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TOTAL POLYPHENOLS, FLAVONOIDS CONTENTS AND ANTIOXIDANT ACTIVITY OF *ROSA* SP. GENOTIPES FROM DIFFERENT ALTITUDE OF ROMANIAN REGIONS

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Abstract. Eight wild rose hip genotypes from different altitudes varying from 3m to 902m were analyzed in order to evaluate the total polyphenols, flavonoids content and the antioxidant activity. The *Rosa spp.*rosehips collected from the Northeastern and the Southeasternof Romanianwere, as follows: *Rosa canina*, *R. caesia*, *R. corymbifera*, *R. micrantha*, *R. nitidula*, *R. rubiginosa*, *R. subcanina,* and *R. vosagiaca*. In some genotypes, the level of flavonoids and polyphenol content increased with the increasing altitude while in other it was observed a decrease. Polyphenol content reached a maximum of 144.36 mg GAE/g DW in *R. rubiginosa* whereas the lowest content of 61.72 mg GAE/g DW was recorded in *R. caesia*. The highest polyphenol content was reached at altitude of 860 m. The amount of flavonoids content ranged between 7.32 mg CE/g DW and 19.45 CE/g DW in *R. caesia* and in *R. nitidula,* respectively. The radical scavenging capacities of Rosa genotypes extracts were not positively correlated with altitude, except the *R. corymbifera* extractswhere the antioxidant activity increased with the increase of altitude.

INTRODUCTION

Rose is one of the most important crops in the floriculture industry being used as cut flowers, potted plant, and garden plants as well as in the food, perfumery, and cosmetics industries for many years (Kazaz et al., 2009).The genus Rosa contains over 100 species that are widely distributed in Europe, Asia, the Middle East and North America (Nilsson, 1997).

In Romania, there are known 29 spontaneous and subspontaneous species of *Rosa* L. genus (Oprea, 2005), widely spread throughout the country, from the sea level to the high altitude.

Rosa canina L.is a member of Rosaceae family. The fruits are well known as a rich source of vitamin C and polyphenols (Tumbas et al., 2012,Oprica et al., 2015), being considered to have one of the highest vitamin C content (30–1300 mg/100 g) among fruits and vegetables (Ziegler et al., 1986). According to Tumbas et al. (2012), vitamin C and flavonoids are responsible for the antioxidant activity of rosehips tea, while only polyphenols contribute to its antiproliferative activity. In addition, fruits of the *Rosa sp*. species, especially rose hip, contain vitamins and minerals, carotenoids, tocopherols, phenolic compounds, flavonoids, fruit acids, tannins, pectin, sugars, organic acids, amino acids and essential oils (De Vries 1980, Razungles et al. 1989, Chai and Ding, 1995, Demir and Özcan 2001; Kadakal et al. 2002, Ercisli S., 2007, Bucsa et al., 2013, Oprica et al., 2016). Because of its significant nutritional and therapeutic benefits, members of *Rosaceae* have been used both traditionally and for medicinal purposes. The bioactive compounds of the rose hips are known to have antibacterial, antidiabetic, anti-inflammatory, antiviral, immunomodulatory, antioxidant, antiulcerogenic and antidiarrheal properties as well as, effects on tumor cells andkidney stones (Orhan and Hartevioğlu, 2013). The composition and distribution of nutrients and high-valuecomponents**,** such as phenolics**,** mainly depends upon genotypes, fruit tissue, as well as, the maturity levels of fruits and to a smaller extent on environmental aspects (Manzoor et al., 2012). By the other hands, ecological factors other than altitude, soil type, temperature, and precipitation, might affect the synthesis and turnover of secondary compounds (Nobel, 1991). In this respect, some factors as seasonality, circadian rhythm, plant development, phenology, temperature, altitude, water availability, UV radiation, nutrients, pollution, mechanical stimuli and attacks by herbivores or pathogens are considered to affect the occurrence of plant metabolites (Harborne, 1993). For example, the fruits of rosehips collected in early autumn have the highest content of vitamin C compared with those collected in the late autumn. Instead, the antioxidant capacity and total phenolics of rose hips have the lower content in the early autumn due to lower levels of colored polyphenols (Pogačnik and Poklar, 2011).

With regard with the harvesting altitude of the rose hips, Rosu et al. (2011) founded that the vitamin C, total sugars and carotene content varied mostly with *Rosa sp*. genotype and only the carotene level appears to be positively correlated with altitude.

