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PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF WATER EXTRACTS FROM *ROSA DAMASCENA* PETALS GROWN IN KAZANLAK VALLEY, BULGARIA

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Abstract

The current study aimed to evaluate the phenolic content, total flavonoid content, as well as the antioxidant activity in water extracts obtained from Rosa damascena petals grown in Kazanlak valley, Stara Zagora region, Bulgaria. The rose samples were collected during May 2019 from the plantations in Skobelevo, Asen, Iasenovo, and Koprinka villages grown under different conditions. The contents of total polyphenols and flavonoids in the water extracts from rose petals were determined. Additionally, the antioxidant activity of rose water extracts was evaluated by four reliable methods: 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays. The highest values of total phenols and flavonoids were found in the rosewater extract from organically grown plantation: 47.09±2.89 mg GAE/g dry weight and 6.87±3.00 mg QE/g dry weight, respectively. The highest radical scavenging activity demonstrated the extracts from organic plantations, while the metal-reducing assays showed higher antioxidant potentials in the extracts from conventionally grown roses. The results demonstrated the potential application of these edible flowers rose extracts as ingredients or additives for food and cosmetic purposes with the potential antioxidative properties.

***Key words**: antioxidant activity, petals, phenolic content, Rosa damascena, water extracts.*

INTRODUCTION

Rosa genus, belonging to the *Rosaceae* family, includes 200 species and more than 18.000 cultivars. One of the most important Rosa species is *Rosa damascene* Mill (Patil et al., 2015), which is the hybrid between *R. gallica* and *R. phoenicia* Mahboubi (2016). This plant is called damask rose because it was originally brought to Europe from Damascus (Patil et al., 2015). Nowadays, Bulgaria and Turkey are the main producers of *R. damascena* essential oil in the world and the Bulgarian *R. damascena* oil is the known best ones (Mahboubi, 2016).
The oil-bearing rose (*Rosa damascena* Mill. f. Trigintipetala Dieck) is an emblematic plant species for Bulgaria. The industrial rose cultivation in Bulgaria involves exclusively the species *R. damascena* Mill f. Trigintipetala Dieck (Kazanlashka roza), due to its higher rose oil content and superior essential oil quality (Kovacheva et al., 2010). Recently, there has been a growing interest in antiviral, antibacterial, anticancer, and antioxidant activities on *R. damascena* products (Mahboubi, 2016). For instance, Baydar and Baydar (2013) reported that fresh and spent flowers extract obtained from *R. damascena* could be a good natural antioxidant source.
In recent years, industrial production of organic oil-bearing roses has become one of the fastest developing sectors of organic agriculture in Bulgaria. Today, all rose plantations and rose flower processing facilities are private property. Organic producers are concentrated in southern Bulgaria. Essential oil crops including roses are produced mainly in the Sub-Balkan Valley as well as in the Thracian Valley, which is considered the most appropriate for oil rose and lavender production (Chalova et al., 2017). The interest in organic products is growing worldwide principally because consumers are concerned about the amounts of agrochemicals, pesticides, synthetic growth stimulants, and antibiotics in foods, as well as in genetic

modifications (Torjusen et al., 2001). The application of an organic source of nutrients is rapidly attracting attention. Organic maturing has beneficial impacts on soil properties and produces safe plants. It is reported in the literature that some vegetables of organic origin present a higher content of flavonoids when compared to the same products coming from conventional cultivation, as in the work of Chassy et al. (2006), where authors compared organic with conventional tomatoes. Ren et al. (2001) determined the content of polyphenols in five commonly consumed in Japan, produced by organic and conventional cultivation. The contents of flavonoid (quercetin) and caffeic acid in organically grown plants were 1.3-10.4 times higher than those found in conventional plants. The effects of organic and conventional cultures on anthocyanins, total phenolics, and antioxidant activity in blueberry fruit were studied (Wang et al., 2008), and the results showed that fruit produced following organic procedure showed a higher content of some concerning conventionally grown plants. Many investigators obtained the best results by using organic compost for several medicinal and aromatic plants (Taie et al., 2010). Besides, Ginova et al. (2013) evaluated total phenols and radical scavenging activity of rose samples collected from seven industrial-scale plantations in Bulgaria during 2009-2010 (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo). But unfortunately, there are not so many studies connected with the influence of different agriculture systems on the antioxidant activity of *Rosa damascena.*

