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THE CONTENT OF TOTAL PHENOLS, FLAVONOIDS AND ANTIOXIDANT ACTIVITY IN ROSEHIP FROM THE SPONTANEOUS FLORA FROM SOUTH ROMANIA

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Abstract

Rosa canina L. is a shrub native to Europe, northwest Africa and western Asia with various uses in naturopathy, nutrition, as a decorative plant or in breeding as rootstocks etc. Although in Romania, Rosehip grows in abundance in wild flora, few know about the valuable biochemical properties that it has and the antioxidant potential of this species. At present, a priority in the human diet, both nationally and internationally, is to identify natural sources of antioxidants. In this study, it was analyzed the content of total polyphenols, flavonoids and antioxidant activity of Rosehips genotypes from the spontaneous flora of Oltenia (Romania). The total phenolic content was determined according to the Folin-Ciocalteu method, the antioxidant activity by DPPH radical (2,2 1 picrilhidrazil diphenyl) and flavonoids by colorimetric method. Polyphenol content was between 35.43-48.07 mg GAE/g, antioxidant activity varied between 99.3 and 363.64 μmTE/100 g sp., and the content in flavonoid was between 211.8-672.67 mg/100g. The best results for total polyphenol content and antioxidant activity was observed in Group I which comprises three genotypes (G6, G9, G11), considered as prospects for the improvement of this species.

Key words*: natural antioxidants, Oltenia, Rosa canina.*

INTRODUCTION

The interest in finding new, cheap and reliable sources of natural antioxidants which could replace synthetic antioxidants used in food or therapeutic products has increased lately. The most used synthetic antioxidants are butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Their use in food products is limited or restricted due to their toxicity and carcinogenicity (Iqbal et al., 2005).

The human body needs antioxidants in order to fight free radicals in the body. Natural antioxidants are often sourced from plants, spices and teas recommended for the daily diet. Current needs in natural food and pharmaceutical products require more thorough knowledge of the biochemical composition of non-agricultural plant species i.e. rosehips (Roman et al., 2013).

A number of scientific studies have shown that *Rosa canina* berries are rich sources of vitamin C, vitamin A, vitamins B1 and B2, vitamin P, nicotinic acid, vitamin K, hydrolysable tannins, citric and malic acid, peptine, volatile oils, fat oil, proanthocyanidins, anthocyanins, flavonoids, carotenoids, mineral substances, traces of vanillin, alpha and beta tocopherol (vitamin E), lecithin, sugars, etc. (Aptin et al., 2013; Barros et al., 2011; Chrubasik et al., 2008; Demir and Ozcan, 2001; Fecka, 2009; İlbay et al., 2013; Nowak, 2006), having anti-inflammatory properties (Hamidi et al., 2015; Roman et al., 2013; Warholm et al., 2003) also antioxidant (Ghazghazi et al., 2010; Koca et al., 2009), anti-mutagenic (Karakaya and Kavas, 1999) and antibacterial ones (Montazeri et al., 2011). Researchers have shown that the use of *Rosa canina* L. as a remedy in traditional folk medicine comes from its high content of phenolic compounds and minerals (İlbay et al., 2013).

The genus *Rosa*, with over 100 species, is part of *Rosacea* family and it is found in Europe, Northwest Africa and Western Asia (Netoiu et al., 2008). The *Rosa canina* species and its inter-taxa were identified in the 60 plant communities and it is characterized by a big abundance-dominance and high constancy.

The underwoods edified by *Prunus spinosa* and *Crataegus monogyna* are highly encountered in the hilly floor and with reduced frequency in the lower mountain sub-floor (Niculescu, 2006).

Phenolic compounds can be classified into a number of subgroups including phenolic acids, flavonoids, isoflavonoids, lignans, stilbenes, and complex phenolic polymers. In terms of structure, phenolic compounds consist of an aromatic ring, bearing one or more hydroxyl substitutes, and range from simple to high polymerised phenolic molecules compounds. Lately, a rich diet in polyphenols has been associated with reducing the risk for cardiovascular disease, cancer and other diseases. These compounds have antioxidant, anti-inflammatory and anti-carcinogenic properties. According to Pandey and Rizvi (2009), polyphenols and flavonoids are antioxidants which provide a significant protection of the humane body against the development of some chronic diseases including cancer, diabetes, neurodegenerative and cardiovascular problems.