In recent years, the attention was focused on natural antioxidants of plants which have been shown to have multiple benefits on human health and nutrition.There is a lot of interest in the antioxidant activity of flavonoids and plant

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phenolic compounds due to their potential in health promotion and disease prevention. For this reason, this study was designed to measure the contents of total polyphenolsand flavonoids as well as the antioxidant activity of eight rosehip genotypes (*Rosa canina, R. caesia, R. corymbifera, R. micrantha, R. nitidula, R. rubiginosa., R. subcanina* and *R. vosagiaca*) collected from the Northeastern and the Southeastern regions of Romania, from altitudes between 3 to 902 m.

MATERIALS AND METHODS

Sample collection and processing

Phytochemical analysis was performed on samples of rose hips representing 8 species of *Rosa spp*., as follow:*Rosa canina* L.S Str., *R. caesia* Sm., *R. corymbifera* Borkh., *R. micrantha* Sm., *R. nitidula* Besser, *R. rubiginosa* L., *R. subcanina* (Christ) Vuk., and *R. vosagiaca* N.H.F. Desp., and identified (Rosu, 2011).Samples were collectedfrom spontaneous flora of Northeast and Southeast Romanian regions from different altitudes ranging from 3 to 902 m (Table 1). Rose hips were harvested from the end of September to mid of October depending on the ripening period. The fruits were picked at the fully ripe mature stageas judged by their colour and the average samples (3 replicates) were randomly chosen from 100 fruits. The selected samples were mixed together for a homogenous distribution. Chemical composition (flavonoid and total polyphenol) and DPPH activity of dry powder obtained from whole wild rose hips fruits (seeds together with the fruit flesh) were analyzed.

Reagents. All the reagents (Folin-Ciocalteau, 2,2-diphenyl-1-picrylhydrazyl or DPPH, aluminium chloride, sodium nitrite, gallic acid and catechine) and solvents used were purchased from Sigma-Aldrich (Madrid, Spain), Fluka (Buchs, Switzerland) and Merk (Darmstadt, Germany).

Preparation of plant extract

Extraction was performed with methanol and about 0.02g of dry rose hip samples were homogenized with 80% methanol. Then it was stirred for 30 minutes and centrifuged at 3000 rpm for 15 minutes at 4°C. The supernatants were used for the further determinations.

Table1. The *Rosa spp*. fruits collected from spontaneous flora of North East and South East Romanian regions.

Taxa under study	*Sampling sites/Altitude (m)*
R. nitidula	Gradinita 1 (902m), Vatra Dornei (807m), Rusca 1 (777m), Rusca 4 (774m), Bistrita 1 (770m), Bistrita 3 (690m), Agigea 1 (3m)
R. caesia	Sadova 1 (885m)
R. vosagiaca	Sadova 6 (884m), Dorna Candreni 2 (830m)
R. subcanina	Sadova 4 (880m), Bicaz Chei 1 (685m), Agigea 2 (12m)
R. canina	Sadova 5 (875m), Dorna Candreni 1 (820m)
R. rubiginosa	Ceahlau 1 (860m)
R. micrantha	Dorna Candreni 4 (850m), Bicaz Chei 3 (668m), Sucevita 2 (595m)
R. corymbifera	Dorna Candreni 3 (840m), Sucevita 1 (691m), Dorna Candreni 5 (830m) Agigea 3 (12m)

Total polyphenols assay

The total polyphenols content was determined by using of modified Folin-Ciocalteau method (Singleton et al., 1999). The appropriately diluted sample was mixed thoroughly with Folin-Ciocalteau reagent.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 milligram gallic acid equivalent per g of dried weight (mg GAE/g DW) (R^2=0.99). Three readings were taken for each sample and the results averaged.

Total flavonoids assay

The flavonoids contentwas measured following a spectrophotometric method (Dewanto et al., 2002). Briefly, methanol extracts 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). The flavonoids content was expressed as mg catechin equivalents per g of dry weight (mg CE/g DW) (R^2 =0.97). Three readings were taken for each sample and the results averaged.

