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PLANT PROTECTION - Article

Local genotypes of dog rose from Interior Aegean region of Turkey as a unique source of pro-health compounds

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ABSTRACT: Rosehip, *Rosa canina* L. fruit, is valued for its flavor, taste, color and aroma, in accordance with its recognition as one of richest sources of pro-health compounds. Screening, preservation and propagation of the most valuable local populations of rosehip are performed for food, pharmacological, and cosmetic applications. Eleven native *R. canina* genotypes from the Interior Aegean region, Turkey, were collected and analyzed regarding organic acids, phenolic compounds, sugars, and DPPH scavenging activity within this study. Regarding biochemical profile of fruits, protocatechuic acid and quercitrin were the most dominant compounds among 12 identified phenolics. The dominant organic acids were malic and citric and fructose and glucose were the dominant sugars. There was no correlation between DPPH scavenging activity and the analyzed chemicals in fruits. Although levels of certain compounds varied significantly between consecutive years, the ranking of genotypes according to the levels of particular chemicals was maintained. Generally, the most promising chemotype regarding biological value was 64US03. The chemical composition and the presence of bioactive compounds make the native to Interior Aegean *R. canina* genotypes a valuable source of bioactive agents preventing oxidative-stress related diseases.

Key words: *Rosa canina* L., germplasm, phytonutrients, biodiversity preservation.

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INTRODUCTION

R. canina is the most widespread species with the broad geographic range of all dog roses (*Rosa* sect. *Caninae*). This species is resistant to environmental stress factors as low soil fertility or a harsh climate, and it readily colonizes wild, edge habitats, fallow pastures, or wastelands. Due to this feature, dog rose grows in valleys, as well as high altitude plateaus, even above 1,500 m altitude (Jürgens et al. 2007). Dog rose is a perennial deciduous shrub, ranging from 1 to 4 m in height, sometimes climbing, with pink or white flowers and leaves composed of 5 or 7 leaflets (Fig. 1). The root system is shallow but laterally spread, providing the plant with excellent adaptability to poor locations (Ercisli 2005; Uzun and Bayir 2009). Dog rose's pseudo-fruits (rosehips), often named "fruits" in the literature, consist of several hairy achenes (30 to 40% of fruit fresh weight) enclosed by a red and fleshy floral cup, the urceolus (60 to 70% of fruit fresh weight) (Jagodzinski et al. 2016).

R. canina has been valued by people since ancient times. In Turkish and European folk medicine, the roots, leaves, branches, and fruits were used as herbal cures against a broad range of sicknesses, including infections and inflammatory illness. Rosehips are soft and delicious during a relatively short session of time. Therefore, local people eat just minor amount of fruits freshly. Drying has been the standard way of preservation, and it is being consumed as herbal tea, which is the most popular way of consumption, with its sweet flavor and high vitamin C content (Tumbas et al. 2012). Fresh or dried rosehips are also used as supplements enriching taste and biological quality of fruit wines, jams, teas, various beverages and, recently, as a component of probiotic drinks, yoghurts and soups (Nadpal et al. 2016). *R. canina* fruits provide the raw material for modern food and pharmaceutical industry as it is a valid source of antioxidant compounds such as ascorbate, β-carotene, glutathione, α-tocopherol, anthocyanins and other phenolics (Tumbas et al. 2012). Antioxidant nutrients of *R. canina* fruits play a significant role in the protection of humans from tissue-damaging effects of reactive oxygen- and nitrogen species, associated with inflammation. It was understood in clinical trials that rose-hip powder reduces symptoms of rheumatoid inflammation and the extract of this powder has anti-inflammatory antimicrobial, diuretic, and anti-allergic activity (Werlemark and Nybom 2010; Oyedemi et al. 2016).

The collection, investigation and preservation of regional plant landraces are crucial prerequisites for maintenance of biodiversity and counteracting the negative impact on bio-resources of human management. Turkey is considered one of the most important centers of *Rosa* spp. biodiversity as well as rosehip production (Ercisli 2005; Jürgens et al. 2007). Recent studies showed considerable variability in the phytochemical composition among *R. canina* genotypes, which offers the prospects for identification of superior chemotypes (Verma et al. 2013; Winther et al. 2016). Roman et al. (2013) demonstrated high diversity of L-ascorbic acid content, flavonoids and total antioxidant activity in the hips of roses from the sect. *Caninae*. The lipid fraction of the rosehip seed contains more than 50% polyunsaturated fatty acids. The major fatty acids in the rosehip are palmitic, stearic, oleic, linoleic, linolenic, and arachidic acids (Fofana et al. 2013). According to Ouerghemmi et al. (2016), a future exploration involving a significant number of *R. canina* accessions collected from different regions can highlight the chemotype markers of this species. Moreover, the significant genetic differentiation within *R. canina* populations at the continental scale suggests lower gene flow with increasing geographical distance. Regional genotypes are useful for →

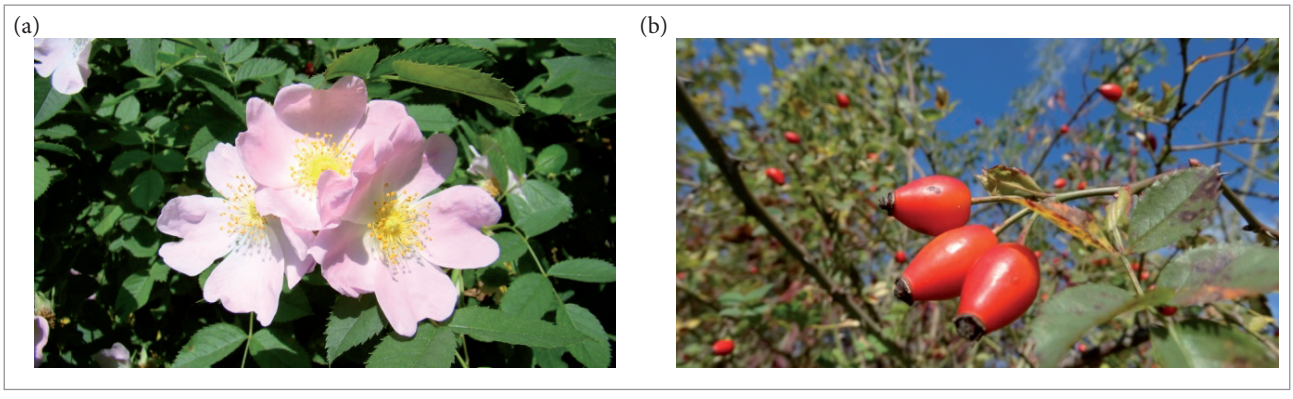

Figure 1. (a) Flowers and (b) ripe pseudo fruits of *R. canina*, so called rosehips.

propagation and breeding purposes in their original areas to preserve local biodiversity (Jürgens et al. 2007; Usanmaz et al. 2018).

