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Original Article

Bioactive Content of Rose Hips of Different Wildly Grown *Rosa dumalis* Genotypes

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Abstract

A rose hip is the fruit of a rose plant and mostly belongs to *Rosa canina* and *Rosa dumalis*. These species are genuine found as wild in natural conditions, while in some countries are cultivated for their hips that are picked and used in a wide variety of preparations. Because they contain a variety of antioxidants, carotenoids, flavonoids, polyphenols, leucoanthocyanins and catechins, rose hips are considered to be a good cancer preventative. The *Rosa* genus has been the focus of many recent studies due to its potential benefic effects on treatment and prevention of several diseases. However, there are few *in vitro* studies concerning its composition and antioxidant capacity. The aim of the present study was to determine bioactive content, including ascorbic acid, total phenolics, total flavonoids, total carotenoids and antioxidant activity of *Rosa dumalis* genotypes growing wild in Erzurum province located eastern Anatolia. The results revealed that fruits of *Rosa dumalis* different genotypes were rich rich in terms of vitamin C, which ranged from 402 to 511 mg/100 g fresh weight base. The total phenolic content varied from 297 to 403 mg/100 g fresh weight. The genotype 'E-09' had the highest total flavonoids content (229 mg quercetin equivalent/100 g fresh weight) and the lowest value was attributed to genotype 'E-04' with 143 mg/ quercetin equivalent/100 g fresh weight. The antioxidant activity of the genotypes was between 12.9-28.6 µg Trolox/ml samples. The results revealed that there was enough diversity among *Rosa dumalis* genotypes for bioactive content and promising genotypes, with high bioactive content, were determined, which can become study material for future breeding activities.

Keywords: biological activity, carotenoids, flavonoids, rose hip

Introduction

There are various neglected and underutilized fruit tree species grown in different parts of the world, while these fruit species could be exploited directly as foods, or used to obtain valuable natural compounds and derivatives (Kaczmarska *et al*., 2015; Mishra *et al*., 2015).

Among these wild edible fruits, rose hip belongs to *Rosa* genus has special emphasize because it has a high vitamin C content among wild and cultivated fruits (Ercisli, 2005).

The genus *Rosa* contains approximately 100 species that are widely distributed in Europe, Asia, the Middle East and North America. The Anatolia region of Turkey is one of the major genetic diversity centers of *Rosa* species (Nilsson, 1997) whereas most of the rose species growing in this area have arisen from seed. Twenty-five rose species have been reported in Turkey (Ercisli, 2005). These 25 species are distributed over more than half the country and the Eastern and Middle Anatolia region has the largest native rose population (Ercisli, 2005). In most parts of Anatolia, wild roses have been gathered for their fruits from scattered sites since ancient times. The domestication of native rose hip species to become a horticultural crop in Turkey began in the 1990s.

Rose hips could be consumed fresh (seldom) or processed in various ways. The fruit of the wild rose, the rose hip, is an excellent source of total phenolics (Hvattum, 2002), vitamin C (Sen and Gunes, 1996), carotenoids (Hornero-Mendez

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and Minquez-Mosquera, 2000), sugars (Uggla *et al*., 2005) and mineral elements (Szentmihalyi *et al*., 2002). The fruits are commonly used to make jam, marmalade, fruit juice, etc. (Uggla and Nybom, 1999), while the dried fruits and roots are excellent for making tea (Sen and Gunes, 1996).

Indigenous knowledge of wild edible fruits is important for sustaining utilization of those plant species. It is important for people to know the prevailing traditional food plants in their areas and how they can be improved for sustainable food security/nutrition. Wild edible plants, including their fruits, are very important for the wellbeing of the populations in rural areas, not only as sources of supplemental food, nutritionally balanced diets, medicine, fodder and fuel, but also for developed methods of resource management, which may be fundamental to the conservation of some of the world's important habitats (Ercisli and Esitken, 2004; Kaczmarska *et al*., 2015; Mishra *et al*., 2015).

