Among all studied extracts obtained from *Calendula officinalis* L. and *Tagetes erecta* L. flowers, their 80% methanol extracts contained the highest level of phenols – 5.1 and 5.24 mg GAE/g fw, respectively (Table 3). These results were in agreement with the previous reports that the most proper solvent for the extraction of phenolic and flavonoids compounds from marigold and tagetes was methanol[9,12,29,33]. The obtained results in current investigation for total phenolic content in marigold 80 % methanol extracts was higher than the reported in literature - 313.40 mg/g dw[12]. In addition, Gong et al.[34] reported that total phenolic content of defatted marigold residue extracts ranged from 8.50 to 62.36 mg GAE/g. The obtained results for total phenolic content in tagetes, bougenvillea, marigold and Jerusalem artichoke were in accordance or near to previous studied about these plants [15,32,35]. The levels of total phenolic content in all edible flower were higher than these in some edible fruits and vegetables[16,21,22]. The total flavonoid contents of the tested edible flowers varied from 0.45 mg QE/g fw (tagetes water extract) to 8.89 mg QE/g fw (80 % methanol Jerusalem artichoke extract) (Table 3). The highest content of total flavonoids were found in all 80 % methanol extracts. *Helinathus tuberosus* L. 80 % methanol extracts showed the highest level of total flavonoids - 8.89 mg QE/g fw, followed by *Tagetes erecta* L and *Calendula officinalis* L. In all investigated edible flowers the lowest levels were established in aqueous extracts, especially *Tagetes erecta* L.- 0.45 mg QE/g fw. The last mentioned fact was in accordance with Hemali and Sumitra[33]. The obtained results for total flavonoid content in Jerusalem artichoke flowers were near to previously reported results for flowers and tubers of these plants[16,35]. The results for higher flavonoids content in methanol extracts from marigold were in accordance with Butnariu and Coradini[29]. According some previous research the flowers of *Bougainvillea glabra* and *Tagetes erecta* were evaluated with higher content of kaempferol ranging from 76 to 87 mg/100 g dw[15]. Therefore, the higher flavonoids

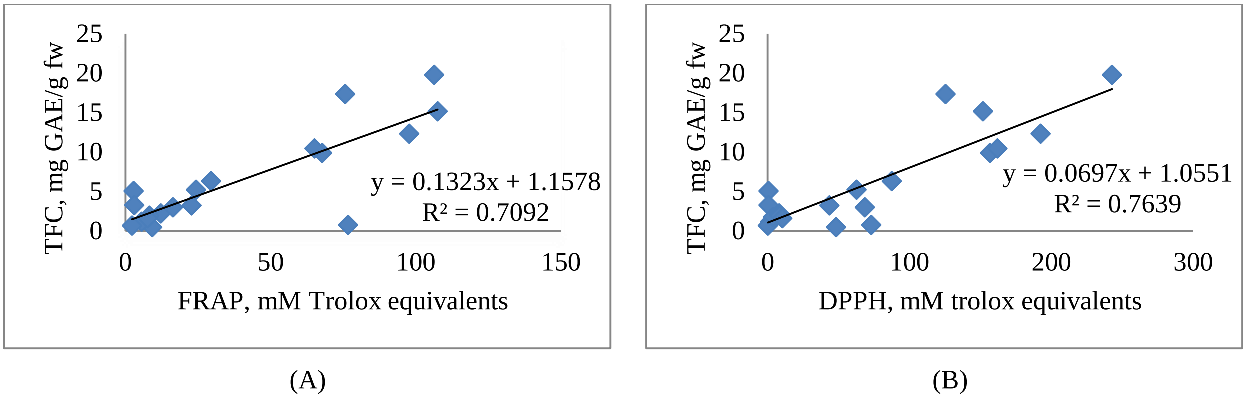

Figure 1: Linear correlations between FRAP values and total phenolic content (A) values; Coefficient of determination (R^2) = 0.7092 as well as DPPH and total phenolic content (B) for the total values edible flowers extracts; Coefficient of determination (R^2) = 0.7639

Table 5: Correlation between total phenolic content (TFC) and antioxidant activities

	TFC	Flavonoids
DPPH	0.8814	0.1459
FRAP	0.8368	0.3613

concentration in our extracts form the above mentioned plants were due to the presence of this compound.

