

Antioxidant Capacity of Petals and Leaves from Different Rose (*Rosa damascena* Mill.) Plantations in Bulgaria

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ABSTRACT

Antioxidant capacity of *Rosa damascena* petals and leaves was studied using samples from seven industrial-scale plantations (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo) in Bulgaria, representing different climatic conditions, during two consecutive growing seasons (2009 and 2010). In addition to the free radical-scavenging ability, the contents of total polyphenols and anthocyanins (in rose petals) were determined. While higher total polyphenolic contents of rose petals were observed in the year 2009 for all plantations, the total anthocyanin concentrations were significantly smaller (except for Mirkovo sample). The antioxidant capacity correlated well, $R = 0.78-0.88$ and $R = 0.97$, with the contents of total polyphenols in rose petals and leaves, respectively. Interestingly, significant correlations between both the radical-scavenging ability ($R = 0.72$) and total polyphenolic content ($R = 0.78$) and rose plantation altitude were observed for the leaf samples. The results obtained show that environmental stresses within the growing regions have significant effects on the polyphenolic content and antioxidant capacity of *Rosa damascena* petals and leaves. This may affect their application as a rich source of polyphenols that might be used as health-promoting ingredients of functional foods or as natural antioxidants, preventing lipid oxidation in food systems.

Key words: *Rosa damascena*, Total polyphenols, DPPH

INTRODUCTION

Rosa damascena Mill. belongs to the family *Rosaceae* and genus *Rosa*, which includes 200 different species and more than 18 000 cultivars¹. *R. damascena* is an important essential oil crop, with Bulgaria and Turkey being the main rose petal processing countries in the world. Rose oil is a highly prized product used in perfumery, cosmetics, food industry and pharmacy^{2, 3}. Growing of *R. damascena* plants under different climatic conditions has been found to affect significantly the rose oil composition⁴.

Plant-derived foods contain a broad spectrum of secondary plant metabolites such as polyphenols that inhibit human low density lipoprotein oxidation, thus are made responsible for the beneficial effects on human health⁵. Recently, industrially distilled (de-aromatized) rose petals were suggested as a rich source of polyphenols, particularly flavonols⁶, which have been demonstrated to exert antioxidant properties both *in vitro*⁷ and *in vivo*⁸. Different rose flower species have been evaluated as caffeine-free sources for preparing rose petal tea, with *R. damascena* being among those exhibiting the highest antioxidant activity⁹. In addition to their antioxidant potential, extracts from rose petals have been reported to display antibacterial activity¹⁰. There are only few studies connected with the polyphenol composition in *Rosa* sp. leaves and their antioxidant properties^{11,12,13}.

The aim of the present study was to evaluate variations in the antioxidant capacity of petals and leaves collected from *R. damascena* plants growing under different climatic conditions. In addition to the radical-scavenging ability, the contents of total polyphenols and anthocyanins (in rose petals) were determined.

MATERIAL AND METHODS

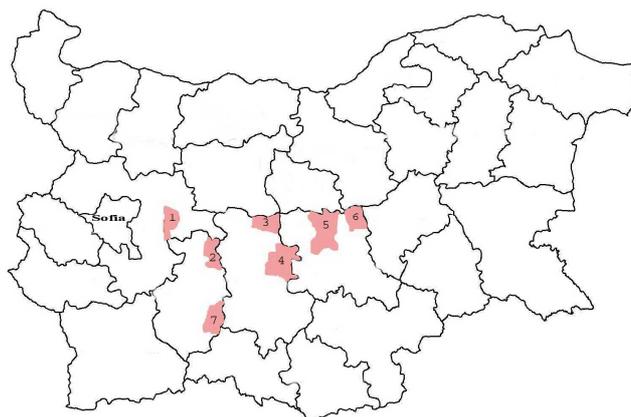
Chemicals

Reagents used for analytical purposes were as follows: DPPH [2,2-diphenyl-1-picrylhydrazyl] and Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid] (Sigma-Aldrich, Steinheim, Germany); Folin-Ciocalteu's reagent (Merck, Darmstadt, Germany); gallic acid monohydrate (Fluka, Buchs, Switzerland). All other reagents and solvents used were of analytical grade.