DPPH Free radical scavenging activity

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The DPPH radical scavenging capacity of each extract was determined according to the method of Molyneux (2004) modified by Shirwaikar et al. (2006). 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 2ml of this solution was mixed with 20 µl of the methanol plant extracts. The control (without any antioxidant) contained 80% methanol and DPPH solution. The decrease in the absorbance of the formed blue to violet reagent (product) was determined after 20 min at 517 nm and the percentage (%) of inhibition activity was calculated using the following formula:

DPPH free radical scavenging activity (%inhibition) = (1-AE/A0)x100

where *AE* is the absorbance of the sample with extract; *A0*is the absorbance of DPPH solution with ethanol.

Statistical analysis

All experiments were carried out with three independent repetitions, the results were calculated as means ± standard errors (SE) and differences between means were assessed using ANOVA test.

RESULTS AND DISCUSSION

The amount of metabolites present in a given plant may be influenced by biological and environmental factorsas well as by biochemical, physiological, ecological and evolutionary processes (Harborne, 1993). Altitude, among other external factors, has also an effect on the contents of secondary metabolites in higher plants. In addition to incurring many climatic differences, altitude influences the quality of radiation. Especially, UV-B radiation is high in alpine sites compared with lower habitats (Barnes et al, 1987). Moreover, Zidorn (2010) showed that enhanced UV-B radiation is probably not the key factor inducing shifts in the phenolic composition in *Asteraceae* studied plants growing at higher altitudes but it is rather the temperature which decreases with altitude.

Total polyphenol content

The total polyphenol content, expressed as gallic acid equivalents (GAE) of the methanolic extracts ofeight rosehips wild genotypes, showed a great variability (Figure 1).Thus, in *R. nitidula* fruits the total polyphenol contents varied in a range of 83.02 GAE/g DW to 142.83 GAE/g DW, even the fruits were harvested from two very close altitudes (807m and 774m, respectively). Among all analyzed genotypes, the *R. rubiginosa* revealed the maximum total polyphenol content of 144.36±4.55 mg GAE/g DW in rosehips, while in *R. caesia* was measured the minimum value of 61.72±4.04 mg GAE/g DW. Both *Rosa sp.* genotypes were collected also, from close altitudes of 860m and 885m, respectively.

The polyphenol content in *R. micrantha* and *R. canina* rosehips increased with an increase in altitude from 106.04 mg GAE/g DW (at 595m) to 114.92mg GAE/g DW (at 850m) and from 95.05 mg GAE/g DW (at 820m) to 100.38 mg GAE/g DW (at 875m), respectively. Moreover, the same trend was observed in *R. corymbifera* rosehips, and the content varied from 96.28 mg GAE/g DW (at 12m) to 110.27 mg GAE/g DW (at 840m). On the other hands, in other genotypes as *R. subcanina* and *R. vosagiaca,*the polyphenol content diminished with the increase of altitude from 119.73 mg GAE/g DW (at 12m) to 77.96 mg GAE/g DW (at 880m) and from 103.67 mg GAE/g DW (at 830m) to 95.66 mg GAE/g DW (at 884m), respectively.

Long-term consumption of polyphenol-rich fruits and vegetables suggests protection of human health according to epidemiological studies and associated meta-analyzes, which is why these secondary metabolites are of high scientific interest (Pandey and Rizvi, 2009, Oprica, 2016).

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The antioxidant capacity of phenolic compounds is mainly due to their redox properties. For this reason, they are believed to be the major phytochemicals responsible for the antioxidant activity of plants (Somaye et al., 2012). At higher altitudes, the higher solar radiation has an impact on secondary metabolite profiles. Turunen and Latola, 2005 reported an increase in phenolic compounds with increasing altitude as a response to increasing UV radiation. The authors showed that the alpine timberline plants are generally, adapted to UV-B, but on the other hand, alpine timberline plants of northern latitudes may be less protected against increasing UV-B radiation than plants from more southern latitudes and higher elevations. Meanwhile, some authors described a positive correlation (R^2= 0.55) between altitude and total polyphenols content in wild bush tea (*Athrixia phylicoides* DC.) (Nchabeleng et al., 2012) and *Hedychium spicatum* Buch. (Sandeep et al., 2011). Giorgi et al., (2010) conducted a detailed evaluation regarding the effect of environmental growth conditions on the antioxidant capacity and total phenolic content of *Achillea collina* collected from two different altitudes (600 and 1050 m). They found that growing at high altitude may constitute an effective way to significantly enhance of yarrow composition quality for both medicinal and nutritional uses.