In Romania, *Rosa canina* species biodiversity has recently begun to be studied, in regions of different heights. Thus, in Transylvania research has been carried out at 440 to 1250 m high (Brasovan et al., 2011; Roman et al., 2013), in the northern and northeastern region of Romania, at 400-1060 m high (Ghiorghita et al., 2012; Ropciuc et al., 2011; Rosu et al., 2011) and in Oltenia at 110-600 m high (Soare et al., 2014a and b).

The purpose of this study was to continue the research begun in Oltenia in order to get a valuable selection of *Rosa canina* genotypes, as potential sources of natural antioxidants.

MATERIALS AND METHODS

The biological material consisted of average samples of *Rosa canina* berries harvested from the indigenous flora of the southern Romania, in Dolj county, i.e. genotypes G1-Poiana Mare, G2-Craiova, G3-Teslui, G4-Filiasi, G5-Carcea and from Valcea County, i.e. genotypes: G6-Mateesti, G7-Tetoiu, G8-Lapusata, G9-Balcesti, G10-Horezu, G11 and G12 - Slătioara. Harvesting was carried at 110-600 m, in the fall of 2014 in full ripening. These areas are characterized by temperate continental climate with dry summers and annual average temperatures of 10-11.5°C (in Dolj County) but also with cool summers without any sudden changes in temperature or humidity, with an annual average temperature of 10.3°C (Valcea County).

In order to achieve the objectives, the content of total polyphenols, flavonoids and antio-xidant activity content has been analyzed.

Chemicals and Reagent

The used Methanol for the extraction was from Sigma-Aldrich. Gallic acid, 1.1-diphenyl-2-picrylhydrazyl, Ghydroxy - 2,5,7,8 - tetramethylchromon 2-carboxylic acid (Trolox) were obtained from Sigma-Aldrich, Germany and Quercetin was purchased from Carl Roth. Folin-Ciocalteu reagent was obtained from Merck, Germany. All the other used chemicals were of analytical grade.

Sample preparation

Extracts for the determination of total phenolic content, total flavonoid content and antioxidant activity were prepared into 80% aqueous methanol (1:10 w/v) at 24°C for 16 h. The resulting slurries were centrifuged at 4000g for 5 min and the supernatants were collected. In addition, for the determination of phenolic compounds was prepared an aqueous extract (1g:50 mL dH_2O) at 7°C, 60 minutes.

Determination of total phenolic content (TPC): Phenolic compounds were determined colorimetric by using the Folin-Ciocalteu method (Singleton et al., 1999) based on the oxidation of phenolic groups with phosphomolybdic and phosphotungstic acids. 2 mL Folin-Ciocalteu's phenol reagent (1:10) and 1.5 mL 7.5% w/v Na_2CO_3 were added to 0.5 mL sample extract. The mixture was allowed to stand at room temperature in the dark for 60 min and then the absorbance was recorded at 765 nm using a Thermo Scientific Evolution 600 UV-Vis spectrophotometer. The total phenolic content (TPC) was calculated using a standard curve prepared