The rose hip is the *pomaceous* fruit of the rose plant that typically is red-to-orange, but might be dark purple-to-black in some species. It is well known as aromatic and medicinal plant. The rose hip has been used both for food and for medicinal purposes throughout Turkey for a long time (Ercisli, 2005). Recently, this plant has been the focus of several studies due to its human health nutraceutical properties and the beneficial health effects observed which might result from specific compounds extracted from the fruit, such as polyphenols, carotenoids, vitamin E, flavonoids, vitamin C with antioxidant potential (Serteser *et al*., 2008; Montazeri *et al*., 2011; Fattahi *et al*., 2012; Roman *et al*., 2013).

The medicinal functions of Rosaceae fruits may be partly attributed to their abundance of phenolics. Phenolics possess a wide spectrum of biochemical activities, such as antioxidant, antimutagenic, anticargionogenic effects, as well as ability to modify gene expression (Tapiero *et al*., 2002; Nakamura *et al*., 2003).

Studies on antioxidants have reported the use of isolated nutrients in the treatment and prevention of diseases. However, a large variety of compounds that can act synergistically in protecting cells and tissues are found in foods (Bianchi and Antunes, 1999). Phenolic compounds such as flavonoids are the most active and common antioxidants present in fruit and vegetables (Nijveldt *et al*., 2001). These compounds have an antioxidant function which result from a combination of chelating properties and scavenging of free radicals as well as inhibition of oxidases and other enzymes (Trueba, 2003; Alonso *et al*., 2004).

Considering the increase in cultivation process of this plant and the lack of scientific studies regarding the bioactive composition of rose hip fruits and its beneficial implications in treatment of diseases, the aim of the present study was to determine the total phenolic, total flavanoids, total carotenoids, vitamin C and antioxidant capacity in fruits of 10 *Rosa dumalis* genotypes in Turkey.

Materials and Methods

Biological material

A total of 100 fresh fruits from pre-selected 10 *Rosa dumalis* genotypes were collected from the naturally growing areas in Erzurum province of Turkey at the end of September 2014. The taxonomic identification of the collected plant materials was done by a plant taxonomist from the Department of Biology, Ataturk University, Erzurum, Turkey. The collected fruit materials were kept in cold chain and promptly transported to laboratory for further analysis. Rose hips without calyxes were washed several times with water and kept in a freezer at -20 °C. Whole rose hips were used for biochemical analysis (vitamin C, total phenolic, total flavonoids, total carotenoids and antioxidant activity assays).

Preparation of fruits

For each genotype, 100 fruits were thawed together as 5 replicate, kept at room temperature and homogenized in a standard food blender.

Vitamin C

Homogenates were assayed for Vitamin C determination. Ascorbic acid (Vitamin C) of samples was quantified with the reflectometer set of Merck Co (Merck RQflex) and expressed as mg/100 g fresh weight.

Extraction

After thawing to room temperature, triplicate of 100 g lots of rose hip fruits from each genotype were homogenized in a blender and they were screened for their total phenolic, total carotenoid, total flavonoid and antioxidant capacity, following a single extraction procedure (Singleton and Rossi, 1965). For this procedure, 3 g aliquots of each homogenate were transferred to polypropylene tubes and extracted with 20 mL of extraction buffer containing acetone, deionized water and acetic acid (70:29.5:0.5 v/v), for one hour.

Total phenolics

Total phenolics were determined colorimetrically using Folin-Ciocalteu reagent as described by Slinkard and Singleton (1977). The results were expressed as mg gallic acid equivalent/100 g FW (fresh weight).

Total flavonoids

The total flavonoids content was measured by the $AlCl_3$ colorimetric assay according to Zhishen *et al*. (1999). An aliquote (0.1 ml) of extracts was added to 10 ml volumetric flask containing 4 ml ddH_2O. Then, 0.3 ml 5% $NaNO_2$ solution was added. After 5 minutes 0.3 ml 10% $AlCl_3$ solution was poured into the flask and maintained for another 6 minutes, after which 2 ml 1M NaOH solution was added. The total volume was completed up to 10 ml with ddH_2O. The solution was mixed and the absorption was measured at 510 nm. The total flavonoids content was calculated using a calibration curve, and then expressed as mg quercetin equivalent/100 g fresh fruit. Samples were analysed in triplicates.

Total carotenoid content

The total carotenoid content was determined by Reyes *et al*. (2007). The spectrophotometer was blanked with acetone and the absorbance of the samples in 1 cm quartz cuvettes was measured at 470 nm. The carotenoids were quantified as β-carotene using a standard curve for this compound (1-4 μg/ml). Results were expressed as mg β-carotene equivalent per 100 g fresh weight.