Plant material

Rosa damascena leaves and petals were collected from the pre-selected sampling sites in seven different plantations (Fig.1) located at the base of several mountains –Stara Planina, Sredna Gora and Rodopa. The samples were tearing off early morning during the flowering season (May/June). The plant materials were dried at room temperature in the laboratory of AgroBioInstitute – Sofia, Bulgaria.

Fig. 1. Locations of the sampling plantations in Bulgaria.



Sample number/ Plantation	Latitude (N)	Longitude (E)	Altitude(m)
1. Mirkovo	42° 42' 0"	23° 58' 58.8"	715
2. Strelcha	42° 30' 11.75"	24° 19' 18.82"	490
3. Moskovets	42° 39' 0"	24° 40' 58.8"	380
4. Zelenikovo	42° 24' 0"	25° 4' 58.8"	310
5. Kazanlak	42° 37' 1.2"	25° 24' 0"	407
6. Gurkovo	42° 40' 1.2"	25° 43' 58.8"	350
7. Bratsigovo	42° 1' 1.2"	24° 22' 1.2"	599

Sample preparation

0.5 g of the finely milled (particle size <0.4 mm) material were transferred into 50 ml volumetric flask using 40 ml of acidified (0.1% HCl) or non-acidified methanol for the rose petals and leaves, respectively. After extraction for 24 h at 10 °C, the flask was filled up to the mark with the corresponding solvent and filtered through a paper filter. Extraction was performed in duplicate.

Chemical analyses

Total polyphenols were assessed according to the Folin-Ciocalteu's reagent procedure¹⁴. Briefly, appropriately diluted extract (0.1 mL) was mixed with 0.5 mL of Folin-Ciocalteu reagent (diluted with distilled water 1:4, v/v) and 1.5 mL of sodium carbonate solution (7.5%, w/v) and the final volume was adjusted to 10 mL with distilled water. The mixture was incubated for 120 min at room temperature before absorption was measured at 750 nm. Total polyphenolic content was expressed as gallic acid equivalents (GAE) in grams per 100 g on a dry weight basis (dwb).

Total anthocyanin content was determined by the pH-differential method¹⁵. The extract was diluted both in buffer pH 1.0 (0.025 potassium chloride) and buffer pH 4.5 (0.4 M sodium acetate). After 30 min of incubation at room temperature, absorption was measured at 520 and 700 nm. Results were calculated using molar absorptivity of 26900 L/(mol cm) and molecular weight of 449.2 g/mol¹⁶ and expressed as cyanidin 3-glucoside equivalents (CGE) in milligrams per 100 g dwb.

Antioxidant capacity was evaluated using the DPPH free radical method¹⁷ modified as follows: 2.25 mL of DPPH methanolic solution (6×10^{-5} M) were mixed with 0.25 mL of extract (diluted with distilled water 1:3, v/v); absorption at 515 nm was measured after 15 min of reaction in cap-sealed cuvettes kept at room temperature. Results were expressed as Trolox, a water-soluble vitamin E analogue, equivalents (TE) in micromoles per 1 g dwb.

All measurements were performed with a Helios Omega UV-Vis spectrophotometer, equipped with a VISIONlite (Version 2.1) software (all from Thermo Fisher Scientific, Madison, WI, USA), using 1 cm path length cuvettes.

Statistical analysis

The results reported in the present study are the mean values of at least three determinations and the coefficients of variation, expressed as the percentage ratio between the standard deviations and the mean values, were found to be < 5% in all cases. Linear regression analysis was performed using the statistical package from Microsoft Excel.

