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Antiradical activities and phenolic compositions of *Rosa canina* L. from Iran and Turkey

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Abstract

Rosa canina L. is a climbing wild rose plant species native to Europe, Northwest Africa, and Western Asia from the Rosaceae family. Antioxidants such as polyphenols and ascorbic acids, as well as carotenoids, Vitamin B and E, are abundant in the flesh of *Rosa canina* fruits. Plant phenolics, particularly flavonoids, have attracted a lot of attention in recent years because of their wide range of biological effects, including antiinflammatory, antiallergic, and antibacterial properties. Functions of flavonoids as antioxidants, free radical scavengers, and divalent cation chelators were recorded in the literature among others. Epidemiologic studies have shown a link between increased flavonoid antioxidant intake and a lower risk of cardiovascular disease and certain types of cancer. In this experiment, dry and fresh fruits of *Rosa canina* from two regions (Turkey and Iran) were investigated according to *in-vitro* antiradical assays such as (2,2′-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging activity and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\bullet}$). The phenolic composition of the extracts was also analyzed by HPLC and spectrophotometric methods.

Keywords

ABTS, DPPH, HPLC, phenolics, *Rosa canina*.

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INTRODUCTION

Rosaceae family, composed of over 100 species, is widely distributed in Europe, Asia, the Middle East, and North America (Nilsson, 1997). *Rosa canina* L. is a perennial shrub plant, which has a height of about 2-3 m with multiple arching stems. Fruits are smooth, deep pink and they ripen through September to October. Fruits can remain fresh on the plant for months and eventually turn black. Plants can reproduce sexually by seed, and vegetatively by suckering and layering (Nilsson, 1997; Pavek, 2012). Turkey is one of the most significant germplasm centers, accounting for approximately 25% of all rose species. These 25 species are native to Turkey and can be found anywhere from sea level to 3000 meters above sea level. *R. canina* is a hardy plant that can withstand harsh environments. It grows wildly in Turkey's numerous regions particularly in central and north-east Anatolia (Ercisli, 2005). Peasants have been gathering rose hips (fruits) from scattered sites in most parts of Anatolia since ancient times as a food source (Ercisli, 2007). In the North West of Iran, rose hips can be found as well because of its significant climate which is needed for the growth of the plant. The pseudo-fruit of *R. canina*, the rose hip (brier hip, brier, dogberry, dog rose, hip fruit, hop fruit, sweetbrier, wild brier), is an un-shaped receptacle containing various achenes. The hip is rich in flavonoids, pectin, vitamin A, B complex, C, and E, as well as minerals such as calcium, iron, selenium, and manganese. Mg, K, S, and Si have also been discovered in trace quantities. Because of its rich content, it is used as a functional food. It can be eaten raw, but there is only a thin layer of flesh and a layer of irritating hairs just below the flesh layer, making it difficult to eat. So it is preferred to be used as juice, wine, tea, jelly, jam, alcoholic beverages, marmalade, and herbal tea. Fruits can be used for cosmetic purposes as well (Yildiz and Alpaslan, 2012; Pavek, 2012).

Rose hips are very rich in phenolic compounds such as phenolic acids (protocatechuic acid, 4-hydroxy benzoic acid, syringic acid, caftaric acid, ferulic acid, 2.5-dihidroxy benzoic acid, vanillic acid, chlorogenic acid, p-coumaric acid, t-caffeic acid, and sinapic acid), flavonoids (catechin, procyanidin-B2, (−) epicatechin, 4-methyl catechol, epicatechin gallate, and t-resveratrol) and gallic acid. The most important chemical component of fruits is vitamin C (ascorbic acid) (Demir *et al.*, 2014).