The aim of the current study was to evaluate the phenolic content, total flavonoid content, as well as the antioxidant activity in water extracts obtained from *Rosa damascena* petals grown in Kazanlak valley, Stara Zagora region, Bulgaria.

MATERIALS AND METHODS

Plant material

The samples were collected from arable areas in Southern Bulgaria, a region called Rose (Kazanlak) Valley. The Rose Valley is situated in the middle of the country between the Stara Planina Mountain (North) and Sredna Gora Mountain (South). The valley is around 90 km long and around 10 km wide and the average altitude 350 m. For the experimental study, six oil rose plantations were selected (Table 1). In three of them have been applied to an organic agriculture system and in the rest of the farms, a conventional agriculture system was used. The google maps coordinates, altitude, soil type, and geographical area are presented in Table 1. Conventional agricultural practices utilized nitrogen fertilizers and pesticides, drip irrigation, and mechanization. Organic crops cannot be genetically engineered, flowers were collected manually, fields were not irradiated, or not fertilized. Additionally, farmland used to grow organic crops is prohibited from being treated with synthetic pesticides and herbicides for at least 3 years before harvest. Rose flowers of *R. damascena* were collected in May 2019 in the morning (6-8 a.m.), in 3, 4, and 5 phases as described by Staikov and Zolotovich (1954). Plant material was air-dried in shade at room temperature and grounded in a mechanical grinder (final powder size less than 400 μm). The samples are stored in a dark and cool room at 16-18°C before the analysis.

Table 1. Locations of the sampling plantations in Kazanlak valley, Bulgaria

Farm	Area	Google maps coordinators Latitude (N) Longitude (E)	Altitude	Soil type
	Conventional agriculture system			
1	Damascena 1, Skobelevo	42.670050 25.196783	520 m	Fluvisols
2	Damascena 2, Skobelevo	42.668950 25.198150	520 m	Fluvisols
3	Koprinka	42.633433 25.329483	400 m	Luvisols
	Organic agriculture system			
4	Skobelevo	42.672067 25.177383	522 m	Fluvisols
5	Asen	42.643567 25.175150	483 m	Fluvisols
6	Yasenovo	42.693767 25.277983	510 m	Fluvisols

Extracts preparation

Rose petals were extracted with distilled H_2O in solid to liquid ratio 1:10 (w/v). The extraction procedure was performed in an ultrasonic bath (SIEL, Gabrovo, Bulgaria, 35 kHz, and 300 W) for 20 minutes, at 65 °C. The obtained extracts were filtered, and the residues were extracted once again under the above-mentioned conditions. The combined extracts were used for further analysis.

Total phenolic contents

Total phenolic content was measured using a Folin-Ciocalteu reagent. Briefly, 1 mL Folin-Ciocalteu reagent (diluted five times) was mixed with 0.2 mL rose petals extracts and 0.8 mL 7.5% Na_2CO_3. The reaction was performed for 20 minutes at room temperature in darkness. The absorbance was measured at 765 nm against the blank. The results were expressed as mg equivalent of gallic acid (GAE) per g dried weight (dw), according to Ivanov et al. (2014).

The total flavonoids content

The total flavonoids content was analyzed using $Al(NO_3)_3$ reagent as previously described (Kivrak et al., 2009). After 40 minutes the absorbance was measured at 415 nm against the blank. The results were presented as mg equivalents quercetin (QE) per g dry weight (dw) according to the calibration curve linear in the range of 10-100 µg/mL quercetin (Ivanov et al., 2014).

