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EVALUATION OF SOME PHYTOCHEMICAL CONSTITUENTS AND THE ANTIOXIDANT ACTIVITY IN SIX ROSE HIPS SPECIES COLLECTED FROM DIFFERENT ALTITUDE OF SUCEAVA DISTRICT

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Key words: Rose hips fruits, polyphenol, flavonoids, anthocyanins contents, antioxidant activity
Abstract: In this study, species of rose hips fruits were assayed for the composition of polyphenol, flavonoids, anthocyanins contents as well as the antioxidant activity. Samples of rose hips representing six species *(Rosa pendulina, R. tomentosa, R. canina, R. rubiginosa, R. corymbifera* and *R. nitidula*) were collected from spontaneous flora of Suceava district. The smallest content of total polyphenols was recorded in *R. nitidula,* both in seeds (3.11mg GAE/g DW) and pulp (23.99 mg GAE/g DW). The maximum concentration of total polyphenols was found in *R. pendulina,* both in seeds (17.68 mg GAE/g DW) and pulp (71.48 mg GAE/g DW). The most abundant anthocyanin content in pulp of all specie studied was in *R. canina (*16.60 mg%). The relevant differences between pulp and seed regarding flavonoid content, about 7-fold were reached at *R. tomentosa* collected from Vatra Dornei. The results revealed that antioxidant activity of pulp extract from *R. pendulina* showed the largest scavenging activity while the lowest scavenging capacity was recorded for *R. nitidula.*

INTRODUCTION

Rosa canina L., known for their fruits (rose hip, brier hip, brier, rose, dogberry, dog rose, hip fruit, hop fruit, sweet brier, wild brier), grows wildly in various regions of Romania having culinary and medicinal values. *Rosa* species have attracted the attention due to their antioxidant, antibacterial and other properties (Nowak and Tuzimski, 2006). It is mostly used for the prevention and treatment of the common cold, gastrointestinal disorders, diabetes, kidney disorders, and other infections (Davis, 1972, Yeşilada, 2002). The main amount of vitamin C is located in the skin whereas the seed contain a great content of oil with antibacterial and antioxidant properties (Georgieva et al., 2014; Cheryll, 2013). In addition, in many European countries the *Rosa* species have long been used as an herbal tea, vitamin supplement or food product because its richness in ascorbic acid. Also, rose hips are a rich source of carotenoids, minerals (K and P), folate, polyphenols and various flavonoids such as anthocyanins (Uggla et al., 2005, Demir et al., 2001). On the other hand, *Rosa canina* L. is a great source of tocopherols, bioflavonoids, tannins, pectin, aminoacids, unsaturated and polysaturated fatty acids, phospholipids and gallactolipids (Chrubasik et al., 2008). However, the seeds representing 30% of the rose hip fruit and are considered a low value by product (Çinar and Dayisoylu, 2005).

More than, rose hips extract is able to scavenge reactive oxygen species (ROS) (Daels-Rakotoarison et al., 2002) and among the various natural scavengers of ROS, polyphenols compounds have received attention. There is considerable interest in natural antioxidants from foods and biological systems because of the potential nutritional and therapeutic benefits (Vitaglione and Fogliano, 2004). In their study about nutritional value and chemical composition of eight rose hips fruit species collected from Transilvania, Roman et al. (2013) found that varieties of *R. canina* can be used as a potential source of natural antioxidants.

The medicinal value of rose hips depends primarily on the content of vitamin C and flavonoids (Kuźnicka and Dziak, 1987). In the last years, the relationship between food and health has become more important and nowadays the consumers demand healthy, tasty and natural functional foods that have been grown in uncontaminated environments.

The goal of this research was to determine the chemical composition (flavonoids, total polyphenol and anthocyanins contents, as well as antioxidant activity) in pulp and seed of six wild rose hips (*Rosa pendulina, R. tomentosa, R. canina L., R. rubiginosa L., R. corymbifera Borkh* and *R. nitidula Besser*) collected from different altitude of Suceava district. Another objective was to compare the content from seed and pulp of wild rose hip species in order to establish which has better antioxidant properties.