RESULTS AND DISCUSSION

In order to evaluate *Rosa damascena* petals as a potential source of natural antioxidants, samples from seven industrial-scale plantations (Kazanlak, Zelenikovo, Moskovets, Bratsigovo, Strelcha, Mirkovo and Gurkovo) were collected during two consecutive growing seasons (2009 and 2010). While higher total polyphenolic contents in the year 2009 were observed for all plantations (Table I), the total anthocyanin concentrations were significantly smaller (except for Mirkovo sample). These results suggest that the contents of polyphenolic antioxidants in rose petals are strongly influenced by environmental, e.g. geographical and edaphic, factors. The latter assumption is especially plausible for the anthocyanins taking into account their functions in plants¹⁸, particularly to act as osmotic adjusters during periods of drought and low temperatures.

Table I. Total polyphenolic (TPP) and anthocyanin (TMA) contents and radical-scavenging ability (DPPH) values of rose petals depending of the growing season.

Sample number/ Plantation	TPP (g GAE/100 g dwb)			TMA (mg CGE/100 g dwb)			DPPH (μ mol TE/g dwb)		
	2009	2010	Mean	2009	2010	Mean	2009	2010	Mean
1. Mirkovo	11.80	6.03	8.92	321.7	256.3	289.0	1193.2	391.2	792.2
2. Strelcha	11.30	7.44	9.37	141.6	220.3	181.0	985.3	476.3	730.8
3. Moskovets	10.60	7.04	8.82	141.6	216.4	179.0	850.0	518.2	684.1
4. Zelenikovo	9.60	6.66	8.13	105.5	274.5	190.0	791.1	465.1	628.1
5. Kazanlak	8.46	6.76	7.61	-	-	-	657.8	415.5	536.7
6. Gurkovo	11.80	7.50	9.65	287.9	340.4	314.2	878.4	513.1	695.8
7. Bratsigovo	11.20	5.85	8.53	179.6	230.3	205.0	960.9	365.8	663.4

In this study the antioxidant capacity was assessed by the DPPH free radical-scavenging method, which is simple, rapid and sensitive¹⁹. Rose petals from Mirkovo and Moskovets showed the highest antioxidant capacity in the year 2009 and 2010, respectively. Interestingly, despite different total polyphenolic and anthocyanin contents of rose petals from Moskovets and Gurkovo, similar antioxidant capacity values were observed. This can be explained by the fact that the radical-scavenging ability depends not only on the concentration of polyphenolic antioxidants, but also on their chemical structure²⁰. Moreover, the existence of synergistic and antagonistic interactions between flavonoids may be taken into consideration²¹.

In accordance with Vinokur et al.²², the antioxidant capacity of rose petals correlated well ($R = 0.78-0.88$) with the contents of total polyphenols (Table II). However, the correlation coefficient for each growing season was higher than that obtained between the mean values, thus demonstrating the importance of the environmental conditions. In general, weaker correlations were found for the total anthocyanins, which may be attributed to their relatively low contents.

Total polyphenolic contents of *Rosa damascena* leaves (Fig. 2) were in the same range that has been previously reported for seventeen rose species²³. In line with the latter study, the antioxidant capacity of leaf samples correlated strongly ($R = 0.97$) with the content of total polyphenols. Interestingly, significant correlations between both the radical-scavenging ability ($R = 0.72$) and total polyphenolic content ($R = 0.78$) and rose plantation altitude were observed (Fig. 3), which underlines the importance of the geographical gradients. This assumption is supported by results from a recent study on bilberry (*Vaccinium myrtillus* L.) leaves, where higher antioxidant capacities and concentrations of soluble phenolics and flavonols have been found at higher latitudes and altitudes²⁴.

Table II. Correlation coefficients (R) between radical-scavenging ability values and contents of antioxidant compounds of rose petals depending on the growing season.

Compound	R		
	2009	2010	Mean
Total polyphenols ($N \geq 21$)	0.83	0.88	0.78
Total anthocyanins ($N \geq 18$)	0.67	0.28	0.48

Fig. 2. Relationship between total polyphenolic (TPP) contents and radical-scavenging ability (RSA) values of rose leaves. Sample numbers as in Fig. 1.

