

# Antioxidant Activity of Some Edible Flowers Water Extracts from Bulgaria

Dorina PETKOVA<sup>1</sup>, Dasha MIHAYLOVA<sup>1\*</sup>, Petko DENEV<sup>2</sup>, Albert KRASTANOV<sup>1</sup>

<sup>1</sup>Department of Biotechnology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria

<sup>2</sup>Institute of Organic Chemistry with Centre of Phytochemistry-BAS, Laboratory of Biologically Active Substances, 139 Ruski Blvd, 4000, Plovdiv, Bulgaria

\*corresponding author: dashamihaylova@yahoo.com

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## Abstract

Recently, there has been a considerable interest in finding natural antioxidants from plant materials to replace the synthetic ones. The edible flowers represent a valuable alternative source of bioactive compounds and have been used as food and herbs with increasing interest. The antioxidant properties of 7 edible flowers of the South Bulgaria, including *Viola tricolor* L., *Cucurbita pepo* L., *Sambucus nigra* L., *Calendula officinalis* L., *Hibiscus rosasinensis* L., *Rosa damascena* Mill., and *Allium ursinum* L. were evaluated. The contents of flower chemicals, such as total phenolics and total flavonoids content, were determined as well. By comparing decoction and infusion as methods of extraction, the decoction ones revealed to be the most appropriate in respect of the evaluated compounds. The results showed that the highest antioxidant activity was found in the *Rosa damascena* and *Viola tricolor* ones. The established total polyphenol content and total flavonoids in the decocts of *Rosa damascena* and *Viola tricolor* were  $56.66 \pm 0.48$  and  $135.82 \pm 1.50$  mg GAE/g dw and  $28.60 \pm 0.43$  and  $15.87 \pm 0.52$  mg QE/g dw, respectively. The present research extends the traditional knowledge and revealed an opportunity to obtain biological active substances of the nature and edible flowers in particular.

**Keywords:** antioxidant activity, edible flowers, water extracts

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## Introduction

Plants and their primary and secondary metabolite constituents are leading to an emerging scientific interest in herbal medicine (Tyler, 2000). Phytochemicals, present in plants, are bioactive compounds of natural origin. The palette of phytochemicals is divided into groups and subgroups. One such group is consisted of polyphenolic compounds, which are able to absorb free radicals (molecules with unpaired electrons in

their outer orbit) and chelate metal ions. Humans are unfortunately constantly exposed to the free radicals and their overproduction in the body can lead to numerous chronic diseases such as atherosclerosis, cancer, diabetes, rheumatoid arthritis, myocardial infarction, cardiovascular diseases, etc. (Sinclair et al., 1991; Kabel, 2014). Among polyphenols, flavonoids are recognized as of great importance based on their capacity to fight against diseases and to help human body. The

ability of flavonoids to act as potent antioxidants depends on their molecular structures, the position of the hydroxyl group and other features in its chemical structure. They are abundantly found in plants as their glycosides (Rajanandh and Kavitha, 2010). Natural antioxidant intake has been associated with diabetes progression delay, reduction of cancer risk, and rejuvenation of the body (Yang et al., 2001; Sun et al., 2002).

The modern diet and highly creative cuisine are recently trends gaining more and more the interest of the consumers. In particular, the perception of modern diet and human health has undergone drastic changes in recent years. Many research papers dealt with problems of modern people eating habits (Shridhar et al., 2015), eating disorders (Shils and Shike, 2014; Weiten et al., 2014), the health aspects (Devcich et al., 2007; Jew et al., 2009), the trends in nutrition (Mihaylova et al., 2018a; Popova and Mihaylova, 2018) etc. This is an open question gathering the researcher's interest constantly. However, eating is not only getting fed and to gain the nutritional potential and the beneficial compounds but to have the spiritual delight. Nowadays, the consumers associate the food more and more with health, spiritual, cultural, and socio-economic aspects of human life.

Traditional nutrition is an approach aiming to provide basic nutrients to the body and the body itself has a perfect nutrition sensing. However, the consumption of edible flowers is an attempt of the various restaurants and chefs to be more attractive compared to others. This "competition" is beneficial to consumers, not only for their senses, but also for all beneficial compounds presented in edible flowers.