MATERIAL AND METHODS

1. Sample collection and processing

During harvest season (end of September and beginning of October 2013) samples of rose hips representing six species *(Rosa pendulina, R. tomentosa, R. canina, R. rubiginosa, R. corymbifera* and *R. nitidula*) were collected from spontaneous flora of Suceava district from different altitudes ranging between 630m and 830 m (Table 1). Rose hips were preserved according to the standard procedures being collected manually without leafs and were mixed together for a homogeneous distribution. The fruits were picked at the fully ripe mature stage as judged by their colour and were randomly chosen from 100 fruits. Some phytochemical analysis (dry weight, flavonoids, total polyphenol, anthocyanins) of powder obtained from the pulp and separate from the seeds of wild rose hip fruits were analyzed. The results were expressed as the average (± SE) of a total of three replicates.

Table 1. Wild *Rosa* species collecting from different altitude and areas

Sampling zona	Taxa under study	Altitude (m)
Campulung Moldovenesc	*R. rubiginosa*	
	R. canina	630 m
	R.pendulina	
	R. tomentosa	
Vatra Dornei	*R. canina*	807m
Dorna Candreni	*R. corymbifera*	830m
	R. nitidula	

2. Phytochemical analysis

2.1. Dry weight content of rose hip samples was determined using gravimetric method by evaporation at mild temperature (105°C) until they reached a steady weight. Thus, it was determined the quantity of dry matter both in pulp and seeds. The results of dry weight were expressed in g % (g/100g fresh weight) (Boldor et al., 1983).

2.2. Extract preparation

Extraction was performed with methanol. Rose hips samples (pulp or pulp and separate seeds) were homogenized with 80% methanol and then they were stirred for 30 minutes (Andjelkovicet al., 2013, modified). After their centrifugation at 3000 rpm the supernatants were used for the next determinations of total polyphenol content, flavonoids content and antioxidant activity.

2.3. Total polyphenols content assay

The total polyphenols content was determined by using a modified Folin-Ciocalteau method (Singleton et al., 1999). The appropriately dilute sample was added Folin-Ciocalteau reagent and mixed thoroughly. After four minutes, 15% Na_2CO_3 was added. The absorbance of resulting bleu-colored solution was read at 765 nm after two hours, against the blank (distilled water). The amount of the total phenolic content was expressed as mg gallic acid equivalent (mg GAE/g DW) (R^2=0.99). Three readings were taken for each sample and the result averaged.

2.4. The flavonoids content assay

The flavonoids content was measured following a spectrophotometric method (Dewanto et al., 2002). Briefly, methanol extract were appropriately diluted with distilled water. Initially, 5% $NaNO_2$ solution was added to each test tube; at five minutes, 10% $AlCl_3$ solution was added and then at six minutes 1.0 M NaOH was added. Finally, water was then added to the test tube and mixed well. Absorbance of resulting pink-colored solution was read at 510 nm against the blank (distilled water). Flavonoids content was expressed as mg catechin equivalent (mg CE/g DW) (R^2 =0.98). Three readings were taken for each sample and the result averaged.

2.5. Total anthocyanin content assay

Anthocyanins were extracted from samples by homogenizing rose hip pulp from each species with acidified 70% ethanol solution. The mixture was centrifuged after 10 min. and the pH was adjusted at correct value 1 with HCl. The

mixture was brought to a volume of 25 mL and the absorbance was measured at 515 nm, using the Shimadzu UV-Visible spectrophotometer (Fuleki and Francis, 1968).

2.6. Antioxidant activity (DPPH free radical scavenging activity) of methanol extract

Antioxidant activity (AA%) of methanol rose hip fruits was determined using stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described by Molyneux, 2004; Shirwaikar et al., 2006. This is based on neutralization of free radicals emitted by the DPPH solution, resulting in a coloured solution. DPPH radicals have an absorption that is maximal at 517 nm, and which disappears with reduction by an antioxidant compound. The DPPH solution in methanol 0,1mM was prepared daily, and 2 mL of this solution was mixed with 20 µL of the methanol plant extracts. After 20 min of incubation at 37°C in the dark, the absorbance was recorded at 517 nm. The control was achieved using 80% methanol and DPPH solution. The experiment was carried out in triplicate. The percentage of radical scavenging activity (AA%) was calculated according to the following equation:

$$AA\ (\%) = (1-A1/A0)x100$$

where A1 is the absorbance of the extract samples and A0 is the absorbance of the control samples (methanol)

2.7. Statistical analysis

All of experiments were carried out with at least three independent repetitions. The results were expressed as the means value and standard errors of mean.