Antioxidant activity (DPPH)

The DPPH scavenging activity assay was registered according to the method reported by Brand-Williams *et al.* (1995). 80 µM of DPPH solution was freshly prepared in 98% methanol and sonicated for 15 min. following, 2,800 µl of DPPH solution were allowed to react with 400 µl sample and the absorption was measured at 515 nm, for 60 min on a spectrophotometer. The chemical kinetics of the extracts was recorded and the antioxidant capacity was calculated using a calibration curve, expressed in µM Trolox/ml. The antioxidant activity was calculated as follows:

$$\%\text{DPPH scavenging activity} = (1\text{-}[A_{sample}/A_{kontrol\ t=0}]).100$$

Statistical analysis

All data were analyzed using SAS software and procedures (Chicago, IL). Analysis of variance tables were constructed using the Least Significant Difference (LSD) methods at 0.01.

Results and Discussion

The results indicated that there were significant differences among *Rosa dumalis* genotypes for all studied physicochemical characteristics of fruits (Table 1).

The average fruit mass and flesh ratio values were determined to be between 2.78 g ('E-06') and 4.24 g ('E-07') and 63.3 ('E-03')-74.4% ('E-05'), respectively (Table 1). Previous studies conducted on different rose hip from genotypes belonging to different *Rosa* species growing in Turkey showed that the average fruit mass and flesh ratio were variable and ranged between 0.61-7.77 g and respetivley 56-80% (Balta and Cam, 1996; Kazankaya *et al*., 2002; Ercisli and Esitken, 2004). The fruit mass and flesh ratio values found in the current study samples were mostly of the same order of magnitude as in previous published data on rose hips.

Soluble solid content (SSC) of genotypes varied from 17.6 ('E-06') to 22.8% ('E-10') (Table 1). Previously SSC content of rose hip fruits was reported between 12-36% (Balta and Cam, 1996; Kazankaya *et al*., 2002; Ercisli and Esitken, 2004; Celik *et al*., 2015). The hereby results on SSC were in agreement with above literatures.

Among the genotypes under study, 4 out of 10 were found to be low thorny while the rest of the genotypes were medium thorny. Previous studies also indicated that the majority of rose hip bushes had medium thorn (Balta and Cam, 1996; Gunes and Dolek, 2010). All above studies indicating that rose hips grown in different parts of Anatolia differ among each other in terms of fruit mass, flesh ratio, thorn and SSC. Growing regions, cultural applications and in particular genetic background may have contributed on the indicated parameters of rose hips.

The bioactive content of the analyzed *Rosa dumalis* genotypes is presented in Table 2. Statistically significant differences were found in the level of vitamin C, total phenolics, total flavonoids, total carotenoids and antioxidant activity among the assayed genotypes ($p < 0.05$) (Table 2).

The vitamin C content of rose hip fruits was very high, ranging from 402 to 511 mg per 100 g fresh weight (Table 2). The edible portion of rose hip fruits was characterized by very high vitamin C content. In previous studies, vitamin C in rose hip fruits reported was quite variable, with an interval of range from 180 to 965 mg per 100 g fresh weight (Balta and Cam, 1996; Kazankaya *et al*., 2002; Ercisli and Esitken, 2004; Roman *et al*., 2013; Celik *et al*., 2015). It is revealed therefore that Vitamin C depends on species, cultivars, maturation stage, altitude etc.

The total phenolic contents of *Rosa dumalis* genotypes were in the range of 297-403 mg GAE per 100 g fresh weight (Table 2). A wide variation was observed on total phenolic content in fruits of rose hip genotypes bu other researchers, variability that ranged between 326 and 818 mg GAE per 100 g fresh weight (Yoo *et al*., 2008; Roman *et al*., 2013). The phenolic content and composition of fruits and vegetables depends on the genetic and environmental factors, as well as post-harvest processing conditions (Landmark and Alm, 2006). Plant phenolics are the largest class of plant secondary metabolites, which, in many cases, serve in plant defense mechanisms to counteract the reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores (Prior *et al*., 1998).