2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

Rose petals water extract (0.15 mL) was mixed with 2.85 mL 0.1 mM solution of DPPH in 100% methanol. The sample was incubated for 15 minutes at 37 °C. The reduction of absorbance was measured at 517 nm in the comparison to the blank containing methanol and % inhibition was calculated (Ivanov et al., 2014).

2,2′-azino-bi's-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay

ABTS radical cation ($ABTS^{+}$) was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. Then 0.15 ml rose petal extract was mixed with 2.85 ml of the ABTS solution previously diluted with methanol (1:30; v/v). After 15 min at 37 ºC in darkness, the absorbance of the formed complex was measured spectrophotometrically at 734 nm (Ivanov et al., 2014).

Ferric reducing antioxidant power (FRAP) assay

The assay was performed according to Benzie and Strain (1996) with slight modification. The FRAP reagent was freshly prepared by mixing 10 parts 0.3 M acetate buffer (pH 3.6), 1 part 10 mM 2,4,6- tripyridyl-striazine (TPTZ) in 40 mM HCl and 1 part 20 mM $FeCl_3 \times 6H_2O$ in distilled H_2O. FRAP (3.0 ml) reagent was mixed with 0.1 mL rose petal extract. After 10 mins at 37 °C in darkness, the absorbance was measured at 593 nm against blank prepared with water (Ivanov et al., 2014).

Cupric reducing antioxidant capacity (CuPRAC) assay

Rose petal extract (0.1 mL) were mixed with 1 mL $CuCl_2 \cdot 2H_2O$, 1 mL Neocuproine (7.5 mL in methanol), 1 mL 0.1 M ammonium acetate buffer and 1 mL distilled water. The solution was incubated at 50 °C for 20 minutes in darkness and the absorbance was measured at 450 nm (Ivanov et al., 2014).

All the results from the determination of anti-oxidant activity were performed in triplicates and expressed as mM Trolox equivalents (mM TE) by dry weight. All the data were expressed as mean ± standard deviation (SD).

Data analysis

The data were expressed as mean ± standard deviation (SD) from three replicates for each sample. To determine the significant differences between values, and used to perform ANOVA statistical analysis. Statistical analysis with all data was also done with Unscrambler (Camo, Norway) software packages.

RESULTS AND DISCUSSIONS

These results for antioxidant activity and phenolic content from the water extract of rose were summarized in Table 2.

The highest values of total phenols and flavonoids were found in the rosewater extract from organically grown plantation from farm 3 Asen area: 47.09±2.89 mg GAE/g dry weight and 6.87±3.00 mg QE/g dry weight, respectively. In general, total phenol content in rose petals decreased in the following order of the collecting area: Asen> Skobelevo> Yasenovo (organic agriculture system)>

Damascena 1, Skobelevo> Damascena 2, Skobelevo> Koprinka (conventional agriculture system). In rose farms with conventional agriculture systems, the level of total phenols ranged from 19.64 to 13.97 mg GAE/g dw (Table 2). The reported in our study values for total phenols were higher than the reported values for rose petals from seven industrial-scale Bulgarian plantations (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo, and Gurkovo) (from 5.85 to 11.80 mg GAE/g dw). Damask rose gave the highest total phenolic content (31.9 mg GAE g-1 sample) (Sommano et al., 2018), which was following our results for organic rose petals. According to Sengul et al., (2017) the total phenolic content of Turkish rose petals (*Rosa damascena* Mill.) was 481.54 µg GAE/mg sample, while Ge and Ma (2013) found that total phenolic content in petals of Yunnan edible roses was 2087.43 ±17.37 mg GAE/100 g fresh weight. The range of total phenols content in rose teas was 50.7 to 119.5 mg gallic acid equivalents (GAE) per gram of dry matter (Vinokur et al., 2006). Our values for total phenols in organically grown roses were also comparable with reported values for phenolic content in Turkish rose petals syrup 64.94 µg GAE/mg sample (Sengul et al., 2017).
The values of total flavonoids were the highest in the organic agriculture system (farm 4 to 6), where the content varied between 4.63 to 6.87 mg QE/g dw. In comparison to the roses from the conventional agriculture system, the content of total flavonoids was twice higher (Table 2).
It was observed that the levels of total phenols and flavonoids in rose petals from an organically grown area demonstrated approximately 45-50 % higher values in comparison with conventionally grown roses (Figure 1).