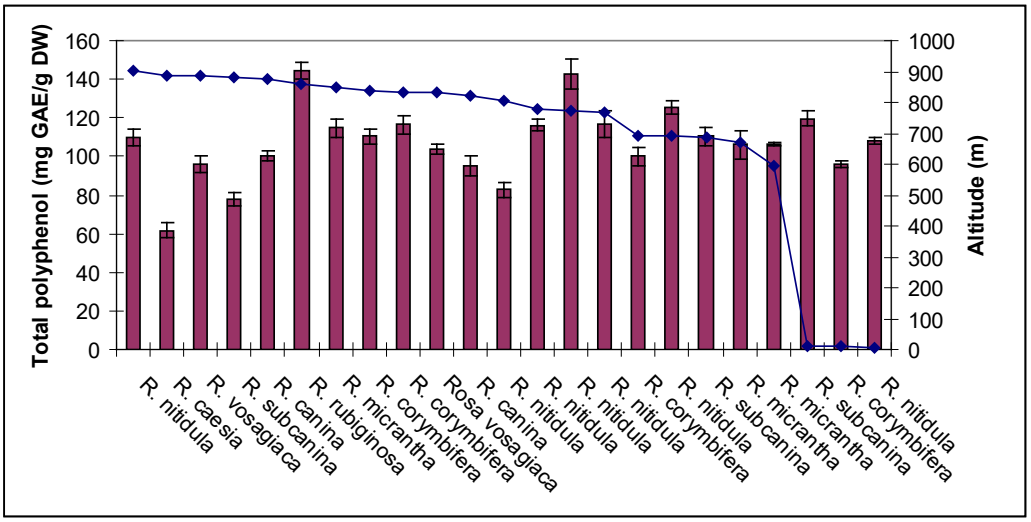

Figure1. Variation of total polyphenol content in eight *Rosa* genotypes collected from different altitude of Romania regions.

Fascella et al. (2019) reported a significant variability in bioactive compounds and antioxidant activity among the rose species collected from spontaneous Sicilian flora. The highest total polyphenol contents (6784.5 and 6241.2 mg GAE/100 g DW, respectively) and highest antioxidant activities were found in *R. canina* and *R. sempervirens* rose hips.

Many studies have reported that rose hips are rich in biologically active compounds (Ercisli, 2007; Chrubasik et al. 2008; Demir et al., 2014; Cunja et al., 2016) with positive effect on health. Barros et al. (2011), also reported the phenolic were the major antioxidant components (247.03-701.65 mg GAE/g 393 of extract). Recently, Koczka et al. (2018) identified the highest total phenolic content (766.0 mg GAE/100 g DW) and antioxidant capacity in ethanolic extracts

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of *R. spinosissima* followed by *R. canina, R. rugosa* and, *R. gallica* and confirmed their potential as functional foods.

Flavonoids content

The content of flavonoids, expressed as catechin equivalent (CE), varied among the wild rosehipsfrom 7.32 to 19.45 mg CE/gDW (Figure 2). The highest content was measuredin*R. Nitidula* rosehips harvested from 774m, while the lowest value was noticed at *R. caesia* from higher altitude (885m).The flavonoids content varies within the genotype and also between genotypes depending on altitude. Therefore, in some genotypes, the content of flavonoids increased (*R. canina, R. micrantha* and *R. corymbifera*) with increasing altitude while in others it was observed a decrease (*R. subcanina*). Between genotypes, the highest content of flavonoids was observed in *R. corymbifera* at 830 m (14.51 mg CE/g DW) while the lowest content at 820m (10.85 mg CE/g DW). With regard to the genotypes *R. micrantha* and *R. canina*the flavonoids content increased with increasing altitude from 12.15 mg CE/g DW (595m) to 15.47 mg CE/g DW (850m) and from 11.49 mg CE/g DW (820m) to 12.53 mg CE/g DW (875m), respectively.In the case of *R. subcanina* genotype, the flavonoids content ranged between 15.54 and 9.38 mg CE/g DW at altitudesof 12m and 880m, respectively. Moreover, in some genotypes like *R. vosagiaca* picked up from the two altitudes, the content was almost identical (12 mg CE/g DW). At *R. nitidula,* between all wild rosehips collected was remarked a no uniform variation of flavonoids content depending on the altitude (902m and 3m) which ranging between 12.02 and 19.45 mg CE/g DW.