Due to the above suggestions, the present work highlights differentiation of fruits' antioxidant potential and phytochemical composition of eleven *R. canina* genotypes collected from Uşak province of Interior Aegean region, Turkey. We hypothesized that *R. canina* of Turkish origin presents great diversity and can be used in breeding programs in order to increase nutrient value as a raw pharmacological material and food resource additive.

MATERIALS AND METHODS

Plant material

Mature fruits of eleven *R. canina* genotypes were selected as the material for the study performed in 2015 and 2016. Fruits were collected from genotypes tagged in the Uşak district of Interior Aegean, Turkey, and named as 64US('Uşak')01, 64US02, 64US03... 64US11. This area is located at 38°40' Lat and 29°23' Long. Taxonomically verified individuals of each genotype, naturally grown in Interior Aegean region, were in similar age and were collected from spatially separated but similar habitats. Collection took place away from settlements, gardens or hedges with *Rosa* spp. locations of non-regional or undetermined provenance more than 300 m, to exclude gene flow from other species or provenances (Jürgens et al. 2007; Jagodzinski et al. 2016). Taking into consideration high heterozygositic and low sexual reproduction status of the species (Gudin 2001), five to seven individuals per genotype were chosen as experimental material for present analysis of the fruits, for future vegetative propagation and maintenance of the genotypes. Fruits were collected from the same plants in successive experimental years. Samples consisted of 500-1000 g of mature fruits (bright red color) and were homogenous in shape, size and color. Samples were stocked in plastic bags at –18 °C for analyses performed with three replications for each genotype. Samples were analyzed in the Central Laboratory, Uşak University, Turkey.

Standards and reagents

Phenolics and organic acids analytical standards of Sigma-Aldrich Company (St. Louis, MO, USA) were used in the analyses. The remaining chemicals were derived from Merck Company (Darmstadt, Germany).

Analysis of phenolic profile

Phenolic compounds (protocatechuic acid, catechin, chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapinic acid, o-coumaric acid hesperidin, eriodictiol, quercetin, and apigenin) were detected in rosehips with the modified method of Rodriguez-Delgado et al. (2001). The plant material was mixed with pure water in equal proportions. The prepared mixture was centrifuged for 15 min at 15,000 rpm. The phenolic acids' determination was carried out with an Agilent 1260 HPLC system equipped with in-line degasser (G 1322A), quat pump (G 1311A), autosampler (G 1313A), column heater (G 1316A) and UV detector (G 1315A). The Agilent HPL ChemStation 10.1 release for the instrument control and data analysis was performed with Microsoft Windows 2000.

Analysis of organic acids

The organic acids (oxalic, citric, tartaric, malic, succinic acid) contents in rosehips were analyzed with the method of Bevilacqua and Califano (1989). The fruits were shattered in a tulle fabric and homogenized, the samples were stored at –18 °C until analysis. Five ml of homogenate were mixed with 20 ml of 0.009 N H_2SO_4 in a silent crusher M (Heidolph, Germany), then homogenized for one hour with a shaker (Heidolph Unimax 1010, Germany). The mixtures were centrifuged for 20 min at 12,000 rpm, the supernatants were filtered through a sieve of 0.45-micron skin, a thick fixation device (ISOLAB, Turkey) and passed through a SEP-PAK C18 cartridge. Measurements of organic acids were carried out with Agilent 1260 HPLC (Agilent, Santa Clara, CA, United States) using a degassing line (G 1322A), quat pump (G 1311A), autosampler (G 1313 A), heating column (G 1316 A) and UV detector (1315A) in wavelenghts of 214 and 280 nm, controlled by the Agilent package program. The plant material derived according to the procedure described above was used to L-ascorbic acid measurement. The samples were combined by centrifugation and oxalic acid 400 **µl** (0.4%) and 4.5 ml 2,6-diclorofenolindofenol solution was added to the supernatant. Data on L-ascorbic acid content were read at the wavelength of 520 nm →

against the blank with the use of a spectrophotometer (Shimadzu, Tokyo, Japan) (Cemeroğlu 2007).

Analysis of individual sugars

The samples were prepared according to the procedure described by Melgarejo et al. (2000) with some modifications used for fructose, glucose and sucrose analyses. Fifteen g of fruit homogenate were centrifuged (2 min, 4 °C, 12,000 rpm). Then the SEP-PAK C18 cartridge was used for supernatant filtration and samples were carried into a flask for measurement. Sugars were determined by HPLC (isocratic program) with μBondapak-NH_2column and refractive index (RI) detector with 85% acetonitrile as a mobile phase.

Analysis of DPPH scavenging activity

DPPH scavenging activity was determined with the use of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with the method described by Dorman et al. (2003). The DPPH solution was prepared before analysis. Then, 1 ml of 10^{-4} M DPPH in methanol solution was transferred to aluminum foil coated glass tubes. Three ml of following sample solutions in methanol at different concentrations (0, 3, 1.25, 6.25, 12.5, 25, 50, 100, 200, and 400 $\mu g \cdot ml^{-1}$) were added to DPPH solution. Instead of the sample solution, 3 ml of pure methanol was added to the control tube as a blank. Samples were stored under darkness at room temperature for 30 min; then the absorbance was measured at 517 nm against methanol. Ascorbic acid and trolox were used as analytical standards (Somparn et al. 2007). The values were expressed as SC50, the concentration of the sample ($\mu g \cdot ml^{-1}$) that causes 50% scavenging of DPPH radical.