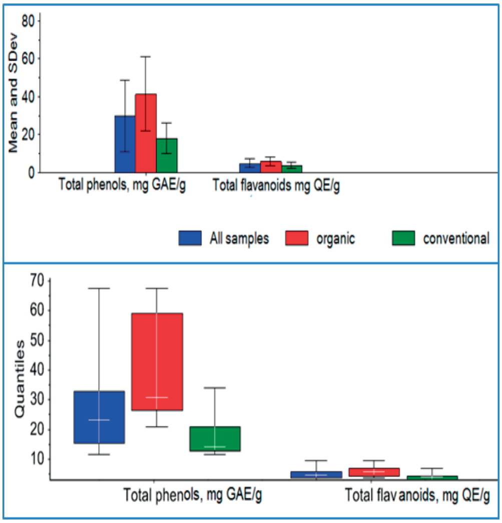

Figure 1. Statistical data of total phenols and total flavonoids of water extracts from *Rosa damascena* petals

Table 2. Total phenols, total flavonoids and antioxidant activity in water extracts from conventionally and organically grown roses

Farm	Area	Total phenols, mg GAE/g dw	Total flavonoids, mg QE/g dw	Antioxidant activity, mM TE/g			
				DPPH	ABTS	FRAP	CUPRAC
Conventional agriculture system							
1	Damascena 1, Skobelevo	19.64±0.08	4.58±1.88	439.38±11.98	379.77±4.81	811.62±91.08	541.59±20.90
2	Damascena 2, Skobelevo	17.62±0.24	3.05±1.71	316.38±2.45	371.11±16.24	803.34±2.70	560.81±19.36
3	Koprinka	13.97±0.13	2.58±0.15	298.68±10.74	320.08±11.04	945.94±12.36	925.85±27.88
Organic agriculture system							
4	Skobelevo	39.01±0.21	4.63±0.99	311.20±5.94	240.97±6.52	237.39±7.58	486.34±13.10
5	Asen	47.09±2.89	6.87±3.00	347.20±8.76	546.13±22.84	322.75±4.25	848.13±5.92
6	Yasenovo	30.50±0.04	5.32±0.27	583.83±13.18	638.44±18.48	1875.80±11.05	563.90±5.04

It was reported previously that quercetin and kaempferol are antimicrobial compounds synthesized by plants in response to pathogen attack (Dixon and Paiva, 1995). Because organically and sustainably grown products were produced by cultural methods utilizing no or very little pesticides, pathogenic pressures may explain the higher total phenolic levels found in the organically and sustainably grown samples (Asami et al., 2003).
The antioxidant activity of rose petals extracts was evaluated by four methods based on the

different mechanisms (DPPH, ABTS, FRAP, and CUPRAC). It was found that organically grown samples (Table 1) demonstrated better radical scavenging activity evaluated by methods base on hydrogen transfer (DPPH and ABTS assays). However, conventionally grown roses showed higher results for antioxidant activity evaluated by methods based on electron transfer as FRAP and CUPRAC (Table 1 and Figure 2). In general, the roses collected from farm 6, Yasenovo area showed the highest result for antioxidant activity evaluated by DPPH, ABTS and FRAP methods from 583.83 to 1875.8 mM TE/g dw. Organic rose petals from Asen and Yasenovo areas demonstrated the highest antioxidant activity evaluated by ABTS method. Vinokur et al. (2006) also reported that rose petal teas from different cultivars exhibited scavenging capacity toward 2,2′-azino-bis-(3-ethylbenzothiazoline)-6sulfonate cation radical (ABTS+) ranging between 712.7 and 1770.7 µM Trolox equivalents (TE) per gram of dry petals. However, reported in the current study results for antioxidant activity of *Rosa damascena* were higher than these of Vinokur et al. (2006) and comparable with reported for rose petals from seven industrial-scale Bulgarian plantations in Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo (Ginova et al., 2013).