Depending on the standard used, the literature data mentioned that flavonoid content for *R. canina* was 14.71 mg/100 g extract (Daels-Rakotoarison et al. 2002), 0.33 ± 0.01 mg RE/ml (Ghazghazi et al., 2010) and 23.6 ± 4.2 mg quercetin/g extract (Montazeri et al., 2011).

In our work, the observed interspecific differences were significant in both of polyphenol (Fig. 1) and flavonoids contents (Fig. 2). Flavonoids have indeed the capacity to absorb the most energetic solar wavelengths (UV-B and UV-A), inhibit the generation of reactive oxygen species (ROS) and then quench ROS once they are formed (Brunettiet al., 2013).

In Romania, Roman et al. (2013) evaluated the amount of total phenols and total flavonoids in eight rose hip extracts from wild Transylvania populations. They reported a total polyphenols content from 575 mg/100 g frozen pulp (var. transitoriaf. ramosissima) to 326 mg/100 g frozen pulp (var. lutetianaf. fallens) and the highest value of total flavonoids of 163.3 mg/100 g frozen pulp (var.assiensis) of *Rosa canina* L. Also, they found a correlation (R^2=0,802) between the ascorbic acid content of several *Rosa canina* L. biotypes and the altitude that suggests that the content in vitamin C increase with altitude.

Also, Soare et al. (2015) determined a total phenolic content between 35.43-48.07 mg GAE/g, the antioxidant activity of maximum 363.64 mTE/100 g sp. and a flavonoid content of maximum 672.67 mg/100g in Rosehips genotypes from the spontaneous flora of Oltenia (Romania).

In a phytochemical study about *Artocarpus gomezianus*fruits collected from different altitudes of Central Western Ghats (Krishnamurthy and Sarala, 2013),the screenings of secondary metabolites revealed the presence of alkaloids, phenols, flavonoids, tannins, steroids and saponins in the fruit samples of all the regions. There was shifting among the flavonoids and phenols in the middle lower and middle higher altitudes. In contrast to phenols, tannins, and

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steroids, the concentration of flavonoids was more in the middle and lower in higher altitudes but lowest in higher and lower altitudes.

Monschein et al. (2010) described the phytochemical compounds of *Calluna vulgaris* collected from different altitude. They found that within phenolic compounds, flavonols showed significant differences in samples collected at different altitudes with increased levels of quercetin glycosides at higher altitudes whereas no significant correlation could be found for caffeoyl quinic acids. Bernal et al. (2013) showed that the overall amount of phenolic acids and neolignan of entire leaves of *Buxus sempervirens* L. increased with altitude while the total amount of flavonoids in leaf cuticles decreased. Another study revealed that the total flavonoid content of *Hypericum perforatum* L. was positively correlated with altitude (Badgonaite et al., 2007, Tekel'ova, 2000).

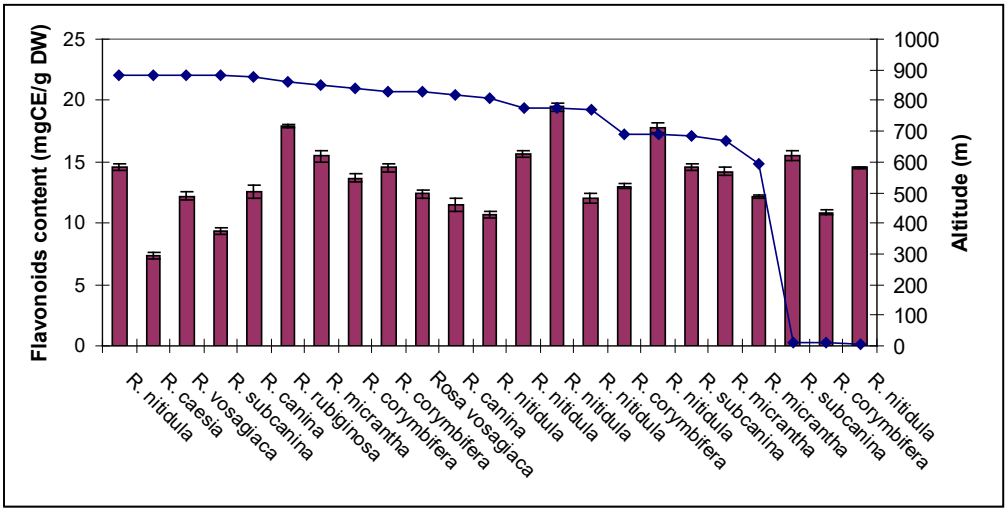

Figure 2. Variation of total flavonoids content in eight *Rosa* genotypes collected from different altitude of Romania regions

DPPH free radical scavenging activity

DPPH is a compound that possesses a nitrogen - free radical and is readily destroyed by a free radical scavenger. For this reason, it was tested the ability of the antioxidant compounds of *Rosa* genotypes who functioning as proton radical scavengers or hydrogen donors.