Statistical data evaluation

The experimental data were statistically analyzed using the STATISTICA 12.0 (StatSoftInc USA) software. Data were analyzed using two-way analysis of variance (ANOVA), differences between means were separated using Tukey's HSD test with $p < 0.05$. The results were expressed as mean values for two years ± standard deviation (SD). Cluster analysis was performed with Euclidean distance, variables were not standardized. *R. canina* genotypes were clustered according to the values of investigated biochemical parameters. Correlation analyses for the relationships between total antioxidant activity and all analyzed biochemical compounds were performed for investigated genotypes (n = 11). Linear coefficients of correlation (r) values were calculated and assessed at $p < 0.05$.

RESULTS AND DISCUSSIONS

Phenolic profile

The major bioactive compounds providing health benefits of *R. canina* fruit hips are phenolics (Roman et al. 2013). Their free radical scavenging ability is facilitated by hydroxyl groups. The composition of phenolics is light yellow pigment and their synthesis is highly depended on climatic conditions (Cemeroglu et al. 2007). In this study, 12 major phenolic components were identified and collated in Tables 1 and 2. Phenolic profile of dog rose fruits was significantly affected by interaction of genotype and year of rosehips collection. Protocatechuic acid dominated in all *R. canina* genotypes, while *p*-coumaric and *o*-coumaric acids were determined in minimal amounts. According to Ouerghemmi et al. (2016), *R. canina* leaf extracts demonstrated a preference for kaempferol and its derivatives. The present study demonstrated that rosehip fruits constitute a good source of catechin and its derivatives. Nadpal et al. (2016) determined five phenolic acids (protocatechuic, p-cumaric, p-hydroxybenzoic, vanillic, and gallic) and seven flavonoids (kaempferol-3-*O*-glucoside, quercitrin, quercitrin-3-*O*-glucoside, hyperoside, epicatechin, catechin, and quinic acid) in rosehips. The analysis of *R. canina* phenolic profile in the present study indicated that genotypes naturally grown in Uşak region were significantly differentiated regarding all analyzed compounds. The maximum content of protocatechuic acid and catechin was measured in genotype 64US03 (cited mean values for 2015 and 2016 are 784.40 and 417.0 $\mu g \cdot g^{-1}$, respectively), while the lowest values were detected in genotypes 64US06 and 64US09. In both experimental years the highest content of chlorogenic acid was noted for 64US07 (with a mean value of 8.12 $\mu g \cdot g^{-1}$); caffeic acid and ferulic acids for 64US08 (mean values of 5.23 and 5.72 $\mu g \cdot g^{-1}$, respectively), while *p*-coumaric acid was detected for 64US10 (with a mean value of 0.140 $\mu g \cdot g^{-1}$). Chlorogenic acid was not detected in fruits of 64US02, 64US05 and 64US11 genotypes. In addition, no *p*-coumaric acid was found in 64US01 genotype, while *o*-coumaric acid, eriodictyol, quercetin and apigenin were not detected in 64US01, 64US06, 64US11, and 64US06 genotypes →

Table 1. Phenolic compounds (μg·g^{-1} FW) in *R. canina* fruits in 2015 and 2016.

Genotypes	Protocatechuic acid	Catechin	Chlorogenic acid	Caffeic acid	p-coumaric acid	Ferulic acid
			2015			
64US01	532.5 ± 1.92 g*	340.0 ± 1.22 d	6.92 ± 0.02 f	4.25 ± 0.02 g	ND	4.26 ± 0.02 e
64US02	346.7 ± 1.25 o	190.6 ± 0.69 r	ND	3.58 ± 0.02 nm	0.038 ± 0.00 j	3.73 ± 0.01 i
64US03	753.0 ± 2.71 b	400.3 ± 1.44 b	3.78 ± 0.01 m	3.66 ± 0.01 lm	0.038 ± 0.00 j	3.35 ± 0.01 m
64US04	362.8 ± 1.31 n	229.5 ± 0.82 m	5.33 ± 0.02 j	4.78 ± 0.02 d	0.038 ± 0.00 j	4.10 ± 0.01 f
64US05	313.6 ± 1.13 p	199.8 ± 0.72 p	ND	3.50 ± 0.01 n	0.048 ± 0.00 h	3.57 ± 0.01 kl
64US06	178.2 ± 0.64 s	87.5 ± 0.32 u	3.16 ± 0.01 o	3.70 ± 0.01 l	0.058 ± 0.00 f	1.95 ± 0.01 p
64US07	514.6 ± 1.85 h	273.1 ± 0.98 j	7.80 ± 0.03 c	4.08 ± 0.01 h	0.058 ± 0.00 f	3.70 ± 0.01 ij
64US08	454.8 ± 1.64 j	295.2 ± 1.06 g	7.66 ± 0.03 d	5.02 ± 0.02 c	0.106 ± 0.00 d	5.49 ± 0.02 b
64US09	99.9 ± 0.36 t	90.5 ± 033 tu	5.56 ± 0.02 i	3.13 ± 0.01 r	0.048 ± 0.00 h	3.73 ± 0.01 i
64US10	541.7 ± 1.95 f	258.4 ± 0.93 k	4.94 ± 0.02 k	2.73 ± 0.01 t	0.134 ± 0.00 b	3.26 ± 0.01 n
64US11	384.0 ± 1.38 m	289.1 ± 1.04 h	ND	3.00 ± 0.01 s	0.038 ± 0.00 j	3.30 ± 0.01 mn
Mean	407.4 ± 30.5 B	241.3 ± 16.2 B	5.64 ± 0.34B	3.77 ± 0.12B	0.060 ± 0.01 B	3.68 ± 0.14 B
			2016			
64US01	576.8 ± 2.08d	368.3 ± 1.33c	7.50 ± 0.03 e	4.61 ± 0.02 e	ND	4.624 ± 0.02 c
64US02	375.5 ± 1.35 m	206.4 ± 0.74 o	ND	3.88 ± 0.01 j	0.042 ± 0.00 i	4.05 ± 0.01 fg
64US03	815.8 ± 2.94 a	433.7 ± 1.56 a	4.09 ± 0.01 l	3.96 ± 0.11 i	0.042 ± 0.00 i	3.63 ± 0.01 jk
64US04	393.1 ± 1.42 l	248.6 ± 0.90 l	5.77 ± 0.02 h	5.18 ± 0.00 b	0.042 ± 0.00 i	4.44 ± 0.01 d
64US05	339.7 ± 1.22 o	216.5 ± 0.78 n	ND	3.80 ± 0.01 k	0.052 ± 0.00 g	3.87 ± 0.01 h
64US06	193.1 ± 0.70 r	94.8 ± 0.34 st	3.42 ± 0.01 n	4.00 ± 0.01 hi	0.062 ± 0.00 e	2.11 ± 0.01 o
64US07	557.5 ± 2.01 e	295.8 ± 1.07 g	8.45 ± 0.03 a	4.42 ± 0.02 f	0.062 ± 0.00 e	4.00 ± 0.01 g
64US08	492.6 ± 1.77 i	319.8 ± 1.15 e	8.30 ± 0.03 b	5.44 ± 0.02 a	0.114 ± 0.00 c	5.95 ± 0.02 a
64US09	108.2 ± 0.39 t	98.0 ± 0.35 s	6.02 ± 0.02 g	3.39 ± 0.01 o	0.048 ± 0.00 h	4.05 ± 0.01 fg
64US10	586.9 ± 2.11 c	280.0 ± 1.01 i	5.36 ± 0.02 j	2.95 ± 0.01 s	0.146 ± 0.00 a	3.54 ± 0.01 l
64US11	415.9 ± 1.50 k	313.2 ± 1.29 f	ND	3.25 ± 0.01 p	0.042 ± 0.00 i	3.58 ± 0.01 kl
Mean	441.4 ± 33.1 A	261.4 ± 17.5 A	6.11 ± 0.36 A	4.08 ± 0.13 A	0.065 ± 0.01 A	3.99 ± 0.16 A