All these results suggested that the contents of polyphenolic and antioxidants in rose petals are strongly influenced by environmental, e.g. geographical and edaphic, factors as previously described by Ginova et al. (2013). Moreover, the current study demonstrated that agriculture practices also strongly influence the content of total phenols and flavonoids in rose petals (Table 1). A similar trend of the increase in phenolic compounds in organically grown samples, as fruits and vegetables were reported by some authors (Ren et al., 2001; Asami et al., 2003; Borguini et al., 2013).

Vickery and Vickery (1981) mentioned that many secondary metabolites as phenolic compounds, for example, act as fungicides and antibiotics to protect the plants from fungi and bacteria. Therefore, higher content of phenolic compounds in organic food occurs because of the possible incidence of pests and pathogens in the organic cultivation method, in which pesticides are not used. An increase in phenolic compounds production is connected with the natural defences of plants (Borguini et al., 2013).

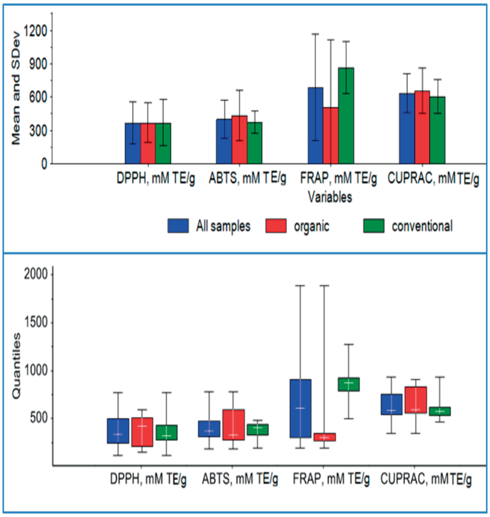

Figure 2. Statistical data of antioxidant activity of water extracts from *Rosa damascena* petals among the different methods

Table 2 presents the correlation coefficient (r) values established based on linear correlations between phenolic content and antioxidant activity, evaluated by four methods. It was established a high correlation between total phenols and total flavonoids in rose flower water extracts, r = 0.78 (Figures 3). The content of total flavonoids exhibited a significant correlation with antioxidant activities in the DPPH and ABTS, with r = 0.58 and 0.69, respectively. That correlation is graphically illustrated in Figure 4. Correlation analysis showed that total antioxidant activity, evaluated by DPPH and ABTS assay, is highly related to the total phenols and total flavonoids (Table 2 and Figure 4). Amongst the methods used for quantifying antioxidant activities, the correlation between ABTS and CUPRAC was 0.53. Büyüktuncel el al. (2014) reported a high correlation between FRAP and CUPRAC; FRAP and ABTS with 0.886 and 0.870, respectively for wine samples. Probably, the low correlation in our study among the rest of the methods could be explained by the use of the water extracts, not organic solvents. According to many authors (Vinokur et al., 2006; Hou et al., 2014; Patil et al., 2015), the antioxidant activity of *R. damascena* is not related to anthocyanin level but is correlated to total phenolic, flavonol contents of *R. damascena*. According to Vinokur et al., 2006, the antioxidant capacity of rose petals is correlated well (r = 0.78-0.88) with the contents of total polyphenols. In our case, total flavonoids expressed as quercetin equivalent is correlated well with radical scavenging activity of water extracts (Figure 4).