Since ancient times, plants and, in particular, edible flowers have found a place in people's diet. However, they are still considered as "modern" and induce increasing interest nowadays. In modern times they can be used in different forms – fresh, dried, in cocktails (in ice cubes as well), canned in sugar, preserved in distillates, etc. (Neugebauerova and Vabkova, 2009). The consumers demand for a reduction of synthetic food preservatives intake have increased throughout the world. In this regard, the contents of common components (proteins, fats, saccharides, vitamins) are not very different from those in other plant organs, e.g., in leaf vegetables (Kovacikova et al., 1997; Aletor et al., 2002;

Kopec, 2004; Upadhyay and Kareel, 2011). New available information in respect of the nutritive quality of flowers is important and contributes to the increasing interest in their consumption (Kopec and Balik, 2008).

Besides their aesthetic appearance which is also preferred by the consumers is their wholesomeness and, last but not least, the suitability for the efficient economic use (Herzog, 1994). Some researchers investigated the possible biological effect of various edible flowers of different origin. Petrova et al., (2016) investigated five edible flowers in respect of biological activity, Romojaro et al., (2013) studied the nutritional and antioxidant properties of wild edible flowers and Rop et al. (2012) studied the mineral content of flowers as potential contributor to human nutrition. In addition there are several review papers on edible flowers potential (Mlcek and Rop, 2011).

In this point of view, the aim of the present research paper was to explore the antioxidant potential of seven edible flowers, which could serve as food ingredient- a topic still growing interest in Bulgaria. In addition their total phenolic and flavonoid contents as afforders of the biological properties were evaluated as well.

## Material and methods

All reagents used in this study were of analytical grade and purchased from Merck Chemicals (Germany) and Sigma-Aldrich (Germany).

### Sample preparation

Seven edible flowers from Bulgaria are subjected to extraction and analyses in the present study - *Viola tricolor* L. (Wild pansy; Violaceae), *Cucurbita pepo* L. flowers (Cucurbitaceae), *Sambucus nigra* L. (Elder; Adoxaceae), *Calendula officinalis* L. (common marigold; Asteraceae), *Hibiscus rosa-sinensis* L. (rose mallow; Malvaceae), *Rosa damascena* Mill. (Damask rose; Rosaceae) and *Allium ursinum* L. (ramsons, wild garlic; Amaryllidaceae). The plant material was either obtained from local shop (Plovdiv, Bulgaria) or purchased from local pharmacies in fragmented and dry condition. The flowers were dried additionally, ground and stored at ambient temperature in air-tight containers prior to extraction. In order to study the most appropriate form of extract to be used for daily consumption by consumers, two different extraction procedures

were performed. Each edible flower sample was extracted with water (ratio of solvent to raw material was 1:20) as follow:

- Infusion - the plant material was infused into boiled water for 30 min;
- Decoction was conducted by boiling of the plant material with the solvent for 30 min;

The obtained extracts were filtered after incubation and stored at 4°C without adding any preservatives until analyses.

#### **Total polyphenol content analysis (TPC)**

The total polyphenol content was analyzed using the Folin-Ciocalteu method of Kujala *et al.* (2000) with some modifications. Each sample extract (1 mL) was mixed with 5 mL of Folin-Ciocalteu's phenol reagent and 4 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub>. The mixture was vortexed well and left for 5 min at 50°C. After incubation, the absorbance was measured at 765 nm. The TPC in the extracts was expressed as mg gallic acid equivalent (GAE) per g dry weight.

#### **Total flavonoid content (TFC)**

The total flavonoid content was evaluated according to the method described by Kivrac *et al.* (2009). An aliquot of 0.5 mL of the sample was added to 0.1 mL of 10 % Al(NO<sub>3</sub>)<sub>3</sub>, 0.1 mL of 1 M CH<sub>3</sub>COOK and 3.8 mL of ethanol. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm. Quercetin was used as a standard and the results were expressed as mg QE/g dw.

#### **Antioxidant activity (AOA)**

##### *DPPH radical scavenging activity*

The ability of the extracts to donate an electron and scavenge DPPH radical was determined by the slightly modified method of Brand-Williams *et al.* (1995). Freshly prepared 4x10<sup>-4</sup> M methanolic solution of DPPH was mixed with the samples in a ratio of 2:0.5 (v/v). The light absorption was measured at 517 nm at room temperature after 30 min incubation. The DPPH radical scavenging activity was presented as a function of the concentration of Trolox. Trolox equivalent antioxidant capacity (TEAC) and was defined as the concentration of Trolox having equivalent AOA expressed as the μM Trolox per g dw.

##### *ABTS radical cation decolorization assay*

The radicals scavenging activity of the extracts against radical cation (ABTS<sup>•+</sup>) was estimated according to a previously reported procedure with

some modifications (Re *et al.*, 1999). The results were expressed as TEAC value (μM TE/g dw).

