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Study on phytochemical and horticultural traits of dog rose (*Rosa canina*) in natural habitat

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Abstract

Dog rose is a wild plant that grows throughout the world particularly in some regions of Iran, as a rich source of antioxidants, vitamins and minerals; it is interested in pharmaceutical and food industries. Thus, its phytochemical and horticultural traits including vitamin C, anthocyanins, antioxidants, flavonoids and total phenols were studied in three habitats – Eshkevarat-Rudsar, Kaleshom-Jirandeh and Rudbar-Kushk valley– at two fruit maturity stages 50% maturity and full maturity. It was found that the interaction between habitat and harvest time was significant for the amount of antioxidant, vitamin C and flavonoid. The highest antioxidant, vitamin C and flavonoid contents were obtained in Rudbar-Kushk valley at 50% maturity, in Eshkevarat-Rudsar at full maturity and in Eshkevarat-Rudsar at 50% maturity, respectively. But, the influence of habitat by harvest stage was not significant on total phenol and anthocyanin content.

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Introduction

Dog rose (*Rosa canina*) belongs to the subfamily of Rosoideae and the family of Rosaceae. Genus *Rosa* includes many species distributed all around the temperate and semi-arid regions of northern hemisphere. Dog rose is a species of rose regarded as a hermaphrodite shrub. Its small rising shrubs are perennial ranging in the height from 1-2 m with strong stems covered with hooked and lunate prickles which are strong in base. Dog rose species have been collected from different parts of Iran for breeding including *R. persica*, *R. foetida*, *R. pimpinellifolia*, *R. hemisphaerica*, *R. canina*, *R. iberica*, *R. damascene*, *R. beggerana*, and *R. orientalis*. Rose species are scattered throughout Iran and in northern regions, but their genetic diversity has not been fully understood yet (Omidbaigi, 2005). Dog rose (*Rosa canina* L.) is a perennial, wild shrub that grows on rocks and in brush in arid regions. Its height varies depending on climatic conditions. Its fruits are a rich source of vitamin C. The main environmental parameters influencing its essential oil quantity and quality include light, temperature, precipitation, day length, latitude, soil characteristics, altitude, and nutrition (Omidbaigi, 2005). Dog rose is the main species of Rosaceae that is used in most parts of Turkey. Thanks to its adaptation to harsh climatic conditions, it can be cultivated under adverse conditions. It is used as ornamental plant in rock parks in most parts of the world (Cutter, 2003).

Studies have shown that the fruits of dog rose are an ample source of invaluable compounds such as carotenoids, polyphenols, vitamin C, antioxidant pigments and strong antioxidant properties. Genus *Rosa* is known as a main product in ornamental plants industry (Senapati and Rout, 2008). Its species are used as cut flowers, pot flowers and horticultural flowers (Guterman *et al.,* 2002). Hips of dog rose have high concentration of vitamin C (300-400 mg per 100 g edible part) as compared to other fruits and vegetables. Hips reportedly have strong antioxidant property, too (Serteser *et al.,* 2008). Antioxidants are compounds that inhibit or eliminate oxygen radicals without being changed or destroyed by these radicals (Hodges, 2003). It was found that the antioxidant property of dog rose is mainly associated with phenols, carotenoids, vitamin C, and tocopherol and is influenced by genotype, climatic conditions, region, and hip harvest time (Ercisli, 2007). Ripe fruits of raspberry had stronger antioxidant activity when compared with fruits at 50% maturity. Also, antioxidant activity was stronger in fruits at 5% and 20% maturity than in fruits at 50% maturity. This increase with the maturity stage is directly related to phenol and flavonoid compounds (Wang *et al.,* 2009). Carotenoids are an extensive group of pigments found in leaves and flowers of the plants. Dog rose fruits have a high amount of carotenoids (Hodisan *et al.,* 1997). Hodisan *et al.* (1997) reported that the major carotenoid compounds in dog rose fruits included beta-carotene, lycopene, beta-cryptoxanthin, and lutein. Dog rose fruits contain a high level of carotenoids varying from 189 to 1192 µg per g dry matter in different species. Mean carotenoid concentration of rose fruits is 651 µg per g dry matter. It has been reported that the fruits of different species including dog rose contain an acceptable levels of phenolic compounds (Ercisli, 2007; Oszmianski and Chomin, 1993). Phenolic compounds are a vast group of secondary metabolites with low molecular weight that are extensively found the plants (Aherne and Obrien, 2002). Dog rose has a considerable amount of flavonoid compounds in its fruits that enhance antioxidant activity of its fruits. It has been revealed that the amount of flavonoid of native Iranian dog rose species is 23.6 mg gallic acid per g (Montazeri *et al.,* 2011). Flavonoid compounds of red raspberry were affected by cultivar and environmental parameters (Rommel and Wrolstad, 1993). Anthocyanins are polyphenolic compounds that are extensively found in plants and cause the color of fruits, leaves and flowers to be blue, purple and red. They are water-soluble pigments widely found in flowers, fruits and vegetables (Clifford, 2000). Rosaceae family can be categorized in terms of anthocyanin attributes, too. 3, 5 diglucoside is the dominant anthocyanin in Rosaceae family (Yuki *et al.,*