Due to the high amount of phenolic compounds, fruit parts of *R. canina* are

being used for different medicinal purposes such as immunosuppressive, antioxidant (Rein *et al.*, 2004), anti-inflammatory (Winther *et al.*, 2005; Lattanzio *et al.*, 2011), anti-arthritic (Ameye and Chee, 2006), antiosteoartrit (Gruenwald *et al.*, 2019), analgesic, antidiabetic (Orhan *et al.*, 2009), cardioprotective, antimicrobial (Deliorman Orhan *et al.*, 2007), gastroprotective, and skin ameliorative activities. According to the German Commission E Monographs, the fruits can be used to treat kidney and lower urinary tract disorders, inflammatory disorders, fever, colds, and infectious diseases such as influenza, as well as gastrointestinal disorders (Blumenthal *et al.*, 1998). The large amount of phenolic compounds in the rose hip has made it a great treatment and prevention for acne, aging and other facial and beauty problems (Patel, 2013).

In the present study, it was aimed to investigate and compare the dry and fresh fruits of *R. canina* samples, collected from two different origins such as Turkey and Iran, for their antiradical activities and phenolic compositions *in vitro*.

MATERIAL AND METHODS

Plant material and reagents

Rosa canina L. fruits were collected from two different places: Ser Mountains of West Azarbayjan of Urmia city of Iran and Lake of Yayla Buldan/Denizli Turkey. All of the chemicals and solvents that were used in the assays were purchased from Sigma Aldrich as analytical grade.

Extracts preparation

5 gr of each sample was extracted with 70% methanol using sonication for 60 mins. After filtration, the samples were evaporated by rotatory evaporator at 45 °C under vacuum. Dried extracts were kept at -18 °C until analyses.

Total phenolics assay

The Folin-Ciocalteau method was used to calculate total phenols as gallic acid equivalents. First of all, 100 µL of the sample was transferred to a 10.0 mL volumetric flask containing 6.0 mL of H_2O, to which 500 µL of undiluted Folin-Ciocalteu reagent was applied. 1.5 mL of 20% aqueous Na_2CO_3 was added after 1 minute, and the amount was made up to 10.0 mL with H_2O. Except for the extract, all of the reaction reagents were present in the controls. The absorbance was estimated at 760 nm after 30 minutes of incubation at 25 °C and compared to a gallic acid calibration curve. Total phenolics were calculated as gallic acid equivalents and presented as the average of three independent studies (Singleton *et al.*, 1999).

High Performance Liquid Chromatography (HPLC) analysis

Phenolic profiles of the extracts were investigated using the HPLC method with photodiode array (PDA) detector (Agilent 1260 infinity). Samples were eluted using C18 reverse phase column (150x0.46 mm, 5µm) with 2.5 % formic acid in water (A solution) and 2.5 % formic acid in acetonitrile (B solution) solutions as a mobile phase. Flow rate was 1 ml/min and the injection volume was 20 microliters. Compounds were identified at 280 nm, 320 nm, and 360 nm wavelengths according to phenolic groups such as benzoic acids, hydroxycinnamic acids, and flavonoids, respectively.

All of the standards and extracts were injected in triplicates, and mean values and standard deviation were calculated. The amount of ascorbic acid in the extracts was calculated using the benzoic acid calibration curve due to the lack of commercial standard (Ph. Eur., 2013).

DPPH radical scavenging activity

Gyamfi et al.'s method was used for assessing the ability of the extracts to scavenge DPPH radical. In Tris-HCl buffer (50 mM, pH 7.4), a 50 µL aliquot of each extract was combined with 450 µL of Tris-HCl buffer (50 mM, pH 7.4) and 1.0 mL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl in methanol. Except for the extract or positive control substance, the controls included all of the reaction reagents. The absorbance was measured at 517 nm after 30 minutes of incubation in dark at room temperature (23°C). Eq 1 was used to calculate the percentage inhibition, and a nonlinear regression algorithm was used to estimate the half maximal inhibitory concentration (IC_{50}) values (SigmaPlot 2001 version 7.0, SPSS Inc., Chicago, IL). As a positive control, butylated hydroxytoluene (BHT) was used. The average of three measurements is used to calculate the results.

