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Original Article

The Determination of Flavonoid Components, Total Phenolic Content, and Antioxidant Capacity in Dog Rose (*Rosa canina* L.) in Lorestan Province

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Abstract

Background and Aim: Dog rose (*Rosa canina* L.) is a valuable deciduous shrub, which has long been used for food and medicinal purposes in various parts of the world. This study was laid out for the quantitative determination of the flavonoid components such as kampferol and quercetin, total phenol content and antioxidant capacity in petals and the fruit of the dog rose (*Rosa canina* L.) ecotype collected in Lorestan province.

Materials and Methods: Petals and fruit samples of wild dog rose were harvested in June and September 2014 respectively. The samples were kept in cold box during their transportation to laboratory. Subsequently, the pericarp and seeds were separated and dried at room temperature. The antioxidant capacities of petals and fruits were determined using DPPH method. The flavonoid components, including kampferol and quercetin, were analyzed by high performance liquid chromatography (HPLC). The total phenol and flavonoids were measured using a spectrophotometric device. The results indicate that the amount of total phenol in petals (1957 $mg.ml^{-1}$) was higher than that of fruits (937 $mg.ml^{-1}$). The amount of total flavonoid in dog rose petals and fruits were 776 and 450 $mg.ml^{-1}$ respectively.

Results: The antioxidant capacity of dog rose was measured and found to be 9.6% in fruits and 66.7% in petals. However, the fruits contained 141 $\mu g.g^{-1}$ of kampferol and petals contained 108 $\mu g.g^{-1}$. The measured quercetin in petals (127$\mu g.g^{-1}$) was higher than that of fruits (66$\mu g.g^{-1}$). In this study, a direct relation was observed between the total phenol and flavonoid contents with antioxidant capacity.

Conclusion: The results of this research showed that there are high amounts of total phenol and flavonoid contents, antioxidant capacity and also kampferol and quercetin in petals and fruits of dog rose grown in Lorestan. These results indicated the significance of dog rose in dietary and medical purposes.

Keywords: Antioxidant capacity, Kampferol, Quercetin and *Rosa canina*

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Introduction

Dog rose (*Rosa canina* L.) is one of the most commonly known species in Rosa family. Most of these species are distributed in Asia, Europe, the Middle East and the United States. Iran is one of the most important centers for Rose germ-plasm. So far, 14 different species of Rosa have been reported in Iran (1, 2).

Reactive oxygen species are one of the most important factors in causing cellular disorders and the incidence of many diseases including cardiovascular, atrophy, stroke, cancer, diabetes, stomach ulcers, eye and neurological diseases (3). Active forms of oxygen also stimulate the cell ageing due to lipid peroxidation (3).

Plants are potential sources of natural antioxidants such as polyphenols and flavonoids that had potential to inhibit of free radicals activity and can reduce the lipids oxidation (4, 5). Natural antioxidants are an important part of the cell's defense structure to confront of harmful effects of free radicals on macromolecules likes proteins, lipids, carbohydrates, and DNA. In this sense, increase the use of plant antioxidants in various forms, such as eating and drinking materials (6).

Various studies have shown that some of plant compounds in fruits and vegetables have antioxidant properties and protect the human body in front of injuries caused by reactive oxygen species (6). The human body has several enzymatic and non-enzymatic antioxidant mechanisms for protecting the vital molecules against the oxidative activity of reactive oxygen species. The alone internal defense system is not enough in front of severe oxidative stress. Therefore, should be using the external antioxidant compounds in order to balance of antioxidants level with production of oxidant compounds (5).

So far, up to 100,000 secondary metabolites, such as terpenes, steroids, alkaloids, flavonoids, and phenolic compounds have been identified in plants (7). Because that Lorestan province is one of the most important natural habitats of dog rose, the flavonoid compounds including kampferol and quercetein

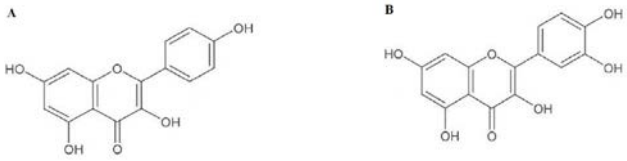

Figure 1. Chemical Structure of Quercetin (A) and Kampferol (B).

(Figure 1) and also antioxidant capacity, total phenol and flavonoid content of this valuable shrub have not been studied in this region. Therefore, the aim of the present study is investigation of flavonoid compounds, total phenol, flavonoid and antioxidant capacity in petal and fruit of dog rose.

Materials and Methods

Plant Materials

The petals and fruits of *Rosa canina* L. were collected in one of the main habitats in Shul-Abad region, Aligudarz, Lorestan province, Iran, in May and September 2013 (figure 2). The collected petals and fruits were dried at room temperature and stored at 4°C.