RESULTS AND DISCUSSIONS

Biosynthesis and accumulation of secondary compounds are regulated internally within the plant and controlled by both external and genetic factors. The composition of plants may be affected by many factors including region, variety, state of ripening, soil type and condition, irrigation and weather (Oktay and Alpaslan, 2012). The most important environmental factors determining photosynthetic rates (and thus synthesis of phenolic compounds) are light and temperature, as long as water and nutrient supplies are adequate (Emmingham and Waring, 1977).

The dry weight content at wild species of *Rosa* sp. fruits studied evidenced no significant differences (Tab. 2). Therefore, in the seeds the content varied very little from 70.21 g% (*R. canina*) to 73.82 g% (*R. pendulina*). In fact, both values of were obtained at fruits collected from the same sampling area, Vatra Dornei (807m). On the other hand, the dry weight amount in the pulp ranged from 28.34 g% (*R. pendulina*) to 37.35 g% (*R. nitidula*), the rose hip being picked up from different altitudes, 807m and 830m, respectively. Similarly, a another study showed that dry weight content varied in other rose hip species from 33.85 g% (*R. villosa*) to 40.35 g% (*R. dumalis* subsp. *boissieri*) (Ercisli, 2007).

In addition, the study of Yildiz and Alpaslan (2012) found in fruits of rose hip a content of dry weight 32.5 g% which is very similar with our results. Moreover, Rosu et al., 2011, found in whole rose hip fruits that dry weight, in climatic condition of 2008, was varied between 27.53g% and 40.22 g% at *R canina* and *R. micrantha*, respectively.

Table 2. Dry weight content in pulp and seeds of wild *Rosa* species from different altitudes (mean ± SD, n = 3).

Sampling area	Species	Dry weight content (g%)		*Altitude (m)*
		pulp	seeds	
Campulung Moldovenesc	*R. rubiginosa*	36.11±0.18	71.07±0.45	630 m
	R. canina	34.74±0.49	71.97± 0.20	
Vatra Dornei	R. pendulina	28.34±0.86	73.82±1.34	807m
	R. tomentosa	29.77±1.23	70.94±0.28	
	R. canina	32.13±0.22	70.21±0.13	

Dorna Candreni	*R. corymbifera*	36.02±0.47	72.00±2.78	830m
	R. nitidula	37.35±0.46	71.93±0.29	

As regard the anthocyanins content, results from this study indicated that in the pulp of rose hip species the minimum was recorded in *R. rubiginosa* (4.81 mg%) while the maximum was observed at *R. canina* (16.6 mg%) (Fig.1). In species *R. pendulina, R. tomentosa, R. canina* and *R. corymbifera* the anthocyanins contents were raised ranging between 13.08 mg% to 16.92 mg%, but in *R. rubiginosa* and *R. nitidula* this was 3 times lower (4.81 and 5.61 mg%, respectively). By the other hand, although the rose hip *R. canina* was collected from two areas with different altitudes (807m and 630m) the values of anthocyanins amount were almost the same (16.60 mg% at those from Vatra Dornei and 16.92 mg% at those from Campulung Moldovenesc). This diminishing of anthocyanins level might be due by the variation in altitude or interspecific differences. In addition, the differences in level of anthocyanins can be the result of rose hip growth region (more specifically, the various altitude) and different *Rosa* genotype which have an impact on this metabolite concentration.