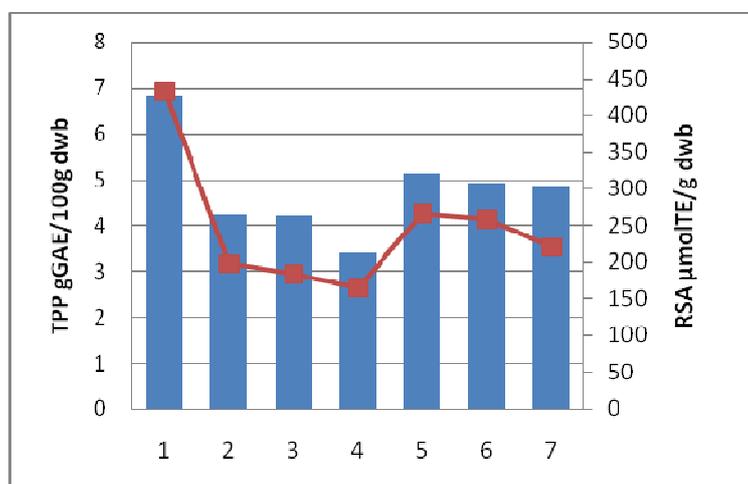
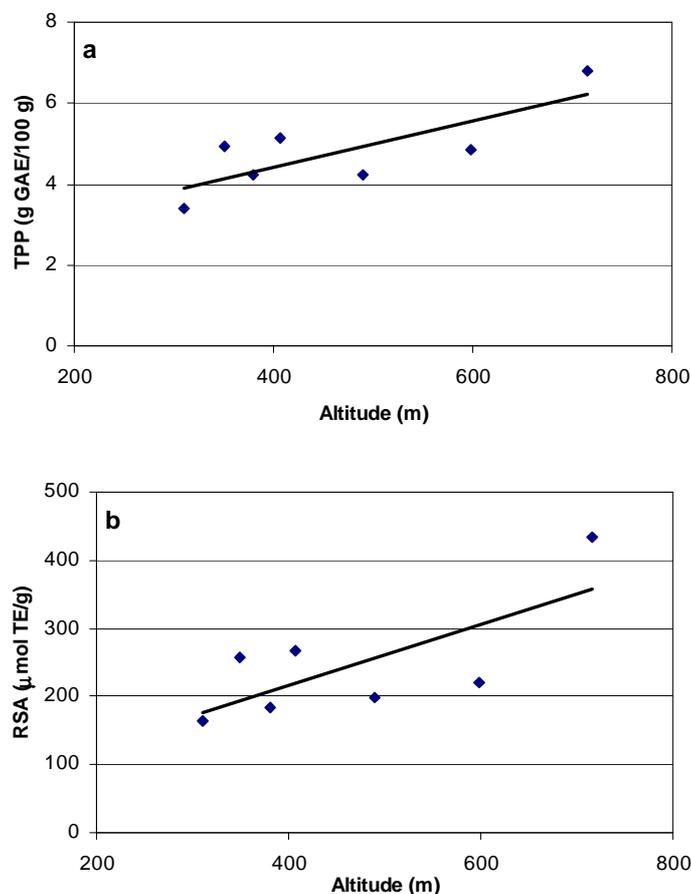


Fig. 3. Effect of altitude on the total polyphenolic (TPP) contents (a) and radical-scavenging ability (RSA) values (b) of rose leaves.



CONCLUSION

In conclusion, the results obtained show that environmental stresses within the growing regions have significant effects on the polyphenolic content and antioxidant capacity of *Rosa damascena* petals and leaves. This may affect their application as a rich source of polyphenols that might be used as health-promoting ingredients of functional foods²⁵ or as natural antioxidants²⁶, preventing lipid oxidation in food systems.

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