The screening of methanolic extractsfrom *Rosa* genotype taken into study revealed a wide variation. The DPPH free radical scavenging capacitiesexpressed as (% inhibition) is ranging from 35.44±0.22 % to 77.87±0.05 % (Table 2). The strong inhibition was also observed for the methanolic extract of *R. nitidula* while the lowest inhibition was observed at *R. subcanina*. Among all those seven *R. nitidula* collected, maximum DPPH radical scavenging percentage was 77.87±0.05% at 774m altitude while the minimum was 48.65±0.11% for genotypes collected from 807m. At this species were not noticed correlations between antioxidant activity and altitude, the values being variable.

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At *R. corymbifera* it was observed an increase of antioxidant activity with the increasing altitude from 53.82±0.39% (at 12m) to 73.97±1.11% (at 830m). On the contrary, at *R. subcanina* the antioxidant activity increased with the decrease of altitude 61.79±0.05% (at 12 m) to 43.37±0.22% (at 880m).

Table 2. Relationship between DPPH radical-scavenging ability and altitude in eight *Rosa* genotypes collected from Romania regions

Taxa under study	*Sampling sites*	*Altitude (m)*	*DPPH free radical scavenging activity (% inhibition)*
R. nitidula	Gradinita 1	902	68,07±0,028
R. nitidula	Vatra Dornei	807	48,65±0,11
R. nitidula	Rusca 1	777	68,25±0,27
R. nitidula	Rusca 4	774	77,87±0,05
R. nitidula	Bistrita 3	690	77,79±0,16
R. nitidula	Bistrita 1	770	54,85±0,27
R. nitidula	Agigea 1	3	63,24±0,44
R. caesia	Sadova 1	885	35,44±0,94
R. vosagiaca	Sadova 6	884	54,65±0,55
R. vosagiaca	Dorna Candreni 2	830	63,64±0,11
R. subcanina	Sadova 4	880	43,37±0,2
R. subcanina	Bicaz Chei 1	685	61,31±0,055
R. subcanina	Agigea 2	12	61,79±0,05
R. canina	Sadova 5	875	65,93±0,78
R. canina	Dorna Candreni 1	820	54,06±0,27
R. rubiginosa	Ceahlau 1	860	76,81±0,44
R. corymbifera	Dorna Candreni 3	840	69,87±0,22
R. corymbifera	Dorna Candreni 5	830	73,97±1,11
R. corymbifera	Sucevita 1	691	66,83±0,055
R. corymbifera	Agigea 3	12	53,82±0,39
R. micrantha	Dorna Candreni 4	850	71,6±0,22
R. micrantha	Bicaz Chei 3	668	54,81±0,33
R. micrantha	Sucevita 2	595	61,23±0,16

Antioxidants are secondary metabolites and their contents in plants depend on varied stress conditions of vegetation (Verpoorte et al. 1999). Antioxidant activity depends on the manner in which the extracts are prepared. For this reason Buřičová and Réblová (2008), found for *R. canina* different values of antioxidant activity using DPPH radical depending on the water (62.7 mg/g) or ethanol plant extracts (6.3 mg/g). On the other hand, Wenzig et al. (2008) found that the radical scavenging activity of the methanolic extracts of *Rosa canina* was 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.

In a study regarding the scavenging activity on DPPH radical on different concentration of *R. canina* infusion, Kilicgün and Altiner (2010) reported that the activity increased

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significantly as a result of increasing concentration. Meanwhile, the scavenging activity dramatically decreased at higher concentrations suggesting that the same plant that optimised antioxidant capacity may also act as a prooxidant in different test systems, depending on its concentration.