Antioxidant activity of edible flowers extracts

Edible plants were widely applied in culinary purpose, because of their aroma and color. However, many ornamental flowers contain high levels of antioxidant compounds, which are often even higher than common horticultural crops[15]. The results for antioxidant activity of four extracts from five edible flowers were presented in Table 4. The DPPH values varied from 0.3 to 242.9 mM TE/g fw, while FRAP values were in range from 2.2 to 107.5 mM TE/g fw. The lowest antioxidant activity evaluated by both assays was reported for water extract of *Calendula officinalis* L. – 0.3 and 2.2 mM TE/g fw. The highest antioxidant activity was established for 95 % ethanol extract obtained from *Geranium macrorrhizum* L. - 242.9 mM TE/g fw for DPPH assay and 106.3 mM TE/g fw for FRAP assay, followed by 80 % methanol extracts from *Helianthus tuberosus* L. - 151.9 mM TE/g fw (DPPH assay) and 107.5 (FRAP assay), respectively. In our previous study *Helinathus tuberosus* L. tubers were evaluated as valuable source of inulin, phenolic compounds and antioxidants[16]. In current research *Helinathus tuberosus* L flowers revealed their potential to be applied for culinary purposes as natural colorants and source of flavonoids and antioxidants. Motahari et al., also demonstrated the well-pronounced radical-scavenging ability of Jerusalem artichoke flowers[35]. To the best of our knowledge for the first time the flower petals of Jerusalem artichoke were evaluated as plant for culinary purposes. Among all investigated edible flowers, the third promising source of antioxidant was *Tagetes erecta* L. Its 95% ethanol extracts showed the best antioxidant activity by DPPH and FRAP assays (Table 4). In agreement with previous reports water extracts possessed the lowest antioxidant activity. This could be explained with the lower levels of polyphenol and flavonoids in them[33]. Several reports discussed the antioxidant activity for Calendula species, *C. officinalis* in particular. Most of them mentioned that the methanol or aqueous methanol extracts from marigold showed the highest antioxidant potential[1,9,36]. However, in our study, 95 % ethanol flower extract from *C. officinalis* displayed the highest radical scavenging activity against DPPH and FRAP among the other extracts from the same plant -1.9 mM TE/g fw and 5.6 mM TE/g fw, respectively. The same tendency was observed only for FRAP assay of *Calendula officinalis* extracts[29]. Similar to our findings, Danila et al.[37] reported the lowest antioxidant activity for Romanian *C. officinalis* water and alcoholic extracts.

Correlation between antioxidant capacity and total phenolic content

The correlation between total antioxidant capacities obtained from DPPH and FRAP and total phenolic contents of five edible flowers extracts assays were presented in Figure 1. The results showed positive linear correlations between total antioxidant activities and total phenolic contents (coefficient of correlation r^2 = 0.83 and 0.88 for FRAP and DPPH values, respectively) (Table 5). These results suggested that the total phenolic compounds contributed significantly to the antioxidant activity of the investigated plant species. In addition, similar observation about dependence of antioxidant potential form TFC were reported by other authors for edible flowers extracts[1,4]. Moreover, these results suggested that antioxidant components in these five edible flowers could reduce oxidants (such as ferric ions) and scavenge free radicals. However, in our study no significant correlation existed between the antioxidant capacity and total flavonoids content (Table 5), that was in accordance with report of Zeng et al.[38] for 19 Chinese edible flowers.

CONCLUSIONS

In the current research some bioactive compounds as natural pigments, total phenolic compounds, total flavonoids content and *in vitro* antioxidant capacities of different extracts obtained from five Bulgarian edible flowers were evaluated. Generally, these plants possessed high antioxidant activity and total phenolic contents. The flowers of *Geranium macrorrhizum* Wild and *Helianthus* *tuberosus* L. were evaluated as the highest source of antioxidant compounds. A significant correlation between the DPPH and FRAP value and the total phenolic content suggested that antioxidant components in these flowers were capable of reducing oxidants and scavenging free radicals. Carotenoids, betalains and phenolic compounds were established in the investigated flowers extracts. These flowers could be applied as potential rich resources of natural antioxidants and colorants for functional foods that help for prevention and treatment of diseases caused by oxidative stress. The current research present new information about the antioxidant activity of these flowers used in human nutrition.

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