2000). Vitamin C is a water-soluble vitamin that is readily oxidized to dehydroascorbic acid (Groff *et al.,* 1995). The essential oil concentration of dog rose fruits in each region is determined by environmental conditions and soil characteristics.

The fruits of dog rose are source of invaluable compounds and these fruits are used for vitamin C supplying in some countries. The habitats of this shrub could impact amount of compounds and quality of fruits. So, the present study was carried out to examine the phytochemical and horticultural properties of dog rose in the mountains of Guilan province, Iran.

Materials and methods

Collection of plant samples

In order to study the phytochemical properties of dog rose, its vitamin C, anthocyanin, antioxidant, flavonoid and total phenol as well as the amount of rooting and essential oil were measured in three habitats (Eshkevarat-Rudsar, Kaleshom-Jirandeh, and Rudbar-Kushk valley with the altitudes of 1552, 1980 and 213 m, respectively). To measure qualitative traits, fruits of dog rose were harvested at two maturity stages: 50% maturity and full maturity.

Vitamin C measuring

Vitamin C was measured by titrating dichlorophenolindophenol. 2 grams of fruit tissue was ground in porcelain mortar by liquid nitrogen and then, 10 cc of 3% metaphosphoric acid was added and was titrated with sodium bicarbonate-contained 2, 6-dichlorophenolindophenol (DIP) until the appearing of pink color. Next, 1.25 g of fruit flesh tissue that had been ground in porcelain mortar by liquid nitrogen was mixed with 5 ml of ethanol:aceton solution (3:7 v/v). Then, it was put in 37°C after one hour and was infiltrated through Whatman filter paper.

Total phenol, total Flavonoid and Antioxidant capacity

Afterwards, the amount of total phenol, total flavonoid and antioxidant capacity of the extracted solution was determined by Du *et al.* (2009)'s method. Total phenol in flesh extracts was measured by Folin-Ciocalteu's method using spectrophotometer (T80+PG Instrument UV/VIS Spectrometer) (Du *et al.,* 2009). So, 50 µl of fruit extract was mixed with distilled water until reaching to the volume of 4 ml and 250 µml of folin (1:1 with water) was added. After one minute, 750 µl of sodium carbonate (20 gr/100 ml) was added. After two hours in room temperate in darkness, extraction absorption was read at 765 nm wavelength. Total flavonoid was measured by Du *et al.* (2009)'s method. First, 150 µl of extract was mixed with 1700 µl of 30% ethanol, 75 µl of 0.5-M $NaNO_2$ and 75 µl of 0.3-M $AlCl_3$. After 5 minutes, 500 µl of 1-M NaOH was added and vortex. After 10-15 minutes, absorption rate was read by spectrophotometer (T80+PG Instrument UV/VIS Spectrometer) at the wavelength of 506 nm. Total flavonoid concentration was measured in terms of Catechins standard (6.25-300 mg/l). Antioxidant capacity of extracts was determined by neutralizing property of 2,2 diphenyl-1-picrylhydrazyl (DPPH) using modified Du *et al.* 's (2009) method. Thus, 100 µl of extract was poured in small falcon tubes and 900 µl of DPPH solution (6.25×10^{-5} M) was added, out of which 50 µl was taken and mixed with 950 µl of DPPH. The resulting solution was rapidly blended and then, it was kept in room temperature in darkness for 30 minutes. After putting the blank and adjusting the instrument at zero, first only DPPH was read. The control and sample absorption rate was read by spectrophotometer (T80+PG Instrument UV/VIS) at the wavelength of 517 nm. The antioxidant capacity of extracts in terms of DPPH inhibitory percentage was calculated by the following equation:

$$\%DPPH_{sc} = (A_{cont} - A_{sample}) / A_{cont} \times 100$$

Where, $\%DPPH_{sc}$ was inhibitory percentage, A_{cont} was DPPH absorption rate, and A_{samp} was absorption rate (sample + DPPH). It should be mentioned that rose hips were used in the measurements.