Other studies like those of Connor et al. (2002) have found a significant interaction between genotype and location when they examined total anthocyanin content in American blueberry cultivars. By the other hand, Lätti et al. (2008), found an extensive variation between the northern and the southern populations of 179 bilberry (*Vaccinium myrtillus*) clones that spanned 1000 km in Finland regarding the anthocyanins content. The authors conclude that the northern climate conditions appear to favour the biosynthesis of more anthocyanins and flavonols, which have been shown to have the greatest antioxidant capacity *in vitro*. Moreover, even though the final colour of the fruit is a combination of different factors, delphinidins are known to give bluish hues in flowers and fruits. Thus, one could speculate that the blue berries growing in the north might be more blue compared with the southern populations.

Anthocyanins water-soluble compounds having a great interest in nutrition and medicine because of their potent antioxidant capacity (Garcia-Alonso et al., 2005). They are also used in dyes industry to replace synthetic pigments by natural ones (Pina et al., 2012).

Generally, environmental factors (temperature and light, latitude and altitude) have an impact on anthocyanin formation. Therefore, when studying the influence of latitudinal, altitudinal or temporal variations on anthocyanin production, environmental factors such as light and temperature are of prime importance (Åkerström, 2010).

The contents of total polyphenols, calculated as mg gallic acid equivalent (GAE) of rose hip methanolic extract was higher in pulp than seeds (Fig. 2). In fact, in pulp the total polyphenols contents ranged from 23.99 to 71.48 mg GAE/g DW whereas in seeds this was reduced varying from 3.11 to 17.68 mg GAE/g DW. Furthermore, the lowest amount was remarked in *R. nitidula*, both in seeds and pulp. On the other hand, *R. canina*, although collected from different areas show the same content of total polyphenols in seeds and values almost similar in pulp (52.34 mg GAE/g DW from Vatra Dornei and 60.39 mg GAE/g DW from Campulung Moldovenesc).

The highest concentration of total polyphenols was found in *R. pendulina*, both in seeds and pulp. Regarding the differences between polyphenolic compounds in rose hip species these could be explained by the genetic variation, because all plants were harvested at ripening.

By the other hand, Scalzo et al., 2005 was reported that the plant genotype, cultivation site and technique affect the total phenolic content in fruit.

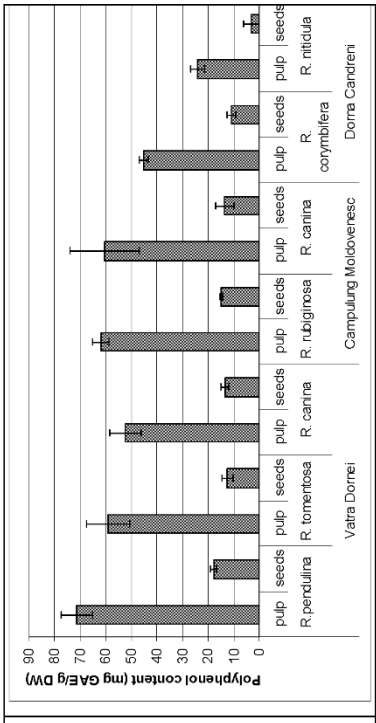

Fig. 2. Total polyphenols content in pulp and seeds of six *Rosa* species from different altitudes (mean ± SE, n = 3).

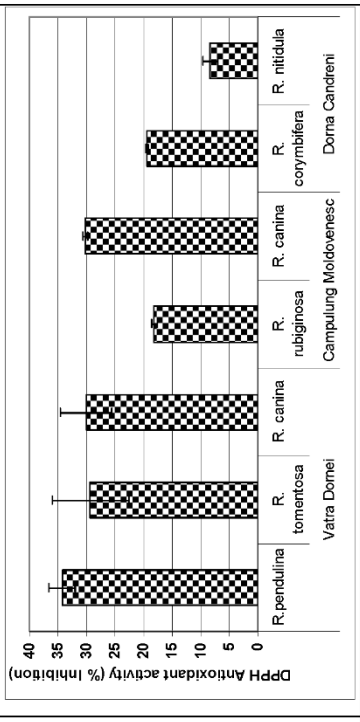

Fig. 4. Free radical scavenging activity (% DPPH inhibition) in pulp of six *Rosa* species from different altitudes (mean ± SE, n = 3).