Percentage inhibition = [(Abscontrol - Abssample) / Abscontrol] × 100 (Eq. 1)

$ABTS^+$ radical scavenging activity

The extracts' ability to scavenge $ABTS^+$ radical was measured via spectrophotometry. 36 mg $ABTS^{+\bullet}$ and 6.6 mg $K_2O_8S_2$ were dissolved in 10 ml distilled water. The mixture was allowed to stand at room temperature for 12-16 hours in the dark before the analysis, and the absorbance was adjusted to 734 nm to 0.7 at ambient temperature. Extracts were diluted with 70% methanol. The absorbance was measured for 30 min with 1 minute intervals at 734 nm after adding 10 µL of extract to 990 µL of $ABTS^{+\bullet}$ solution. The total antioxidant activity was calculated using a prepared Trolox calibration curve (Re *et al.*, 1998).

RESULTS AND DISCUSSION

The samples were divided into two portions. One portion was kept at -18 ºC and the other portion was dried at the room temperature. Fresh and dry fruits from two different locations were extracted with 70% methanol. The extraction yields are given in Table 1. According to the Table 1, the yield of the Turkey sample was higher than Iran's yield. Total phenolics within the extracts were measured by Folin Ciocalteau reagent and the calculated gallic acid equivalent total phenolics are shown in Table 1. The order of total phenolics amounts of the extracts can be given as IF > TF > TD > ID. In the Folin Ciocalteau colorimetric assay, the gallic acid calibration curve was used for the calculation of total phenolics amounts of the extracts.

Table 1: Extraction yield and total phenolics data for *Rosa canina* samples.

Sample *	Extraction Yield (%)	Total phenolics ($mg_{GAE}/g_{extract}$)	DPPH• (IC_{50} mg/mL)
IF	9.27	16.241 ± 0.016**	0.210 ± 0.008
ID	17.03	3.654 ± 0.006	2.095 ± 0.232
TF	21.60	8.552 ± 0.021	0.344 ± 0.016
TD	57.76	6.161 ± 0.013	0.813 ± 0.074

*IF, Iran Fresh fruit; ID, Iran Dry fruit; TF, Turkey Fresh Fruit; TD, Turkey Dry fruit. **mean ± SD (n=3)

The chemical composition of the extracts of both samples was investigated using reverse phase HPLC. Due to HPLC analysis, three different substances could be identified such as benzoic acid, luteolin, and ascorbic acid, within the extracts (Table 2, Figure 2). Although ascorbic acid was the major chemical component in all of the extracts, only luteolin and benzoic acid could be detected in only one of the extract (Table 2). According to the Table 2, the amount of ascorbic acid was varied between 24.18 % and 151.89 %. The previous results within the literature for total ascorbic acid obtained from wild growing *R. canina* were calculated between 2189.7 mg/100g and 2404.0 mg/100g. The ascorbic acid amounts can be varied between species and due to the temperature, geographic area, cultivation and storage time (Taneva *et al*., 2016).

Table 2: HPLC analysis of the extracts of *Rosa canina* samples

Samples*	Luteolin (%)	Benzoic acid (%)	Ascorbic acid (%BAE**)
ID	____	____	47.28 ± 0.33
IF	1.20 ± 0.04***	____	24.18 ± 0.16
TD	____	____	151.89 ± 0.84
TF	____	4.26 ± 0.05	57.42 ± 0.24

*see Table 1; **BAE, benzoic acid equivalent;
***mean ± SD (n=3)

R. canina is a remarkable plant among other *Rosa* species due to its high phenolic content. Also, the concentration of ascorbic acid in *R. canina* is higher than other species *(Czyzowska et al.,* 2015). Furthermore, the comparison between *R. canina, R. sempervirnes, R. coccinea* in an experiment showed that the amount of bioactive compounds having the antioxidant activity was higher in the *R. canina* (Kerasioti *et al.,* 2019). In another experiment about the chemical composition of fruits of *R. canina*, *R. dumalis* subsp. *boissieri*, *R. dumalis* subsp. *antalyasis*, *R. villosa, R. pulverulenta* and *R. formis*, the highest phenolic content was observed in *R.canina* (96 $mg_{GAE}/g_{extract}$) (Ercisli, 2007).

DPPH radical scavenging activities of the extracts of both plant samples were tested at room temperature and the calculated IC_{50} values are given in Table 1.

A moderate correlation (r^2=0,61) was calculated between radical scavenging activities and total phenolics amounts in all of the extracts. According to the Table 1, the most active extract was fresh fruit extract of the Iran sample. These results correlated with the previous papers related with IC_{50} values of *Calendula* extracts (Kerasioti *et al.,* 2019).