The method of the difference in absorption in different pH's was used for measuring total anthocyanins of the fruits (Wroslstad, 1976). Acidic methanol (methanol:hydrochloric acid with the ratio

of 1:1 v/v) was the soluble used for extracting anthocyanins. To extract total anthocyanins, 0.5 g of rose hip was ground in mortar by liquid nitrogen and then, 3 ml of methanol in hydrochloric acid was added. The samples were put in extraction buffer for 24 hours. Then, they were centrifuged at the speed of 10 000 rpm for 15 minutes. After separating the upper liquid, they were read by spectrophotometer. Two wavelengths of 520 and 700 nm were used for reading total anthocyanins.

Results and Discussion

Antioxidant capacity

Analysis of variance (Table 1) indicated that habitat significantly affected antioxidant content of dog rose fruits ($p \leq 0.05$). In addition, the effect of maturity stage and the interaction between habitat and maturity stage was significant on antioxidant content ($p \leq 0.01$). Means comparison for the interaction between habitat and maturity stage for antioxidant content revealed that the highest antioxidant capacity (56.8 DPPH %) was obtained in Rudbar-Kushk valley at 50% maturity stage and the lowest one (45.85 DPPH %) in Kaleshom-Jirandeh region at full maturity.

Geographical conditions – month to month and year to year – can impact the variations of antioxidant capacity of fruits (Lee and Coates, 1997). It can be explained by the variation of the concentrations of flavonoids (Wang and Zheng, 2001). It was reported that maturity stage plays a key role in increasing antioxidants content (Denis *et al.,* 2010). Moderate temperatures (25-30°C) increase antioxidants content. Also, the growth of the plant under cool (12-18°C) or very hot (over 35°C) temperatures reduces antioxidants content (Wang and Zheng, 2001).

Table 1. Analysis of variance of the effect of habitat and fruit growth stage on studied traits.

S.O.V.	df	Antioxidant	Flavonoid	Vitamin C	Anthocyanin	Total phenol
Habitat (A)	2	6.2746^{*}	6.7754^{**}	6.3899^{**}	1.2010^{ns}	3.2317^{ns}
Fruit growth stage (B)	1	1.2405^{**}	22.4509^{**}	86.6634^{**}	0.6269^{ns}	1.6393^{ns}
A×B	2	7.3357^{**}	59.5343^{**}	9.7029^{**}	0.6815^{ns}	2.0132^{ns}
Error	12	30.869	1457.639	8792.583	236.170	46620.722
Total		10.821	13.82	13.82	17.59	9.66
Coefficient of variation		10.821	13.82	13.82	17.59	9.66

*, ** and ^{ns} show significance at 1% and 5% probability level and non-significance, respectively.

Flavonoid

According to the results of analysis of variance (Table 1), flavonoid content of dog rose fruits was significantly influenced by habitat, maturity stage and their interaction ($p \leq 0.01$). Means comparison for the interaction between habitat and maturity stage (Fig 2) revealed that the highest flavonoid content (226 mg/l) was observed in fruits at 50% maturity in Eshkevarat-Rudsar region and the lowest one (150.167 mg/l) belongs to fruits at full maturity in Rudbar-Kuskh valley.

According to these results, it can be stated that the fruits of dog rose contain much more flavonoid at 50% maturity stage than at full maturity stage although like other traits, this trait is affected by growth region, too. Connor *et al.* (2002) found that antioxidant content of blueberries harvested in Minnesota, Michigan and Oregon significantly varied with the location and harvest year which can be associated with local environmental conditions, e.g. the difference in temperature, ultraviolent radiation, water stress and nutrients availability. When fruits approach maturity, plants undergo complicated changes resulting in their phytochemical variations, one of which is the change in flavonoid and phenolic compounds content which are presented as continuous decline (Wang and Zheng, 2001) and/or the increase at the end of maturity stages (Serrano *et al.,* 2005). The high concentration of flavonoid compounds in immature fruits when compared with the fruits at 50% and full maturity can be related to final maturity stages during which flavonoid compounds are compressed and converted to complicated forms of phenolic compound like tannins and lignin (Ben-Ahmed *et al.,* 2000).

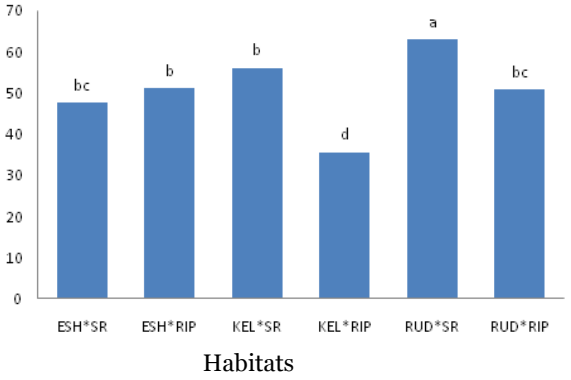

Fig. 1. Interaction between habitat and harvest time for antioxidant content of dog rose (ESH: Eshkevarat-Rudsar; KEL: Kaleshom-Jirandeh; RUD: Rudbar-Kushk valley; SR: 50% maturity; RIP: full maturity).