The Preparation of Plant Extracts for Antioxidant Property Analysis and Total Polyphenol Content

Five g of dry plant material was homogenized in 50 mL of solvent solution [methanol 80%] for 72h. The mixture was crossed from filter paper and then used to measure the total phenol, flavonoid and antioxidant capacities (8).

Estimation of Total Polyphenol Content

The plant extract (0.5mL) was mixed with 2.5 mL of 10% Folin-Ciocalteu reagent, and incubated at room temperature for 2 min. Samples were centrifuged prior to absorbance measurement. After cooling, the absorbance was measured at 765 nm with spectrophotometer. An external standard curve was prepared using gallic acid (10–250 $mg.ml^{-1}$; Roth, Karlsruhe, Germany). Each determination was performed in triplicate (9).

Flavonoid Measurement

The total flavonoid content was measured by aluminum chloride chromatography. 0.5 ml of metabolic extract was added to 0.1 ml of aluminum chloride 10% in methanol and 0.1 ml of 1M potassium acetate (2.4 ml/10 ml of distilled water). Subsequently, 1ml of distilled water was added to a Falcon tube. The

Figure 2. Flowering and Fruit in Dog Rose at the Time of Sampling to Measure its Composition in Lorestan Province.

mixture was placed in the dark condition for half an hour at room temperature and finally absorbed at 415 nm.

Free Radical-Scavenging Ability by the Use of a Stable DPPH• Radical

A volume of 50 ml of various methanolic dilutions of the rose extracts and of ascorbic acid were mixed with 150 ml of a 100mM methanolic solution of DPPH. After 30 min, absorbance of the samples was read at 517 nm in a microplate reader. Quercetin (EC50 1.56 $mg.ml^{-1}$) was used as a positive control. Each dilution was tested in triplicate (10).

Measurements of Kampferol and Quercetin by HPLC

The liquid chromatographic method used for the determination of kampferol and quercetin consisted of an isocratic elution procedure with UV-Visible detection at 290 nm. Separations were carried out on a 5 mm RP C18 column of 250 mm×4.6 mm (Spherical, Optimal ODS-H, Capital HPLC, UK) fitted with a 5 mm RP C18 guard column of 20 mm 4.6 mm (Spherical, Optimal ODS-H, Capital HPLC, UK). The mobile phase employed was a mixture of 0.5% NaH2PO4 (pH 2.25 with H3PO4). The flow rate of the mobile phase was 1.2 mL min1, and an injection volume of 20 mL was used in the quantitative analysis. Each composition was determined based on the comparison of the retention time with the standard sample peak and its value based on the comparison of under curve area of peak (11).

Results and Discussion

The results for total phenol in petals and fruit of dog rose are shown in figure 3. According to the results, total phenol in petals and fruits of dog rose were 1957 and 937 $mg.ml^{-1}$ respectively. These results indicated that dog rose petal had a higher total phenol compared to its fruit. Higher total phenol in petal shows higher antioxidant capacity in this organ. Dog rose petal had total phenol 2 times higher than fruit which shows the higher potential for phenol compound production.

The results for the total flavonoid measurement in petals and fruits of dog rose are shown in figure 4. Based on the results, the amount of total flavonoid in fruits and petals of dog rose were 450 and 776 $mg.ml^{-1}$ respectively. Organs with higher flavonoid contents had a higher antioxidant capacity that was obtained in dog rose petal. Dog rose petals notably produced flavonoid 43% higher than fruits.

The results for the measurement of free radicals' inhibition (total anti-oxidant capacity) using DPPH in petals and fruits of dog rose are shown in figure 5. Based on the results, the antioxidant capacity in fruits and petals of dog rose were 9.6% and 64.6%

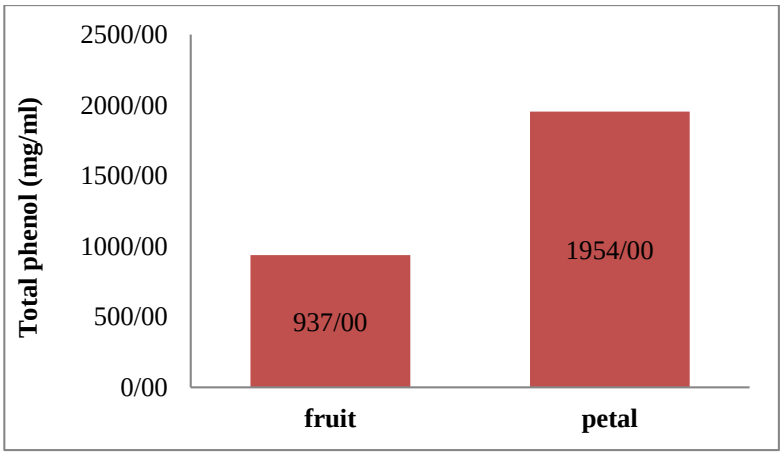

Figure 3. Comparison of Total Phenol in Petals and Fruit of Dog Rose Based on Gallic acid.