*Means of three replicates ± SD; data were subjected to two-way ANOVA; means within a column followed by different letters (capital letters for main effects and lowercase letters for interaction effects) are significantly different at $p \leq 0.05$ according to Tukey's HSD test; ND = not detected.

(Table 2). In both experimental years the highest contents of sinapinic acid, eriodictyol and quercetin were noted for 64US01 with mean values of 6.52, 13.5, and 3.76 μg·g^{-1}, respectively. Genotype 64US03 contained the highest amount of *o*-coumaric acid (1.63 μg·g^{-1}, on average), hesperidin, while 64US04 was characterized by the highest content of apigenin (2.67 μg·g^{-1}). Demir et al. (2014) determined that the gallic acid, catechin, chlorogenic, *p*-coumaric acid, ferulic acid and sinapic acid contents in *R. canina* fruits varied significantly among the genotypes. Our findings are familiar with results retrived by Nowak (2005), measuring caffeic acid with a range of 2.1 mg·100 g^{-1} in *R. coriifolia* and 8.3 mg·100 g^{-1} in *R. rugosa*. Öztürk et al. (2007) reported the acidic contents for *R. canina* that protocatechuic acid, vanillic acid, catechin, chlorogenic acid, *p*-coumaric acid and ferulic acid contents in rosehips were 1.4, 6.9, 3.1, 8.5, 24.9 and 23.9 mg·100 g^{-1}, respectively. Similar ranges of phenolic acids and other phenolic compounds in *R. canina* genotypes were determined by Nowak (2005) and Fecka (2009). Investigations performed by Winther et al. (2016) showed that the sum of flavonoids was a little higher in rosehip shells in comparison with seeds, while the content of rutin was 3-times higher in seeds, but levels of rutin glycoside, quercitrin, in seeds and shells were similar. Environmental factors, as well as maturity stage of fruits, may affect the synthesis and conversions of phenolics, so the variation of flavonoids in *R. canina* fruits is of great importance for chemotaxonomy of this species (Nadpal et al. 2016). Analysis of phenolic profile during consecutive →

Table 2. Phenolic compounds (μg·g^{-1} FW) in *R. canina* fruits in 2015 and 2016.