#### **Ferric reducing antioxidant power assay (FRAP)**

The FRAP assay was carried out according to the procedure of Benzie and Strain (1999). The FRAP reagent was prepared fresh daily and was warmed to 37°C prior to use. The absorbance of the reaction mixture was recorded at 593 nm after incubation at 37°C for 4 min. The results were expressed as μM TE/g dw.

#### **Copper reduction assay (CUPRAC)**

CUPRAC assay was performed according to the method of Ak and Gülçin (2008). To a test tube were added 1 mL of CuCl<sub>2</sub> solution (1.0x10<sup>-2</sup>M), 1 mL of neocuproine methanolic solution (7.5x10<sup>-3</sup>M), and 1 mL NH<sub>4</sub>Ac buffer solution (pH 7.0), and mixed; 0.1 mL of herbal extract (sample) followed by 1 mL of water were added (total volume of 4.1 mL), and mixed well. Absorbance against a reagent blank was measured at 450 nm after 30 min. Trolox was used as standard and total antioxidant capacity of herbal extracts was measured as μM TE/g dw.

#### **Oxygen Radical Absorbance Capacity (ORAC)**

Oxygen Radical Absorbance Capacity (ORAC) method - The method developed by Ou *et al.* (2002) was used with some modifications. This method measures the ability of an antioxidant to neutralize peroxid radicals. The method is based on the inhibition of the decline of fluorescence of fluorescein during its oxidation in the presence of an antioxidant. The thermal decomposition of 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a peroxid radical generator. The results are expressed in μM Trolox equivalents per gram of extract. The excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

#### **Hydroxyl Radical Averting Capacity (HORAC)**

Hydroxyl Radical Averting Capacity (HORAC) method - The method was developed by Ou *et al.* (2001), and measures the ability of an antioxidant to form complexes in conditions of Fenton reaction, caused by the interaction between Co (II) and H<sub>2</sub>O<sub>2</sub>. The results are expressed in μM gallic acid equivalents per gram of extract. The excitation wavelength of 485 nm and emission wavelength of 520 nm were used.

**Table 1.** Total phenolic content and total flavonoid content of edible flower water extracts

Sample/Assay	Extraction method	TPC, mg GAE/g dw	TFC, mg QE/g dw
V. tricolor (VT)	infusion	33.06 ± 0.27	15.89 ± 0.23
	decoction	56.66 ± 0.48	28.60 ± 0.43
C. pepo (CP)	infusion	11.94 ± 0.20	2.80 ± 0.05
	decoction	13.87 ± 0.29	3.33 ± 0.08
S. nigra (SN)	infusion	31.50 ± 0.32	10.22 ± 0.24
	decoction	34.61 ± 0.15	11.51 ± 0.24
C. officinalis (CO)	infusion	16.49 ± 0.20	3.40 ± 0.14
	decoction	19.44 ± 0.04	4.26 ± 0.23
H. rosa-sinensis (HR)	infusion	32.43 ± 0.42	4.31 ± 0.14
	decoction	38.49 ± 0.15	4.94 ± 0.56
R. damascena (RD)	infusion	70.29 ± 0.62	9.36 ± 0.05
	decoction	135.82 ± 1.50	15.87 ± 0.52
A. ursinum (AU)	infusion	17.36 ± 0.11	1.11 ± 0.03
	decoction	19.92 ± 0.14	1.49 ± 0.04

### Statistical analysis

The presented results are average from two independent experiments carried out in triplicates. The results were expressed as mean ± SD, analyzed using MS Excel 2003 software.

### Results and discussions

In the present study seven edible flowers species, belonging to seven botanical families, being of potential interest for consumers, were examined. Aiming a comparative assessment of the biological activity and antioxidant potential in particular, two extraction methods were performed. The results in respect of the total phenolic content and total flavonoid content in the investigated infusions and decocts are presented in Table 1. Among the infusions the values varied from 11.94 ± 0.20 to 70.29 ± 0.62 mg GAE/g dw regarding the TPC and the highest results were established in the *R. damascena* and *V. tricolor* extracts - 70.29 ± 0.62 and 33.06 ± 0.27 mg GAE/g dw, resp. The lowest content was established in the *C. pepo* flowers infusion - 11.94 ± 0.20 mg GAE/g dw. The TPC values of the decocts were confirming the established trend of the highest total phenolic content in *R. damascena* extract followed by the *V. tricolor* (135.82 ± 1.50 and 56.66 ± 0.48 mg GAE/g

dw, resp.). In comparison Dudonné et al. (2009) reported 124.86 ± 1.54 mg GAE/g dw TPC in *R. damascena* extract obtained with stirring at 50°C. *Rosa damascena* flower petals were assumed with antioxidant potential related to the phenolic and flavonoid contents (Baydar et al., 2013) and the water extracts were even recommended as tea and antioxidant rich beverage in particular (Vinokur et al., 2006).