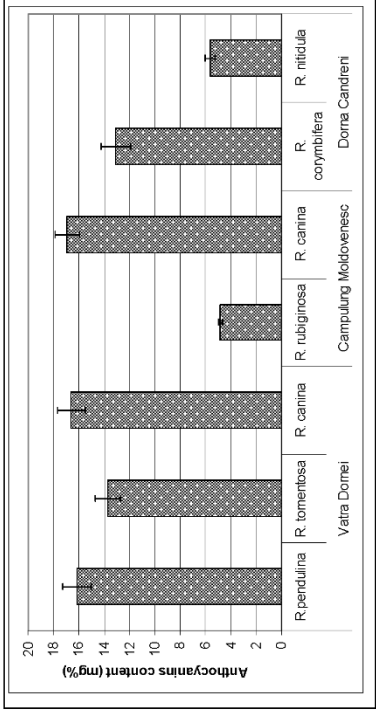

Fig. 1. Anthocyanins content in pulp of six *Rosa* species from different altitudes (mean ± SE, n = 3).

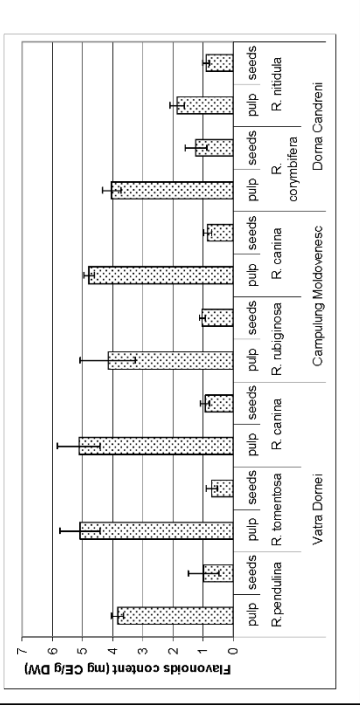

Fig. 3. Total flavonoids content in pulp and seeds of six *Rosa* species from different altitudes (mean ± SE, n = 3).

Phenolic compounds are important constituents because of their free radical scavenging ability facilitated by their hydroxyl groups.

By the other hand, phenolics possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, anticarcinogenic effects as well as ability to modify gene expression (Nakamura et al., 2003). The total phenolic concentration could be used as a basis for rapid screening of antioxidant activity by inactivating lipid free radicals or prevent decomposition of hydroperoxides into free radicals (Pokorny et al., 2001). Rose hips are known to have a high phenolic content (Hvattum, 2002) but *R. rubiginosa* seeds are high in polyphenols and show strong antioxidant activity (Moure et al. 2001).

Our findings are in agreement with those of Ercisli (2007), which found a polyphenols content at *R. canina* by 96 mgGAE/100g. On the other hand, although more reduced, the values of polyphenols are in agreement with those of Yildiz and Alpaslan, (2012) which found a content polyphenols 9982 mg GAE/100g FW in rose hip. Ocksook et al., (2007) reported that total phenolic concentrations of the extracts of *R. nutkana*, *R. pisocarpa* and *R. woodsii* rose hip were 12.20 mg GAE/l, 8.77 mg GAE/l and 6.974 mg GAE/l, respectively.

In the study of Roman et al., (2013) the total polyphenols content of the *R. canina L.* hip extracts registered values between: 575.0 mg GAE/100 g for RC1 (var. *transitoria* f. *ramosissima* from Bistrita-Nasaud, Agiesel) and 326.5 mg GAE/100 g for RC6 (var.*lutetiana* f. *fallens* from Satu-Mare, Petea). In their research on chemical composition of 'Karpatia' *R. pomifera* fruits, Milala et al., (2013), found that among the polyphenolic compounds, the flavanols predominated, both in the flesh and the seeds; their mean quantity was 2783 mg/100 g DW and 842mg/100g DW, respectively.

As in the case of total polyphenol, the contents of flavonoid, calculated as mg catechin of rose hip methanol extract, was higher in pulp than seeds (Fig. 3). The wild rose hips fruits investigated contain an amount of flavonoids which varied in seeds between 0.72 and 0.98mg CE/g DW while in pulp from 1.86 to 5.11mg CE/g DW.