The relation between the polyphenols content (mg GAE/g DW) and the antioxidant capacity (% inhibition) was determined by using linear correlations. There was a good linear correlation ($R^2 = 0.896$) between the total polyphenols content and the scavenging radical of rose hip methanolic extract (Fig. 3). So, the radical scavenging capacity of each extract could be related to their concentration of phenolic hydroxyl groups. Our results were in accord with those of Roman et al. (2013) which found a correlation of the polyphenolic compounds to DPPH in rose hip fruits and a close values of coefficient $R^2 = 0.713$.A good correlation was also found, between the flavonoids content (mg catechin/g DW) and the radical scavenging capacity ($R^2 = 0.883$) (Fig. 4).

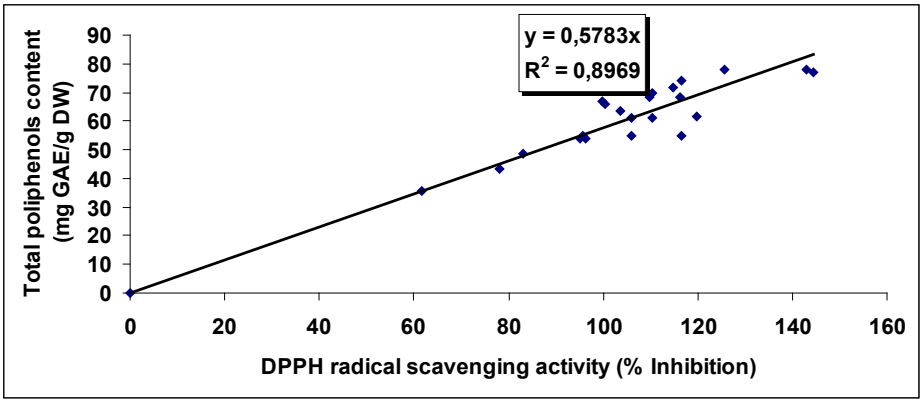

Figure 3.Correlation between total polyphenols and DPPH radical scavenging activity

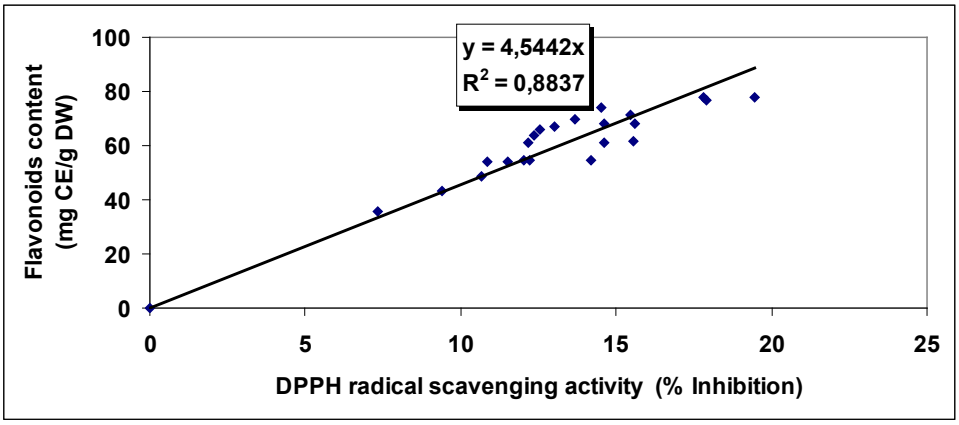

Figure 4.Correlation between flavonoids content and DPPH radical scavenging activity

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CONCLUSIONS

The altitudinal variations of bioactive compounds are not easily studied due to the variety of possible additional factors, which can also lead to secondary metabolites profile variation.

The antioxidant compounds and antioxidant activity of eight eight rosehip genotypes collected from different altitudes of Northeast and the Southeast Romania regions have registered a large variation from 3m to 902m. The polyphenol and flavonoids content increased with the increase of altitude in the same genotype (*R. canina, R. micrantha,* and *R. corymbifera*). Regarding the decrease of polyphenol content with the increase of altitude, there were identified two genotypes *R. vosagiaca* and *R. subcanina*. In case of decrease of flavonoids content with the increase of altitude, there was only *R. subcanina*.

Generally, these wild rosehips fruits had high antioxidant capacities with an average of inhibition around 62%.The radical scavenging capacities of Rosa genotypes extracts were not positively correlated with altitude than at R. *corymbifera* where it was observed an increase of antioxidant activity with the increase of altitude.

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