Genotype	Sinapinic acid	O-coumaric acid	Hesperidin	Eriodictyol	Quercetin	Apigenin
			2015			
64US01	6.65 ± 0.04 a*	ND	9.99 ± 0.06 i	13.77 ± 0.07 a	3.83 ± 0.02 a	2.15 ± 0.01 d
64US02	3.86 ± 0.02 h	0.58 ± 0.00 g	11.52 ± 0.06 f	6.65 ± 0.04 m	1.57 ± 0.01 m	1.77 ± 0.01 ij
64US03	5.05 ± 0.03 e	1.66 ± 0.01 a	15.03 ± 0.08 a	11.55 ± 0.16 d	2.47 ± 0.00 f	2.12 ± 0.01 de
64US04	5.74 ± 0.03 c	0.60 ± 0.00 f	10.57 ± 0.06 h	7.29 ± 0.04 k	1.75 ± 0.01 jk	2.73 ± 0.01 a
64US05	2.69 ± 0.01 m	0.58 ± 0.00 g	7.67 ± 0.04 l	8.81 ± 0.05 i	2.92 ± 0.02 c	2.58 ± 0.01 b
64US06	1.77 ± 0.01 n	0.42 ± 0.00 l	3.75 ± 0.02 m	ND	1.49 ± 0.01 no	ND
64US07	0.48 ± 0.02 p	0.57 ± 0.00 h	15.05 ± 0.08 a	9.87 ± 0.05 f	1.80 ± 0.01 j	1.17 ± 0.01 n
64US08	3.41 ± 0.02 j	0.67 ± 0.00 cd	12.91 ± 0.03 c	9.74 ± 0.05 fg	2.02 ± 0.01 h	2.04 ± 0.01 f
64US09	3.32 ± 0.01 jk	0.55 ± 0.00 j	7.99 ± 0.04 k	2.56 ± 0.01 o	1.14 ± 0.00 p	1.82 ± 0.01 hi
64US10	1.41 ± 0.01 o	0.68 ± 0.00 c	11.91 ± 0.06 e	13.11 ± 0.06 b	2.84 ± 0.02 d	1.91 ± 0.01 g
64US11	4.81 ± 0.02 f	0.38 ± 0.00 m	10.98 ± 0.06 g	0.44 ± 0.00 p	ND	1.33 ± 0.01 m
Mean	3.56 ± 0.32 A	0.67 ± 0.06 A	10.67 ± 0.56 A	8.38 ± 0.76 A	2.18 ± 0.14 A	1.95 ± 0.09 A
			2016			
64US01	6.39 ± 0.04 b	ND	9.59 ± 0.05 j	13.23 ± 0.07 b	3.68 ± 0.02 b	2.07 ± 0.01 ef
64US02	3.70 ± 0.02 i	0.56 ± 0.00 i	11.06 ± 0.06 g	6.39 ± 0.03 n	1.51 ± 0.01 mn	1.71 ± 0.01 k
64US03	4.85 ± 0.03 f	1.60 ± 0.00 b	14.44 ± 0.01 b	11.09 ± 0.07 e	2.37 ± 0.06 g	2.04 ± 0.01 f
64US04	5.52 ± 0.03 d	0.58 ± 0.00 g	10.15 ± 0.05 i	7.01 ± 0.04 l	1.69 ± 0.01 l	2.63 ± 0.01 b
64US05	2.59 ± 0.01 m	0.56 ± 0.00 i	7.37 ± 0.04 l	8.05 ± 0.04 j	2.80 ± 0.01 d	2.48 ± 0.01 c
64US06	1.71 ± 0.01 n	0.40 ± 0.00 l	3.61 ± 0.02 m	ND	1.43 ± 0.01 o	ND
64US07	0.46 ± 0.00 p	0.55 ± 0.00 j	14.47 ± 0.08 b	9.49 ± 0.05 gh	1.72 ± 0.01 kl	1.13 ± 0.01 n
64US08	3.27 ± 0.02 kl	0.65 ± 0.00 e	12.41 ± 0.07 d	9.36 ± 0.05 h	1.94 ± 0.01 i	1.96 ± 0.01 g
64US09	3.19 ± 0.02 l	0.53 ± 0.00 k	7.67 ± 0.04 kl	2.46 ± 0.01 o	1.11 ± 0.01 p	1.74 ± 0.01 jk
64US10	1.35 ± 0.01 o	0.66 ± 0.00 de	11.45 ± 0.06 f	12.59 ± 0.07 c	2.72 ± 0.01 e	1.83 ± 0.01 h
64US11	4.63 ± 0.02 g	0.36 ± 0.00 m	12.38 ± 0.06 d	0.50 ± 0.00 p	ND	1.49 ± 0.01 l
Mean	3.42 ± 0.31 B	0.65 ± 0.06 B	10.42 ± 0.54 B	8.02 ± 0.73 B	2.10 ± 0.14 B	1.91 ± 0.08 B

*Means of three replicates ± SD; data were subjected to two-way ANOVA; means within a column followed by different letters (capital letters for main effects and lowercase letters for interaction effects) are significantly different at $p \leq 0.05$ according to Tukey's HSD test; ND = not detected.

growing seasons showed higher content of protocatechuic acid, catechin, chlorogenic acid, caffeic acid, *p*-coumaric acid and ferulic acid in rosehips collected in 2016, while sinapinic acid, *o*-coumaric acid, hesperidin, eriodictyol, quercetin and apigenin were determined in higher amounts in rosehips collected in 2015. Although the present study showed significant differences between experimental years concerning phenolic compounds concentration, the differences between genotypes were highly repeatable in following years. So, it could be concluded that genetic factor was of the major source of variation in genotypes.

Organic acids contents

Significant differences were also found among organic acid contents in rosehips of investigated genotypes collected in consecutive vegetation seasons (Table 3). The highest content of oxalic acid was determined in rosehips of 64US09 and 64US10 genotypes (mean values for consecutive years were 0.582 and 0.610 g·100 g^{-1}, respectively), while the highest level of citric acid was detected in fruits of 64US02, 64US08 and 64US09 genotypes (with mean values of 2.150, 2.150, and 2.080 g·100 g^{-1} for 2015 and 2016, respectively) and the lowest in 64US11 (with 1.540 g·100 g^{-1}). The highest amounts of tartaric and malic acids were determined in 64US03, while succinic acid in 64US01 and 64US04 in both investigation years. Adamczak et al. (2012) reported similar values, citric and ascorbic acid contents in rosehips were 3.16 and 1.06 g·100 g^{-1}, respectively. Özrenk et al. (2012) determined citric, oxalic, tartaric, malic, and succinic acids contents ranging between 1.56-3.15%, 0.32-0.62%, 0.073-0.155%, 0.76-4.39%, and 0.028-2.465%, respectively. The L-ascorbic acid content →

Table 3. Organic acids (g·100 g^{-1} FW) in *R. canina* fruits in 2015 and 2016.