using gallic acid and expressed as mg of gallic acid equivalents (GAE)/1 g dry weight.
Determination of total flavonoids content: was quantitatively determined by using colorimetric methods at 500 nm with chromogenic system of $NaNO_2$-Al $(NO_3)_3$-NaOH according to Abeysinghe et al. (2007). 0.5 ml of the sample extract was transferred into a 10ml volumetric flask. Furthermore, a 0.6 mL of 5% sodium nitrite ($NaNO_2$) was added and the mixture was shaken and left for 6 min. Secondly, 0.5 mL of 10% $Al(NO_3)_3$ was added to the volumetric flask, shaken, and was left to stand for 6 min. Finally, 3.0 mL of the 4.3% NaOH was added to the volumetric flask. Subsequently, water was added up to the scale. The mixture was then shaken and left to stand for 15 min before determination.
The total flavonoid concentration in methanol extract was calculated from quercetin (Q) calibration curve and expressed as quercetin equivalents (Q)/100g .
DPPH (2.2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The capacity of sample extracts to reduce the radical 2.2-diphenyl-1-picrylhydrazyl was assessed using the method of Babbar et al. (2014) with some modification. A 0.075 mM (final concentration) DPPH solution in methanol was mixed with sample extracts and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded for 20 min at 2 min intervals. The absorbance of the remaining DPPH radicals was measured at 519 nm. The normal color of DPPH will turn into yellow when its singlet electron is paired with a hydrogen atom coming from a potential antioxidant. A blank reagent was used to study stability of DPPH over the test time. The scavenging activity of extracts was evaluated as a percentage of DPPH discoloration using the formula: % scavenging = [A0-(A1–AS)]/ A0]100, where A0 is the absorbance of DPPH alone, A1 is the absorbance of DPPH + extract and AS is the absorbance of the extract only. The Trolox calibration curve was plotted as a function of the percentage of DPPH radical scavenging activity. The final results were expressed as micromoles of Trolox equivalents (TE)/100g. (µmol TE/g fw). All determinations were performed in triplicate, and all results were calculated as mean.
The results were interpreted statistically by analyzing the variance and PCA (Principal Component Analysis). Different letters on columns refer to significant differences between genotypes at 5% level according to LSD.

RESULTS AND DISCUSSIONS

Phenolic compounds are an important group of biologically active substances present in the berries of *Rosa canina*, including tannins, flavonoids, phenolic acids and anthocyanins (Demir and Özcan, 2001; Ercisli, 2007). Türkben et al. (2010) identified different genotypes of *Rosa canina* with different concentrations of phenolic acids (ellagic acid, ferulic acid, caffeic acid, p-coumaric acid, p-hydroxybenzoic acid, protocatechuic acid), ascorbic acid and flavonols (quercetin, kaempferol, myricetin, (+) - catechin). Pogacnik-Ulrich (2011) found that the total phenolics (TP) antioxidant capacity (AOC) and vitamin C content of rosehips vary according to the area and harvesting season. Thus, during the ripening in late autumn the concentration of vitamin C decreases, while the TP and AOC increase.
In the present study, the total polyphenol content in aqueous extract, varied for the *Rosa canina* genotypes between 35.43 mg GAE/1g to 48.07 mg GAE/1g d.w. (Table 1). In the case of the variance analysis of the content of phenols, it was calculated with a significant difference of 3.53 mg/1g for a significance threshold of 5%. In this way, the genotypes G 11 and G12 have the highest medium values, differing significantly from all the other genotypes except the genotype G10. Genotype G10 gets significant difference compared to genotypes G1-G7 and genotypes G1 and G2 have significant negative differences in comparison with all the other genotypes, a significant difference being needed between the two of them.
In another study carried out in Romania by Roman et al. (2013) for the rosehips harvested

in different areas and heights (ranged from 275 m to 1250 m ranged) a total amount of polyphenols content varying from 575 mg/100g frozen pulp to 326 mg/100 g frozen pulp was reported.

There are many studies that confirm the presence of phenolic compounds in the berries of *Rosa canina* in specialized literature, Thus, Ilbay et al. (2013) found a 48.59±0.29 mg content GAE/g DM extract. The total content of phenols reported by Denev et al. (2013) in the berries of *Rosa canina* was of 1934.3 ± 4.3 GAE/100 g and by Montazeri et al. (2011) was of 424.6 ± 1.8 mg GAE/g extract.

Yilmaz and Ercilsi (2011) after studying four taxa of Rosa (*Rosa pisiformis, Rosa canina, Rosa villosa i Rosa dumalis subsp. antalyensis*) have found a total content of phenols from 78 to la102 mg GAE/g DW. Also, Aptin et al. (2013) reported total phenol content of rosehips harvested in North of Iran, by 26.54 mg gallic acid/ml .

The chemical composition of rosehips is influenced by the interaction of several factors: genotype, height, climatic conditions, harvesting time, etc., all these explaining the data differences reported within the scientific studies. Some authors have found that the interaction between the habitat and the harvesting period influences the amount of antioxidant, vitamin C and flavonoids. In Rudbar-Kushk valley at 50% maturity, in Eshkevarat-Rudsar at full maturity and in Eshkevarat-Rudsar at 50% maturity, i.e. there have been obtained the highest value of antioxidant, vitamin C and flavonoid contents, but the influence of habitat on harvesting time was not significant for anthocyanin and total phenol content (Rahimabadi et al., 2013).