Table 2. Pearson's correlation coefficients (r) between total phenols, total flavonoids content and antioxidant activities of water extracts from *Rosa damscena*

	Total phenols, mg GAE/g	Total flavonoids, mg QE/g	DPPH, mM TE/g	ABTS, mM TE/g	FRAP, mM TE/g	CUPRAC, mM TE/g
Total phenols, mg GAE/g	1.00					
Total flavanoids, mg QE/g	**0.78**	1.00				
DPPH, mM TE/g	**0.51**	**0.58**	1.00			
ABTS, mM TE/g	0.39	**0.69**	0.43	1.00		
FRAP, mM TE/g	-0.38	-0.18	0.33	0.37	1.00	
CUPRAC, mM TE/g	0.39	0.42	0.14	**0.53**	0.05	1.00

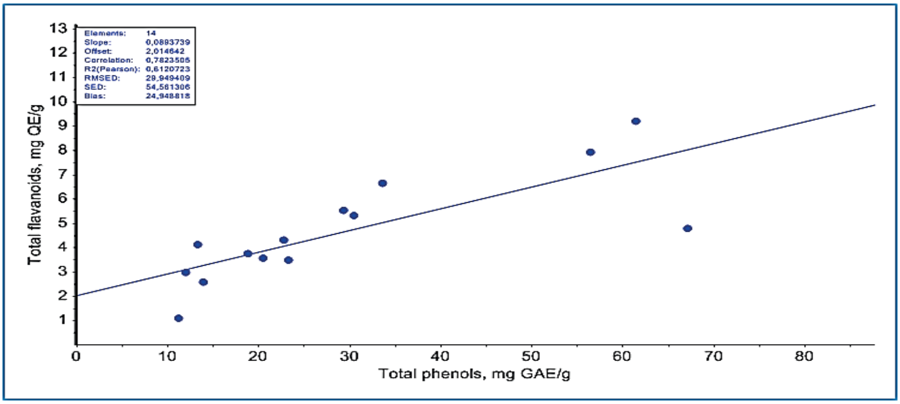

Figure 3. Scatter plots of correlation between total phenols and total flavonoids

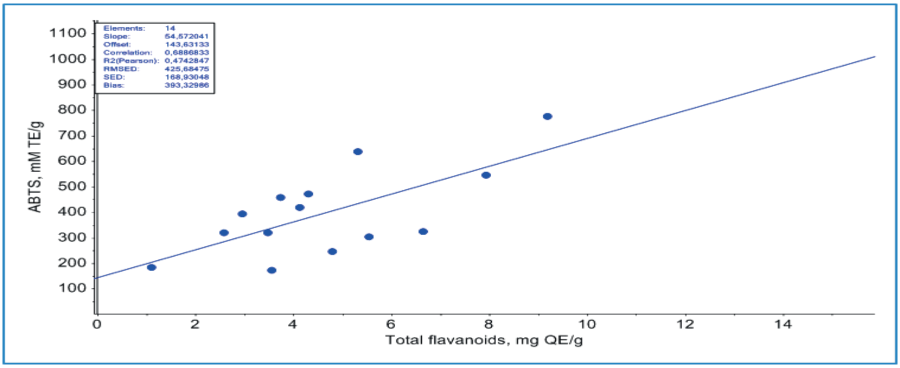

Figure 4. Scatter plots of correlation between total flavonoids and ABTS assay

CONCLUSIONS

In conclusion, the current results demonstrated that antioxidant properties and phenolic compounds of oil-bearing rose petals grown at conventional and organic agriculture systems. A significant variation was found in antioxidant activity and phenolic compounds between two agriculture practices. The radical-scavenging activity in rose petals was mostly due to the high levels of total flavonoids, followed by total phenols. Although the amount of these compounds varied between samples, organically grown samples exhibited high levels of radical scavenging activity. The metal-reducing assays showed higher antioxidant potentials in the extracts from conventionally grown roses. The results demonstrated the potential application of these rose water extracts as antioxidative ingredients or additives for food and cosmetic purposes.

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