The flavonoids content in pulp of *R. tomentosa* and *R. canina* colected from Vatra Dornei as well as, *R. canina* from Campulung Moldovenesc had almost close values, 5.08mg CE/g DW, 5.11mg CE/g DW and 4.77mg CE/g DW, respectively. Moreover, although the species *R. canina* was collected from areas with different altitude (807m and 630m) the variation between flavonoids content from pulp and seeds had close values, these being about 5.4-fold and 5.6-fold from Vatra Dornei and Campulung Moldovenesc, respectively. On the other hand, the highest differences between pulp and seed regarding flavonoid content, about 7-fold were reached at *R. tomentosa* collected from Vatra Dornei. By contrast, *R. nitidula* has recorded the lower content and the differences between pulp and seed was about 2-fold.

The most common flavonoids in plants are quercetin and kaempferol and usually occur as glycosides. In rose hips there are mainly glycoside derivatives of quercetin: quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside) and hyperoside (quercetin-3-O-galactoside) (Nowak and Tuzimski, 2006).

As regards free radical scavenging activity (% DPPH inhibition) the percentages found in fruits of wild *Rosa* species harvested from different altitudes were varying between 8.31% and 34.22% (Figure 4). Therefore, the pulp extract from *R. pendulina* showed the highest scavenging activity and the lowest scavenging capacity was recorded for *R. nitidula.* The antioxidant activity of *Rosa* species collected from Vatra Dornei (807m) was not varied very much (29.32%-30.07%). The difference of antioxidant activity of *Rosa* species were remarked at *R. rubiginosa* and *R. canina* from Campulung Moldovenesc (18.17% and 30.20%) as well as at *R. corymbifera* and *R.*

nitidula from Dorna Candreni (8.31% and 19.31%). Even though *R. canina* fruits were picked up from two different altitude 807 m (from Vatra Dornei) and 630m (from Campulung Moldovenesc) the DPPH radical scavenging capacity was almost the same (30.07% and 30.20%, respectively).

The antioxidant capacity may be widely used as a parameter for medicinal bioactive components. The characteristic feature of antioxidants to scavenge DPPH free radical is accepted and is therefore most often selected as a reliable tool to evaluate the free radical scavenging capacity of different plant extract.

The variations of antioxidant capacity of fruits can be impacted by geographical conditions (month to month and year to year) (Lee and Coates, 1997). Wenzig et al. (2008) demonstrated that the radical scavenging activity of the two rose hips (*R. canina* L.) extracts correlated very well with their total phenolic content, while ascorbic acid contributes only little to the radical-scavenging activity due to its low concentration present in the extracts.

CONCLUSIONS

The contents of total polyphenols, flavonoids and antocyanins as well as antioxidant activity in fruits of six wild *Rosa* species (*R. pendulina, R. tomentosa, R. canina, R. rubiginosa., R. corymbifera* and *R. nitidula*) collected from different altitudes of Suceava district were compared. The results revealed that in *R. nitidula* species were remarked the smaller contents of anthocyanis, total polyphenols and flavonoids.

Concerning the anthocyanin content the highest level was recorded in pulp of *R. rubiginosa* while the smallest was observed at *R. canina*; the difference being approximately three fold higher in rose hip, even if they were collected from the same altitude 630 m. As regards the total polyphenol and flavonoids contents showed a large variation among Romanian rose hip fruits studied. In case of *R. canina* rose hip fruits the total polyphenol content increased with the decrease of altitude while the flavonoids contents diminished with the reduction of altitude. Antioxidant activity of rosehips of studied species is shaped by the presence of anthocyanins, total polyphenols and flavonoids. The maximum capacity of neutralizing DPPH radicals was shown by extracts obtained from *R pendulina* whereas the minimum by *R. nitidula.*

According to the results of the present study it seems that rose hips can be recommended for improving the health because of hight contents of anthocyanins (*R. canina*), total polyphenols (*R. pendulina*), flavonoids (*R. canina*).

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