In respect of TFC the results for the infusions varied from 1.11 ± 0.03 to 15.89 ± 0.23 mg QE/g dw. The highest content was established in the *V. tricolor* and *S. nigra* infusions (15.89 ± 0.23 and 10.22 ± 0.24 mg QE/g dw). The lowest values were determined in the *A. ursinum* and *C. pepo* flowers infusions (1.11 ± 0.03 and 2.80 ± 0.05 mg QE/g dw). In comparison, Hamissou et al. (2013) established quite similar TFC in water extract of *C. pepo* fruits- 8.67 ± 1.59 mg GAE/g fw. The results in respect of the investigated decocts were in accordance with the infusions ones. However, the highest flavonoid content was established in *V. tricolor* (28.60 ± 0.43 mg QE/g dw) showing the contribution of those phenolic compounds to the total phenolic compounds value. Several biological activities were reported by Hellinger et al. (2014) as regard the *V. tricolor* extracts, mainly

**Table 2.** *In vitro* antioxidant activity of edible flowers water extracts according to DPPH, ABTS, FRAP and CUPRAC, ORAC and HORAC assays

Sample/ Assay	Extraction method	DPPH, $\mu\text{M}$ TE/g dw	ABTS, $\mu\text{M}$ TE/g dw	FRAP, $\mu\text{M}$ TE/g dw	CUPRAC, $\mu\text{M}$ TE/g dw	ORAC, mM TE/g dw	HORAC, $\mu\text{M}$ GAE/g dw
VT	infusion	149.34 $\pm$ 1.56	160.95 $\pm$ 0.73	252.08 $\pm$ 3.15	309.51 $\pm$ 5.91	45.47 $\pm$ 1.82	12.93 $\pm$ 1.13
	decoction	204.54 $\pm$ 1.40	283.19 $\pm$ 3.87	477.58 $\pm$ 4.60	572.26 $\pm$ 4.43	19.08 $\pm$ 1.04	64.46 $\pm$ 3.28
CP	infusion	30.47 $\pm$ 0.71	75.97 $\pm$ 0.68	82.46 $\pm$ 0.61	119.39 $\pm$ 2.23	14.74 $\pm$ 0.88	6.53 $\pm$ 0.42
	decoction	39.89 $\pm$ 0.40	87.94 $\pm$ 1.23	103.40 $\pm$ 0.18	135.97 $\pm$ 2.01	8.54 $\pm$ 0.92	19.79 $\pm$ 0.47
SN	infusion	140.44 $\pm$ 6.67	147.97 $\pm$ 1.68	224.44 $\pm$ 4.00	312.49 $\pm$ 2.96	40.31 $\pm$ 1.53	11.22 $\pm$ 0.21
	decoction	175.16 $\pm$ 0.56	171.18 $\pm$ 7.11	269.85 $\pm$ 5.02	387.60 $\pm$ 6.96	11.59 $\pm$ 1.54	72.64 $\pm$ 0.11
CO	infusion	30.65 $\pm$ 1.35	77.96 $\pm$ 0.43	81.35 $\pm$ 1.42	108.84 $\pm$ 0.97	25.14 $\pm$ 0.07	6.30 $\pm$ 0.69
	decoction	53.71 $\pm$ 0.17	54.46 $\pm$ 2.74	124.40 $\pm$ 4.98	176.12 $\pm$ 3.39	5.95 $\pm$ 0.36	20.94 $\pm$ 1.58
HR	infusion	122.84 $\pm$ 12.14	207.39 $\pm$ 1.11	409.46 $\pm$ 2.48	373.57 $\pm$ 11.14	17.47 $\pm$ 0.43	9.29 $\pm$ 0.57
	decoction	218.55 $\pm$ 4.64	304.00 $\pm$ 1.76	484.02 $\pm$ 1.17	456.05 $\pm$ 4.17	8.89 $\pm$ 0.69	37.67 $\pm$ 0.50
RD	infusion	472.55 $\pm$ 8.37	641.10 $\pm$ 11.37	904.40 $\pm$ 8.47	425.91 $\pm$ 9.79	127.13 $\pm$ 9.03	20.66 $\pm$ 0.62
	decoction	1031.70 $\pm$ 14.50	1599.28 $\pm$ 51.57	1804.88 $\pm$ 11.07	1615.28 $\pm$ 29.29	41.55 $\pm$ 2.32	172.72 $\pm$ 3.94
AU	infusion	4.12 $\pm$ 0.12	21.03 $\pm$ 0.08	35.47 $\pm$ 2.23	19.80 $\pm$ 0.58	19.36 $\pm$ 0.36	5.35 $\pm$ 0.32
	decoction	13.40 $\pm$ 0.06	23.83 $\pm$ 0.91	45.54 $\pm$ 0.18	69.23 $\pm$ 0.96	2.93 $\pm$ 0.55	61.06 $\pm$ 2.43