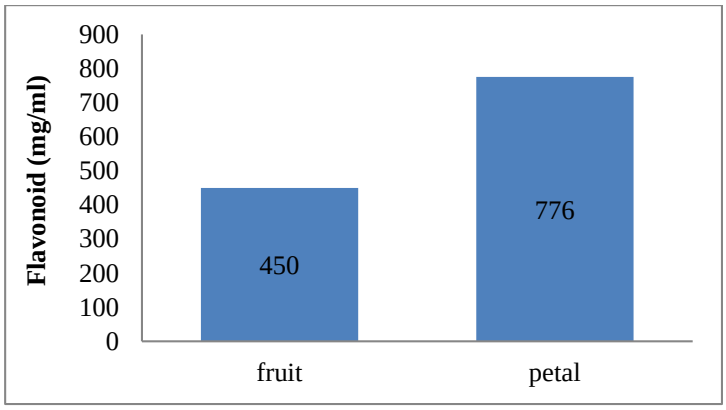

Figure 4. Comparison of Total Flavonoid Content in Petals and Fruit of Dog Rose.

respectively. These results indicated that antioxidant capacity in dog rose petals was 6.7 times higher than fruits.

The results of HPLC analysis that was carried out to identify kampferol and quercetin in fruits and petals of dog rose are indicated in figures 6 and 7 respectively. The inhibitory time for the identification of kampferol and quercetin in petals and fruits of doge rose were 8.05 and 9.9 min at 290 nm.

For the purpose of identification, the retention time of kampferol and quercetin peak in samples were compared with the standard norm in each injection. The type and amount of each substance in the samples were determined based on the retention time and the under peak curve area, and then they were compared with the standard norm. Based on HPLC, the results indicated that the kampferol in dog rose fruits and petals were 141 and 108 $\mu g.g^{-1}$ respectively (figure 8). The kampferol compound in dog rose fruits was higher than petals up to 24%.

Based on HPLC, the results showed that the quercetin in fruits and petals of dog rose were 66 and 127 $\mu g.g^{-1}$ respectively (figure 9). The quercetins in dog rose petals were significantly higher than fruit up to 49%.

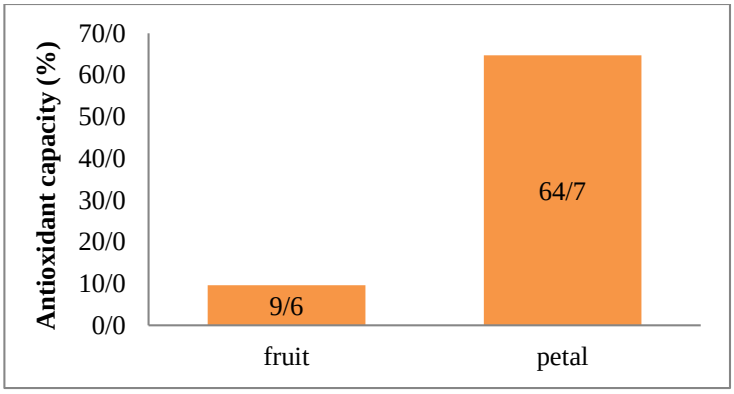

Figure 5. Comparison of Free Radicals Inhibition in Petals and Fruits of Dog Rose Using DPPH Method.

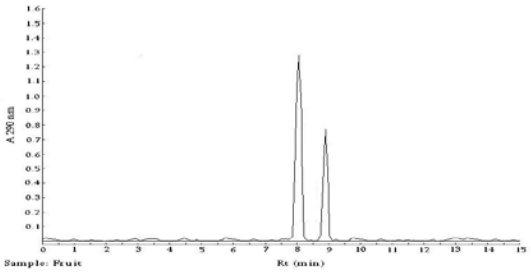

Figure 6. Chromatogram Analysis of Flavonoid, Kampferol and Quercetin in Fruits by HPLC.

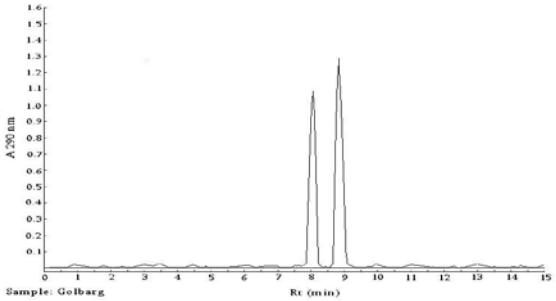

Figure 7. Chromatogram Analysis of Flavonoid, Kampferol and Quercetin in Petals by HPLC.