Genotype	Oxalic acid	Citric acid	Tartaric acid	Malic acid	Succinic acid
			2015		
64US01	0.433 ± 0.002 gh*	1.864 ± 0.008 fg	0.124 ± 0.005 g	2.215 ± 0.010 e	2.276 ± 0.010 a
64US02	0.350 ± 0.001 n	2.214 ± 0.010 a	0.185 ± 0.001 c	2.101 ± 0.010 f	0.876 ± 0.004 i
64US03	0.608 ± 0.003 b	1.947 ± 0.008 e	0.216 ± 0.001 a	3.234 ± 0.014 a	0.762 ± 0.003 k
64US04	0.464 ± 0.002 f	1.977 ± 0.009 de	0.144 ± 0.001 e	3.111 ± 0.014 b	2.307 ± 0.010 a
64US05	0.412 ± 0.001 ij	2.081 ± 0.009 c	0.072 ± 0.000 n	1.154 ± 0.005 lm	1.947 ± 0.008 e
64US06	0.422 ± 0.002 hi	1.792 ± 0.008 hi	0.144 ± 0.001 e	1.185 ± 0.001 l	0.041 ± 0.005 o
64US07	0.350 ± 0.002 n	1.895 ± 0.008 f	0.093 ± 0.000 j	1.906 ± 0.008 h	1.009 ± 0.004 g
64US08	0.494 ± 0.002 e	2.214 ± 0.010 a	0.082 ± 0.000 l	1.689 ± 0.007 j	0.422 ± 0002 m
64US09	0.600 ± 0.003 bc	2.142 ± 0.009 b	0.113 ± 0.000 h	1.936 ± 0.008 gh	1.957 ± 0.008 de
64US10	0.628 ± 0.003 a	1.669 ± 0.007 j	0.144 ± 0.001 e	1.195 ± 0.005 l	2.112 ± 0.009 c
64US11	0.371 ± 0.002 m	1.586 ± 0.007 k	0.134 ± 0.001 f	3.069 ± 0.013 bc	0.134 ± 0.001 n
Mean	0.467 ± 0.017 A	1.944 ± 0.035 A	0.132 ± 0.007 A	2.072 ± 0.131 A	1.258 ± 0.148 A
			2016		
64US01	0.407 ± 0.002 jk	1.756 ± 0.008 i	0.116 ± 0.001 h	2.086 ± 0.009 f	2.144 ± 0.009 bc
64US02	0.330 ± 0.001 o	2.086 ± 0.009 c	0.175 ± 0001 d	1.978 ± 0.009 g	0.825 ± 0.004 j
64US03	0.572 ± 0.002 d	1.833 ± 0008 gh	0.204 ± 0.001 b	3.046 ± 0.013 c	0.718 ± 0.003 l
64US04	0.437 ± 0.001 g	1.862 ± 0.008 fg	0.136 ± 0.001 f	2.929 ± 0.013 d	2.173 ± 0.001 b
64US05	0.388 ± 0.002 l	1.959 ± 0.008 e	0.068 ± 0.000 o	1.086 ± 0.005 n	1.833 ± 0.008 f
64US06	0.398 ± 0.002 kl	1.688 ± 0.007 j	0.136 ± 0.001 f	1.116 ± 0.005 mn	0.039 ± 0.000 o
64US07	0.330 ± 0.001 o	1.785 ± 0.008 i	0.087 ± 0.000 k	1.795 ± 0.008 i	0.951 ± 0.004 h
64US08	0.476 ± 0.002 f	2.086 ± 0.009 c	0.078 ± 0.000 m	1.591 ± 0.007 k	0.398 ± 0.002 m
64US09	0.563 ± 0.002 d	2.018 ± 0.009 d	0.107 ± 0.000 i	1.824 ± 0.007 i	1.843 ± 0.008 f
64US10	0.592 ± 0.002 c	1.571 ± 0.007 k	0.136 ± 0.001 f	1.125 ± 0.005 mn	1.989 ± 0.009 d
64US11	0.349 ± 0.001 n	1.494 ± 0.007 l	0.126 ± 0.000 g	2.891 ± 0.013 d	0.126 ± 0.001 n
Mean	0.440 ± 0.016 B	1.831 ± 0.033 B	0.124 ± 0.007 B	1.952 ± 0.123 B	1.185 ± 0.140 B

*Means of three replicates ± SD; data were subjected to two-way ANOVA; means within a column followed by different letters (capital letters for main effects and lowercase letters for interaction effects) are significantly different at $p \leq 0.05$ according to Tukey's HSD test; ND = not detected.

is one of the most important features in rosehip analyses. The investigations of Novajan et al. (2008) demonstrated that ascorbic acid had the highest stability in untreated dog rose fruits. Investigated genotypes of *R. canina* seemed to be an excellent source of L-ascorbic acid. In the present study, the highest content of L-ascorbic acid was detected in fruits of 64US03 genotype in both years of experiment (1754.7 mg·100 g^{-1}, mean value for 2015 and 2016) and the lowest in 64US01 genotype (324.1 mg 100·g^{-1}, mean value for 2015 and 2016). These results are in conformity with the relevant research data retrieved from other regions of Turkey (106 to 2712 mg·100 g^{-1}), Bulgaria (112 to 360 mg·100 g^{-1}) or Iran (211 to 417 mg·100 g^{-1}) (Novajan et al. 2008; Roman et al. 2013; Demir et al. 2014). Differences among genotypes in organic acids content may be due to local climate and soil conditions and altitude, as well as genetic factors (Barros et al. 2011; Roman et al. 2013). In conditions of the present experiment, significantly higher level of all organic acids was found in rosehips collected in first year of investigations. The results of this study confirmed the traditional use of rosehips as food rich in vitamin C content. Additionally the repeatable differences between genotypes in following years highlighted the significance of genetic factor regarding the accumulation of these bioactive compounds.