provided by the flavonoids content (Vukics *et al.*, 2008 a, b; Piana *et al.*, 2013) and thus defining their application as food, as medicinal agents and as functional food (Koike *et al.*, 2015).

The results regarding the antioxidant activity of the studied water extracts - infusions and decoctions were presented in Table 2. Flowers all around the world have been investigated extensively for their antioxidant potential (Zheng *et al.*, 2018). Since the plant parts explored differ notably and some variability on nutritional composition of edible flowers influencing their properties is reported, only relative comparison is possible.

All conducted six assays (DPPH, ABTS, CUPRAC, FRAP, ORAC and HORAC) confirmed *R. damascena* extract with the highest potential both among the infusions and decoctions, which is in agreement with claimed by Ađar (2010) significant antioxidant and radical scavenging capacity of *R. damascena* extracts. However, the results were in predominance for the decoctions in accordance with the reported by Mihaylova *et al.*, (2018b)

and could be due to the more effective extraction under heat treatment.

According Ginova *et al.*, (2013) the antioxidant potential varied depending on the growing season. The authors reported values toward DPPH<sup>•</sup> of rose petals from Bulgaria between 536.7 and 792.2  $\mu\text{M}$  TE/g dwb, which corresponds with the established in the present study for infusion and decoct - 472.55  $\pm$  8.37 and 1031.70  $\pm$  14.50  $\mu\text{M}$  TE/g dw, resp.

Interestingly based on ORAC and HORAC values along the prevalence of *R. damascene*, *V. tricolor* and *S. nigra* decocts revealed high antioxidant activity. The slight differences among the results could be referred to the various mechanism of the methods used. Furthermore, these findings were in close agreement with the recommendation of application of several based on different mechanism methods for objective *in vitro* antioxidant evaluation (Apak *et al.*, 2013).

The lowest results in respect of antioxidant activity were recorded for the *A. ursinum* and *C. pepo* flowers extracts. The potential of *A. ursinum* infusion toward DPPH<sup>•</sup> (4.12  $\pm$  0.12  $\mu\text{M}$  TE/g dw)

was comparable to the reported by Sapundjieva et al. (2012) for ramsons leaves - 9.94  $\mu\text{M TE/g}$ , but still lower, suggesting the presence of different quantity of biologically active substances in the flower parts of the plant. Indeed, the leaves of this plant species are known with high content of biologically active compounds (Sapundjieva et al., 2012; Mihaylova et al., 2014; Popova and Mihaylova, 2018). However, considering the fact that wild garlic flowers extract consisted of low TPC and TFC, low antioxidant activity is expectable.

Possible influence on the biological activity and the phytochemical composition of the investigated edible flowers could have the time of harvest, soil condition and geographical location in addition to the plant organ explored as suggested by Sobolewska et al. (2013). Furthermore, based on the numerous potential health benefits associated to edible flowers, particular attention to their harvesting and preservation, due to their high perishability is recommendable (Fernandes et al., 2017)

### Conclusions

The present research extends the traditional knowledge and revealed an opportunity to obtain biological active substances from nature, and in particular from edible flowers. Seven edible flowers species, belonging to seven botanical families, being of potential interest for consumers, were examined - *Viola tricolor*, *Cucurbita pepo*, *Sambucus nigra*, *Calendula officinalis*, *Hibiscus rosa-sinensis*, *Rosa damascena* and *Allium ursinum*. However, *R. damascena* flower petals extracts revealed the highest antioxidant activity both for infusion and decoction corresponding to the highest total phenolic content. In respect of the total flavonoid content *V. tricolor* water extracts were evaluated as most potential.

Taking into account both antioxidant activity and the phenolic content of the edible flowers analyzed, they can be considered as food ingredient with potential benefit effects. In addition, they could be used as nutritional supplements in functional foods with high antioxidant activity and as natural antioxidants in order to replace synthetic ones along with their aesthetic effect. However, attention should be given to the specific phytochemical composition establishment.

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