Total flavonoid contents of *Rosa dumalis* genotypes ranged from 143 ('E-04') to 229 ('E-09') mg quercetin equivalent per

Table 1. Physicochemical characteristics of rose hips of different wildly grown *Rosa dumalis* genotypes

Genotypes	Fruit mass (g)	Flesh ratio (%)	Thorn	Soluble solid content (SSC, %)
'E-01'	2.86	66.9	Medium	20.2
'E-02'	3.98	70.8	Low	18.1
'E-03'	3.87	63.3	Low	21.4
'E-04'	3.02	67.8	Medium	18.3
'E-05'	4.13	74.4	Low	19.7
'E-06'	2.78	73.0	Medium	17.6
'E-07'	4.24	71.6	Medium	18.3
'E-08'	3.73	73.1	Low	21.4
'E-09'	3.67	66.2	Medium	20.7
'E-10'	3.70	69.4	Medium	22.8
LSD_{05}	0.23	4.3	Medium	1.9

Table 2. Bioactive content and antioxidant activity of rose hips of different wildly grown *Rosa dumalis* genotypes

Genotypes	Vitamin C (mg/100 g)	Total phenolic content (mg GAE/100 g)	Total flavonoid content (mg QE/100 g)	Total carotenoid (mg β-carotene equivalent/100 g)	DPPH (µg Trolox/ml)
'E-01'	463	306	157	68	27.1
'E-02'	497	355	130	72	15.2
'E-03'	503	348	169	58	21.4
'E-04'	448	366	190	62	25.3
'E-05'	478	403	229	85	12.9
'E-06'	415	388	215	60	17.7
'E-07'	402	378	201	53	19.0
'E-08'	486	341	209	70	14.1
'E-09'	511	350	218	59	16.5
'E-10'	469	297	143	47	28.6
LSD_{05}	26	4.6	16	8	2.9

100 g (Table 2). It was thus clear that the genotype influenced the extent of total flavonoid accumulation in fruits of *Rosa dumalis* in the current study. Total flavonoid content of *Rosa canina* fruits was reported previously between 101-163 mg quercetin equivalent per 100 g in Romania (Roman *et al.*, 2013). The current results were within these ranges and it can be concluded that besides other fruits, Rose hip might be also a good source of total flavonoids.

In the hereby experiment, the total carotenoid contents were greatly different among the *Rosa canina* studied genotypes and varied from and 47 to 85 *β*-carotene equivalent per 100 g fresh weight basis (Table 2). Results obtained for Rose hip genotypes indicated that rose hip fruits are also a good source of carotenoids, not only of flavonoids. Andersson *et al.* (2011) reported that rose hip fruits were very rich in terms of carotenoids and they found that carotenoid content varied greatly between harvesting times, species and year of cultivation (biotic stress), and interactions between factors. Carotenoids are important antioxidants and valuable bioactive compounds contributing to the health benefits of different foods, and rose hips are known for high bioactive content. Carotenoids are the most widely distributed group of pigments, naturally accumulating in large quantities in particular horticultural crops; carotenoids are known for their structural diversity and various functions including the brilliant red, orange and yellow colors of edible fruits (Socaciu, 2008). Carotenoids are principal pigments responsible for color, one of the most important aspects of fruit external quality.

The antioxidant capacity was highly differed among *Rosa dumalis* genotypes in DPPH assay. The genotype 'E-05' had the highest antioxidant capacity value (12.9 µg Trolox/ml) (Table 2). The lowest antioxidant activity was observed in genotype 'E-10' with 28.6 µg Trolox/ml (Table 2). This parameter is very important from the nutritive point of view because rose hip fruits are among the latest fruits harvested in Turkey (in late autumn), when there are no much fresh ripe fruit in this period. It is worth to note those rose hips are a valuable source of some biologically active compounds, including antioxidants.

Conclusions

The data of ten different genotypes of *Rosa dumalis* fruit samples, obtained within the present study, indicated that wild grown *R. dumalis* fruits represent rich sources of antioxidants, with high levels of phenolic compounds, carotenoids, vitamin C and flavonoids. Therefore, utilizing rose hips as sources of phytochemicals could offer enormous opportunities for the functional food industry. The results of the present investigation clearly indicated that the bioactive content vary within *Rosa dumalis* genotypes. The variation in bioactive content noticed in the hereby investigation can be useful in food production, health industry and future breeding programs.

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