Vitamin C

Results of analysis of variance of vitamin C content (Table 1) showed that the effect of habitat, maturity stage and their interaction was significant on vitamin C content at 1% probability level. The highest vitamin C content was observed in Eshkevarat-Rudsar region in plants at full maturity. Also, the lowest one was obtained from plants at 50% maturity in Kaleshom-Jirandeh region. These results proved the significant impact of region and habitat climate on vitamin C content of dog rose.

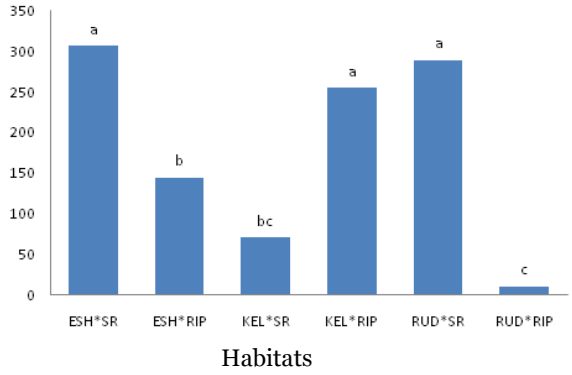

Fig. 2. Interaction between habitat and harvest time for flavonoid content of dog rose.

Vitamin C content can vary with year. These variations are more affected by climatic conditions (Olsson *et al.,* 2004). Cherry tomato fruits grown under cold climate and harvested at different times of the year exhibited a decline in vitamin C content from 44 to 16 mg per 100 g (Raffo *et al.,* 2006). The variations of vitamin C content of fruits depended on

various factors including cultivar, species, growth conditions, regional conditions, and harvest conditions. It is also influenced by environmental temperature variations, photosynthesis process, relative moisture, oxidative stresses and exposure to solar radiation. Also, it can show a decline, an increase or no changes at fruit maturity stage (Ben-Ahmed *et al.,* 2000). Soares *et al* (2007) reported that vitamin C content was increased at maturity stage which can be related to the breakdown of starch to glucose and the subsequent increase in vitamin C biosynthesis at fruit maturity stages.

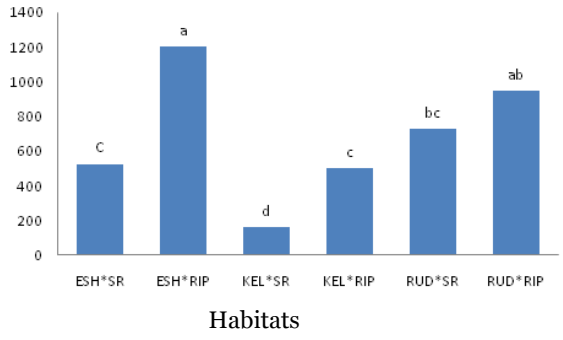

Fig. 3. Interaction between habitat and harvest time for vitamin C content of dog rose.

Anthocyanin and Total Phenol

Table 1 (analysis of variance) revealed that total phenol and anthocyanin contents were not significantly influenced by habitat, growth stage and their interaction. Like all other chemical compounds, the amount of polyphenols in fruits and vegetables are affected by many intrinsic and extraneous factors such as cultivar, harvest time, plant age, maturity stage, environmental conditions, and genetic attributes (Singh *et al.,* 2007). Various reports show that the fruits of different plants including dog rose contain appropriate amounts of phenolic compounds (Oszmianski and Chomin, 1993).

Conclusion

It was found that the interaction between habitat and vitamin C and flavonoid contents. The highest levels of antioxidant, vitamin C and flavonoid were obtained in Rudbar-Kushk valley at 50% maturity, in Eshkevarat-Rudsar at full maturity and in Eshkevarat-Rudsar at 50% maturity, respectively. However, the interaction between habitat and harvest time was not significant for phenol and anthocyanin content. According to the results of the present study and other studies, it seems that dog rose hip can be recommended for improving the health. Additionally, it is recommended to compare vitamin C and antioxidant capacity of dog rose with those of other chromatic fruits. It is suggested to examine other dog roses of Guilan province, Iran as a basis for ornamental roses.

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