On the other hand, the antiradical activities of the extracts were also investigated with $ABTS^+$ radical scavenging assay. Antiradical activities of *R. canina* extracts are shown in Figures 2 and 3 at two different concentrations (0.06 mg/ml and 0.03 mg/ml) due to time and absorbance as kinetically. According to the results, only ID and TD extracts showed reverse ranking at low and high concentrations. The antiradical activity orders of the extracts are as follows; TD>ID>TF>IF for 0.03 mg/mL and ID>TD>TF>IF for 0.06 mg/mL.

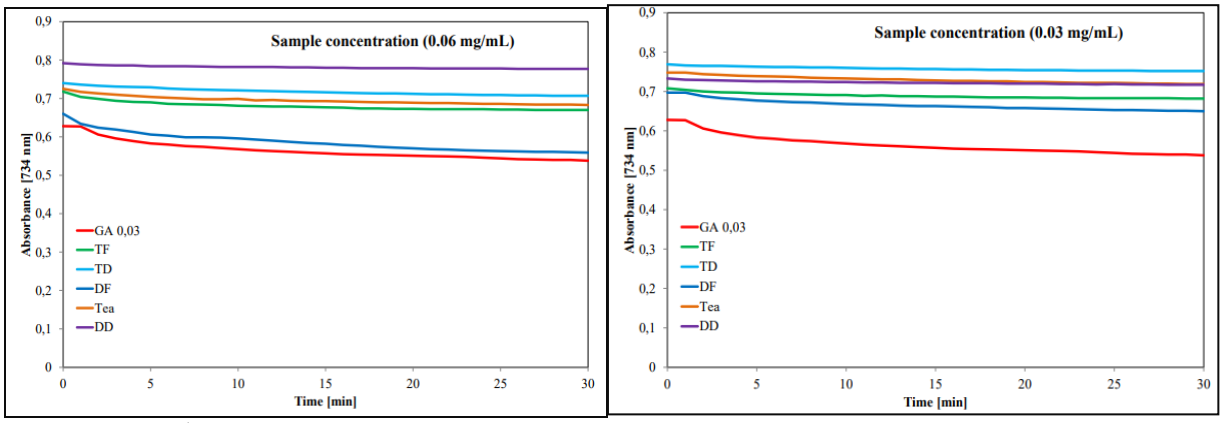

Figure 1: $ABTS^{+\bullet}$ scavenging activity of *R. canina* extracts in 0.03 and 0.06 mg/mL concentrations.

The high radical scavenging ability, established by using $ABTS^{+\bullet}$ assay, could be explained due to the high content of ascorbic acid. The antioxidant activity of 50% ethanol extract was mainly due to the high level of total phenolic content, which is similar outcome in comparison with another research that was conducted to evaluate the radical scavenging ability (Montazeri *et al.,* 2011).