Sugars profile

Sugars, as well as organic acids, contribute in the taste and flavor of rosehips. Significant differences were determined →

among rosehips of investigated genotypes collected in consecutive years in terms of sugars content (Table 4). Analysis of main effects showed that rosehips collected in 2015 contained higher amounts of fructose, glucose and sucrose. In both experimental years the highest level of fructose was found in fruits of 64US03 genotype (15.28 g·100 g^{-1}, mean value for 2015 and 2016), while the lowest was (7.42 g·100 g^{-1}) in genotype 64US01. According to Barros et al. (2010), fructose was the most abundant sugar in dog rose fruits. In the present study, mean content of fructose (11.14 g·100 g^{-1}) was slightly higher than glucose (10.69 g·100 g^{-1}), but markedly higher than sucrose (0.39 g·100 g^{-1}). Sucrose was not detected in 64US01, 64US04, 64US05, 64US08 or 64US11 genotypes within the experimental period. The highest value for glucose was detected in rosehips of 64US05 genotype (13.87 g·100g^{-1}, mean value for 2015 and 2016) and the lowest was in 64US01 genotype (7.89 g 100·g^{-1}, mean value for 2015 and 2016). Özrenk et al. (2012) indicated that fructose, glucose, and sucrose contents in rosehips of *R. canina* grown wild in Erzincan region in Turkey ranged between 7.96-14.76%, 8.06-12.94% and 0.17-0.88%, respectively. Demir et al. (2014) reported that glucose and sucrose contents in *R. canina* fruits were 17.11 and 18.84 g·100 g^{-1}, respectively. Present results showed that it was possible to designate genotypes characterized by the highest values of these compounds regardless of the weather conditions in the following years. Moreover, the consumption of *R. canina* fruits could also be evaluated as a source of food due to their →

Table 4. Sugars (g·100 g^{-1} DW), vitamin C (mg·100 g^{-1} DW) and DPPH scavenging activity (SC50, µg·ml^{-1}) of *R. canina* fruits in 2015 and 2016.

Genotypes	Fructose	Glucose	Sucrose	Vitamin C	DPPH scavenging activity
			2015		
64US01	7.64 ± 0.03 o*	8.13 ± 0.04 l	ND	333.8 ± 1.46 t	101.1 ± 0.44 s
64US02	9.41 ± 0.04 k	11.49 ± 0.05 e	0.670 ± 0.00 a	667.7 ± 2.91 o	130.1 ± 0.57 m
64US03	15.74 ± 0.07 a	12.62 ± 0.05 c	0.422 ± 0.00 e	1807.3 ± 7.87 a	262.4 ± 1.44 a
64US04	12.51 ± 0.05 e	9.42 ± 0.04 j	ND	1242.6 ± 5.42 g	188.9 ± 0.82 g
64US05	11.47 ± 0.05 g	14.29 ± 0.06 a	ND	1368.5 ± 5.97 e	233.7 ± 1.02 e
64US06	9.52 ± 0.04 jk	11.89 ± 0.04 d	0.350 ± 0.00 g	955.2 ± 4.16 j	151.0 ± 0.66 k
64US07	13.95 ± 0.06 c	10.66 ± 0.05 gh	0.288 ± 0.00 i	877.9 ± 3.83 k	117.4 ± 0.51o
64US08	10.36 ± 0.05 i	13.51 ± 0.06 b	ND	1027.8 ± 4.48. i	111.7 ± 0.49 p
64US09	12.58 ± 0.05 e	8.66 ± 0.04 k	0.124 ± 0.00 k	1595.0 ± 6.95 c	138.7 ± 0.60 l
64US10	14.68 ± 0.06 b	10.50 ± 0.05 h	0.597 ± 0.00 c	531.7 ± 2.32 r	168.1 ± 0.73 i
64US11	8.45 ± 0.04 m	9.86 ± 0.04 i	ND	777.0 ± 3.87 m	257.2 ± 1.12 b
Mean	11.48 ± 0.45 A	11.00 ± 0.33A	0.409 ± 0.05 A	1017 ± 76.1 A	169.1 ± 9.89 A
			2016		
64US01	7.20 ± 0.03 p	7.65 ± 0.03 m	ND	316.4 ± 1.37 t	95.2 ± 0.41 t
64US02	8.87 ± 0.04 l	10.83 ± 0.05 g	0.398 ± 0.00 f	628.8 ± 2.74 p	122.6 ± 0.53 n
64US03	14.82 ± 0.06 b	11.89 ± 0.05 d	0.631 ± 0.00 b	1702.0 ± 7.42 b	247.2 ± 1.08 c
64US04	11.79 ± 0.05 f	8.88 ± 0.04 k	ND	1170.2 ± 5.10 h	177.9 ± 0.77 h
64US05	10.81 ± 0.05 h	13.46 ± 0.06 b	ND	1288.8 ± 5.62 f	220.1 ± 0.96 f
64US06	8.96 ± 0.04 l	11.19 ± 0.05 f	0.330 ± 0.00 h	899.5 ± 3.92 k	142.3 ± 0.62 l
64US07	13.13 ± 0.05 d	10.04 ± 0.04 i	0.272 ± 0.00 j	826.7 ± 3.60 l	110.6 ± 0.48 p
64US08	9.76 ± 0.04 j	12.73 ± 0.05 c	ND	967.9 ± 4.22 j	105.2 ± 0.46 r
64US09	11.84 ± 0.05 f	8.16 ± 0.04 l	0.116 ± 0.00k	1502.0 ± 6.54 d	130.7 ± 0.57 m
64US10	13.82 ± 0.06 c	9.88 ± 0.04 i	0.563 ± 0.00 d	500.7 ± 2.18 s	158.3 ± 0.69 j
64US11	7.95 ± 0.03 n	9.28 ± 0.04 j	ND	731.7 ± 3.19 n	242.2 ± 1.06 d
Mean	10.81 ± 0.42 B	10.36 ± 0.31 B	0.385 ± 0.04 B	958 ± 71.7 B	159.3 ± 9.3 B

*Means of three replicates ± SD; data were subjected to two-way ANOVA; means within a column followed by different letters (capital letters for main effects and lowercase letters for interaction effects) are significantly different at $p \leq 0.05$ according to Tukey's HSD test; ND = not detected.

elevated carbohydrate contents. This rosehip compensatory use of rosehips was also reported by Barros et al. (2010).