The results in terms of content in flavonoids, for the studied genotypes are presented in Table 1, it ranged between 211.8 in G2 and 672.6 mg 100 in G9.

As it concerns the variance analysis, a significant difference of 51.70 mg/100 g for a 5% significance threshold was calculated. The best ranked was genotype G9 which had significant differences in comparison with all the other genotypes, followed by genotype G10 which had positive differences distinctly significant compared to all genotypes, except genotype G9 where there is a negative difference. Between genotypes G11, G7 and G8 there were no significant differences; between the mentioned ones and those ranked higher or lower there have been significant positive or negative differences, as it was the case. For genotypes G1 and G2 there were significant negative differences in comparison with all other genotypes classified higher compared to these two genotypes, mentioning that there was no significant difference between these two.

Our results are similar to those obtained by Yoo et al. (2008) who reports a flavonoid content of 400 mg catechin/100 g fw for *Rosa rubiginosa* and Barros et al. (2011) in a study carried out to get a chemical analysis of *Rosa canina* berries at different stages of maturity, reports a content of flavonoids of 9.8 mg/1 g extract for the ripened rosehips and extraction progress of 43.19%.

Demir et al. (2014) reports a flavonoids content of 9.48 mg rutin/g dry weight for *Rosa canina* berries in a study of five different rosehips species grown in Turkey. Furthermore, our results are higher than those reported by Roman et al. (2013) (101.3 mg/100 g to 163.3 mg/100 g frozen pulp), while the lowest concentration in flavonoids was reported by Adamczak et al. (2012), who found a medium content in flavonoid of 41 mg/100 g dw in the rosehips harvested in different parts of Poland.

Knowing the variation of the antioxidant and antibacterial activity is very important in choosing the plant material that can be used in food production, health industry and future breeding programs (Yilmaz and Ercisli, 2011). Montazeri et al. (2011), after the in vitro study in order to know the variation of the antibacterial and antioxidant activity of different extracts of *Rosa canina* berries harvested in Iran, it is suggested a possible use of the *Rosa canina* methanol extract as a source of natural antioxidant and antimicrobial agents. The antioxidant activity of *Rosa canina* studied berries varied from 99.3 to 363.44 μmol TE/1g f.w. In the case of the

variance analysis, a significant difference of 48.13 μmol TE/1g sp (DPPH) has been calculated for a significant threshold of 5%. Thus, G1 has a significant positive difference compared to all other genotypes for this significance threshold. Genotypes G11 and G3 are characterized by significant differences compared to the last two genotypes classified, whilst the last genotype classified has significant negative differences compared to all other genotypes (Table 1).

In the study carried out by Roman et al. (2013) on rosehip berries from Transylvania (Romania), the antioxidant activity varied from. 63.35 μM TE/100g frozen pulp to 127.8 μM TE/100g frozen pulp. By Denev et al, (2013) in the *Rosa canina* berries originating in Bulgaria, the antioxidant activity was of 201.14 μmol $TE.g^{-1}$. Moreover, Montazeri et al. (2011) identified high activity of DPPH indicated by the methanol extract of 11.58 μg/ml, found in the berries harvested from Kandeloos village, Noshahr Mountains, Mazandaran province, Iran, and Yilmaz and Ercisli (2011) identified an antioxidant activity of 91.4% for the *Rosa canina* berries, which were samples from Erzurum in Eastern Anatolia, a region in Turkey. Duda-Chodak et al. (2011) in a study evaluating the antioxidant activity in fifteen popular herbal products reports the lowest ability to scavenge the ABTS radical for the *Rosa canina* berries of 20.6 mg TE/1g dry weight (82.4 (μM TE/1g dw) and Egea et al. (2010) report an antioxidant activity of 416.64 (μM TE/1g fw) for the *Rosa canina* berries and Demir et al. (2014) report an antioxidant activity of 35.51 μM TE/g dry weight.