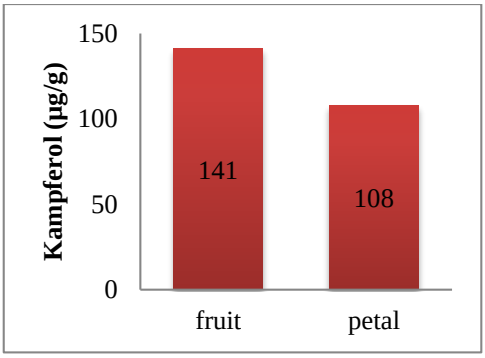

Figure 8. Comparison of Flavonoid Kampferol in Fruits and Petals of Dog Rose Using HP.

The results of the present study indicated that antioxidant capacity in dog rose is correlated with such compounds as phenols and flavonoids. However, plant organs with higher phenolic compound had higher antioxidant capacity due to antioxidant properties of phenolic compounds. The antioxidant activity of medicinal plants is directly related to the amount of phenolic and flavonoid compounds (12). Antioxidant capacity in medicinal plants is also related to secondary metabolites that are in line with the present findings. It was also noted that plants with higher phenolic and flavonoid compounds could have antioxidant properties. In this regard, Jaimand *et al.* (13) measured the amount of quercetin in different organs of Yarrow by HPLC method. They revealed that the highest and lowest quercetin concentration could be founded in Yarrow flower buds (2164 ppm) and stems (23 ppm) respectively. They also indicated that flavonoids are polyphenols affected by environmental conditions, which is consistent with present findings. However, our findings showed that flavonoid compounds in petals were higher than dog rose fruits. In this regard, some researchers have stated that the amount of quercetin in dry and non-mature fruits, leaves and flowers of the locust tree is extractable and its amount in locust flowers is higher than leaves. These results are consistent with the findings of the present study (14). Extraction of flavonoids by solvent at short time improves the quality of these compounds due to the lack of heat. The most suitable solvents for the extraction of locust flavonoids are methanol and water

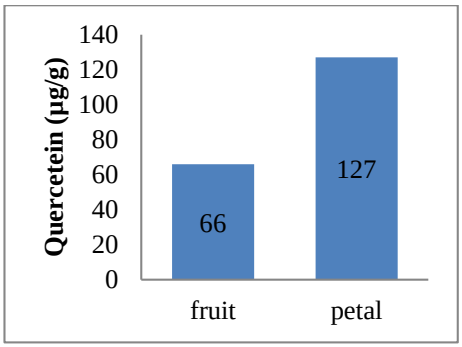

Figure 9. Comparison of Quercetin in Fruits and Petals of Dog Rose Using HPLC.

(14). In this research, 85% methanol solvent, which was a suitable solvent for the extraction of these compounds, was used for the extraction of flavonoids. It is easy to dissolve flavonoids whose extract and measurement is comfortable.

Flavonoids are biologically and medically of great significance because they are capable of having various positive functions such as blood purification, immune boosting, blood cholesterol regulation, blood pressure regulation, cancer prevention. Moreover, they have strong antioxidant, anti-radical and anti-inflammatory properties, and also have protective effects in the human body. Among flavonoid compounds, quercetin and kampferol have the most significant antioxidant properties. Flavonoids are the most prominent types of natural compounds that have been considered for special medicinal properties. In the near future flavonoids, as a kind of medicine, can play a significant role in the treatment of certain diseases. Medicinal plants are potential sources of natural antioxidants. Hence, in recent years, major studies have been conducted to investigate the potentials of plant products as antioxidant compounds against human diseases induced by the activity of free radicals (15). According to previous reports, the use of natural and herbal antioxidants has a remarkable impact on health of the human body. In these materials, some compounds such as polyphenols and flavonoids have the potential to inhibit the activity of free radicals, which could reduce the lipids oxidation (5). In the present study, methanolic extracts of both petals and fruits of dog rose had inhibitory activity against free radicals. However, the phenol present in dog rose extracts plays a significant role for the observed DPPH radical-scavenging activity. This finding is also confirmed by another research (16).

Conclusion

In the present study, two flavonoid combinations, namely kampferol and quercetin, were extracted from petals and fruits of dog rose using HPLC method. It was found out that the use of this method to measure these compounds could be useful. Furthermore, each of these organs has different amounts of both flavonoids. Based on the results of the present research, the consumption of dog rose fruits and petals can treat certain human diseases due to the high total phenol content, flavonoids, and consequently high antioxidant capacity, as well as highly valuable compositions of kampferol and quercetin. Moreover, there is higher antioxidant capacity in petals of dog rose compared to its fruits. The traditional use of petals pertains to the pharmaceutical industry. It is also used in the production of some drugs for the treatment and prevention of certain diseases.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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