DPPH scavenging activity

Particular biochemical constituents of rosehip fruits develop different antioxidant activities, depending on their chemical structure. Phenols act as main reducing agents in plant cells, hydrogen donors, singlet oxygen quenchers or metal chelators, but the capacity for scavenging free radicals by phenols' classes (phenolic acids, flavonols, anthocyanidins, stilbenes) differs significantly (Roman et al. 2013). Antioxidant activity of rosehips seems to be stronger in comparison to the other extensively consumed wild fruits (Barros et al. 2010, Barros et al. 2011, Nadpal et al. 2016). In the present study, the significant variability was recorded for DPPH scavenging activity of rosehip genotypes, and generally was higher in first year of investigations. In both experimental years, 64US03 genotype demonstrated the highest DPPH scavenging activity (254.8 $\mu g \cdot ml^{-1}$, mean value for 2015 and 2016); while 64US01 genotype was the lowest (98.1 $\mu g \cdot ml^{-1}$). Demir et al. (2014) detected that DPPH scavenging activities of *R. canina*, *R. dumalis*, *R. gallica*, *R. dumalis* subsp. *boissieri* and *R. hirtissima* were measured as 278.90, 181.45, 161.25, 165.01 and 185.33 $\mu g \cdot ml^{-1}$, respectively. Roman et al. (2013) indicated that the radical scavenging capacity of *R. canina* extracts was related to the concentration of phenolic hydroxyl groups. On the other hand, Nadpal et al. (2016) did not find a clear correlation between the biochemical compounds content and antioxidant assays. They postulated that a strong synergistic effect of phenolics, flavonoids and ascorbic acid, as well as other uninvestigated bioactive compounds, could be responsible for the DPPH scavenging activity. Cosmulescu et al. (2017) concluded that the antioxidant activity of *Rosa* spp. fruits was linked to individual phenolic compounds rather than to total phenolic content. In the cited study the correlation coefficient (r) between total phenolics and antioxidant activity was 0.847. Present results did not show a significant correlation between individual as well as total phenolics and antioxidant activity (Table 5). For all analyzed phenolic compounds, the correlation coefficients were not significant. We can conclude that the DPPH scavenging activity of investigated *R. canina* fruits was attributed not only to the amount of individual bioactive components, such as phenolics, vitamin C, pigments, but also to the interaction of these compounds.

Table 5. Correlation coefficients (r) at particular significance level (p) between total antioxidant activity and chosen biohemical compounds.

	Sum of phenolics	Protocatechuic acid	Catechin	L-ascorbic acid
r	0.2113	0.2086	0.2486	0.4931
p	0.533	0.538	0.461	0.123

Hierarchical cluster analysis was performed to assess the similarities and differences between the investigated *R. canina* genotypes regarding their chemical composition. According to the results presented in Fig. 2, the 64US03 was clustered separately. It can be seen that rosehips of this genotype stood out alone concerning protocatechuic acid, catechin, *o*-coumaric acid, tartaric and malic acids, fructose, sucrose, ascorbic acid and DPPH scavenging activity. 64US03 can be considered as promising material for future investigations including biochemical, genetic as well as agronomic trials. 64US04 and 64US05; 64US07 and 64US08; 64US02 and 64US11; and 64US01 and 64US10 had formed separate clusters. A common characteristic of *R. canina* genotypes shed new light on differentiation of their chemical composition and led to evaluate predispositions of particular genotypes for future investigations.

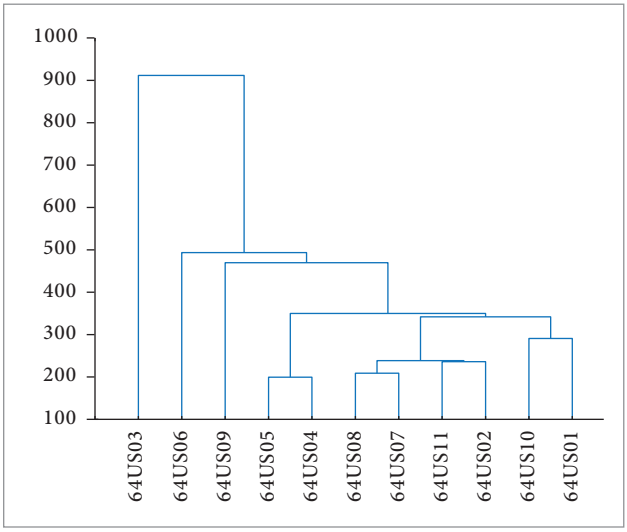

Figure 2. Cluster analysis of *R. canina* genotypes based on the biochemical parameters.

CONCLUSION

In this study, the *R. canina* genotypes from Interior Aegean (Uşak) region of Turkey were evaluated concerning their →

phytochemical profile. In this respect, significant differences were found between genotypes and experimental years regarding all analyzed chemical compounds. However, genetic factor seemed to be more important in determining all investigated rosehips' quality parameters than environmental conditions in vegetation periods, because differences between genotypes were highly repeatable in consecutive years. The liquid chromatography analysis resulted in quantification of 12 phenolic compounds in rosehips, with protocatechuic acid and quercitrin as the most dominant. Fructose and glucose were dominant sugars while ascorbic, malic and citric acids were dominant organic acids. Antioxidant activity of *R. canina* fruits was attributed not only to the amount of individual bioactive components, such as phenolics, vitamin C and pigments, but also to the interaction of these compounds. Results of the present study confirmed the traditional use of rosehips as food product rich in vitamin C and with its potential health and nutritional benefits. The 64US03 genotype was found as promising for future investigations, as the best source of high quality rosehips. The high levels of phenolics and organic acids found in *R. canina* genotypes justified its use as a source of bioactive agents preventing oxidative-stress related diseases for commercial usage in food and pharmaceutical industries.

AUTHOR'S CONTRIBUTION

Conceptualization, Okatan V.; Methodology, Okatan V.; Investigation, Okatan V., Çolak A. M., Güçlü S. F. and Korkmaz N.; Writing – Original Draft, Sękara A. and Okatan V.; Writing – Review and Editing, Sękara A.; Funding Acquisition, Okatan V., Çolak A. M., Güçlü S. F., Korkmaz N. and Sękara A.; Resources, Okatan V. and Sękara A.; Supervision, Sękara A.

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