Table 1. The analysis of variance and the calculation of the significant differences for the chemical compounds analyzed

Genotype	Total phenolic content (mgGAE/1g) Mean	Antioxidant activity μmol TE/100g (DPPH) Mean	Total flavonoid content (mgQ/100g) Mean
G1	35.43d	363.64a	206.13g
G10	45.71ab	188.8cd	593.51b
G11	47.85a	242.54b	534.13c
G12	48.07a	99.3e	302.26ef
G2	36.43d	232.5bc	211.8g
G3	40.5c	246.42b	279.62f
G4	40.71c	236.67bc	387.09d
G5	42.14c	149.01d	344.68de
G6	42.14c	206.15bc	378.6d
G7	42.14c	218.62bc	522.82c
G8	42.86bc	224.5bc	500.2c
G9	43.50bc	222.24bc	672.67a
Average	42.29	219.198	411.1258
Standard error	8.70	1621.84	1871.82
Standard deviation	1.70	23.25	24.98
LSD 5%	3.53	48.13	51.70

Means followed by the same letter in each column are not significantly different according to LSD Test at 5 % level.

Dog rose is a major source of acids, phenolic compounds (Aptin et al., 2013), flavonoids, anthocyanins and ascorbic acid (Ercisli, 2007; Chrubasik et al., 2008).

In order to identify sources of valuable genes, with the purpose of the establishment of some plantations the analysis of the main components was also performed.

From Table 2 one can see that out of the three factors analyzed the first two of them influence the total variance with a percent of 82.555%, these two factors being the content

of phenol in aqueous extract mg/1g) in a percent of 48.674% and the antioxidant activity µmol TE/100g (DPPH) in a percent of 33.881%. The other factor analyzed i.e the flavonoid content (mgQ/100 g) influenced the variance by 17.445%.

Table 2. Eigen values and component score coefficients

Component	Initial Eigen values			Component Score Coefficient Matrix	
	Total	% of Variance	Cumulative %	1	2
Total phenolic content (mgGAE/1g)	1.460	48.674	48.674	0.619	0.202
Antioxidant activity µmol TE/100g sp	1.016	33.881	82.555	0.565	-0.219
Total flavonoid content (mgQ/100g)	0.523	17.445	100.000	0.012	0.930

The graph in Figure 1 was carried out for the first two components, namely the content of total phenolic compounds (mg/1g) (PCA 1) and antioxidant activity µmol TE/100g (PCA 2). Thus, in this graph one can see that we can identify four groups with the following characteristics:

• Group I has 3 genotypes with high values both for the for the total phenolic content and for antioxidant activity (both positive components).

• Group II has 4 genotypes with high values for the total phenolic content extract and low ones for the antioxidant activity.

• Group III has 2 genotypes with low values (negative) for both components.

• Group IV has 3 genotypes and has high values for the antioxidant activity and low values for the total phenolic content.

Principal Component Analysis was applied by Atoosa Danyaei et al., 2012 for 37 *Rosa damascena* genotypes, also by Marjorie Mercure and Anne Bruneau, 2008) for 11 morphological types used in the study of natural hybridization between *Rosa blanda* and *Rosa rugosa* to show the share of each within the total variation.

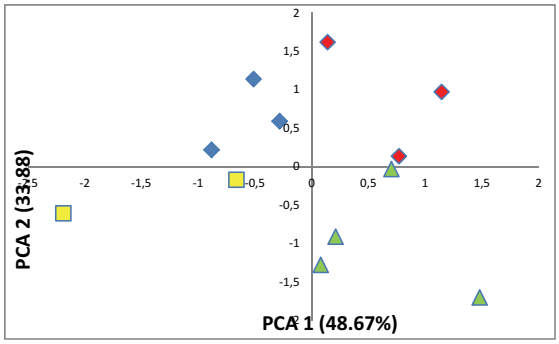

Figure 1. Plot of the first two principal components (PCA1 and PCA2). Eigenvalues for each two principal component are listed in parentheses.

CONCLUSIONS

Based on the results obtained in terms of the content of the active compounds of the *Rosa canina* berries harvested in different areas of Oltenia region a high variation was found.

After the PCA analysis, the best results in terms of total polyphenol content and antioxidant activity were observed in Group I which is formed of three genotypes: G6, G9, G11, which are thought as a chance for the improvement of this species. The three genotypes are to be found in Valcea County at a 360-600 m height. These genotypes are possible sources of natural antioxidants.

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