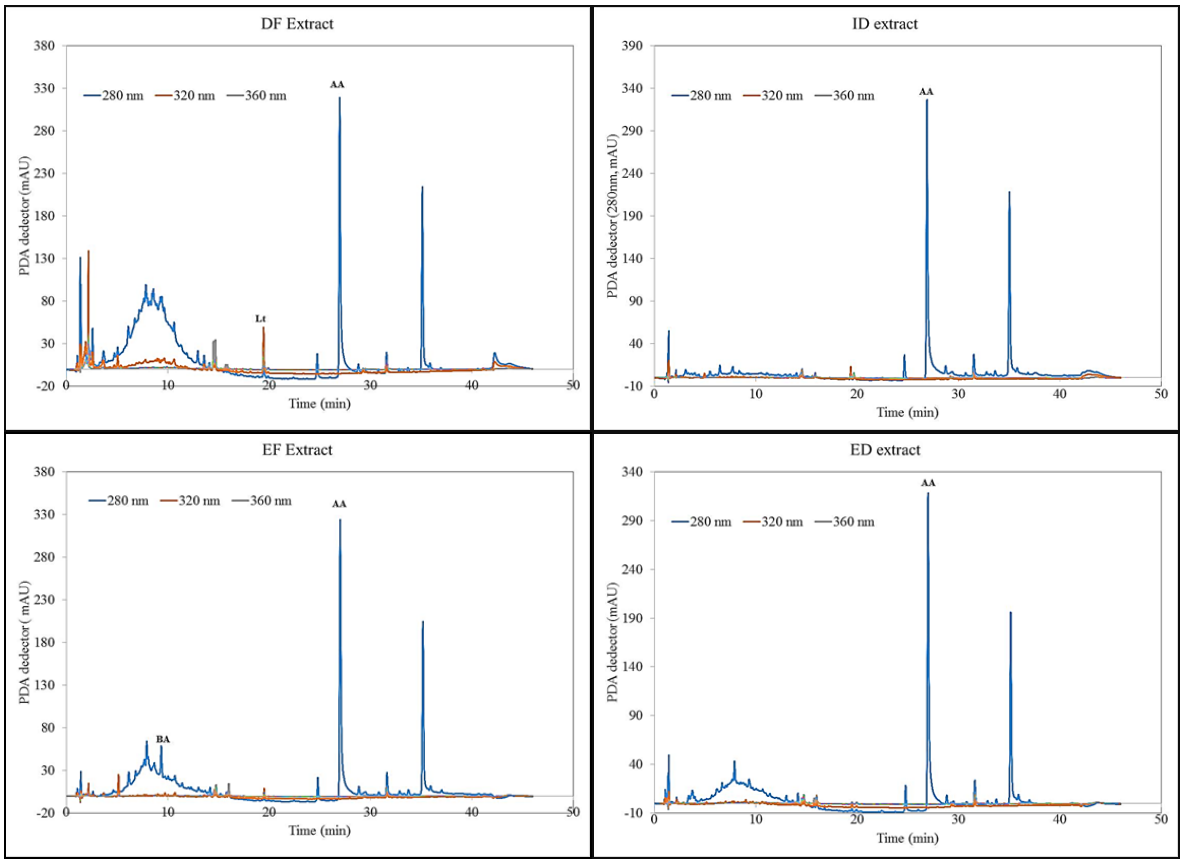

Figure 2: HPLC chromatograms of extracts.

REFERENCES

Ameye LG, Chee WSS (2006). Osteoarthritis and nutrition. From nutraceuticals to functional foods: A systematic review of the scientific evidence. *Arthritis Research and Therapy* **8**(4): 1–22.

Blumenthal M, Busse WR, Goldberg A, Gruenwald J, Hall T, Klein S, Riggins CW, Rister R (1998). Therapeutic Guide to Herbal Medicines. *In The Complete German Commission E Monographs* (p. 369).

Czyzowska A, Klewicka E, Pogorzelski E, Nowak A (2015). Polyphenols, vitamin C and antioxidant activity in wines from *Rosa canina* L. and *Rosa rugosa* Thunb. *Journal of Food Composition and Analysis* **39**: 62–68.

Deliorman Orhan D, Hartevioglu A, Kupeli E, Yesilada E (2007). In vivo anti-inflammatory and antinociceptive activity of the crude extract and fractions from *Rosa canina* L. fruits. *J Ethnopharmacol* **112**(2): 394–400.

Demir N, Yildiz O, Alpaslan M, Hayaloglu AA (2014). Evaluation of volatiles, phenolic compounds and antioxidant activities of rose hip (*Rosa* L.) fruits in Turkey. *LWT - Food Science and Technology* **57**(1): 126–133.

Ercisli S (2005). Rose (*Rosa* spp.) germplasm resources of Turkey. *Genet Resour Crop Evol* **52**(6): 787–795.

Ercisli S (2007). Chemical composition of fruits in some rose (*Rosa* spp.) species. *Food Chem* **104**(4): 1379–1384.

Gruenwald J, Uebelhack R, Moré MI (2019). *Rosa canina* – Rose hip pharmacological ingredients and molecular mechanics counteracting osteoarthritis – A systematic review. *Phytomedicine* **60**(March): 152958.

Hvattum E (2002). Determination of phenolic compounds in rose hip (*Rosa canina*) using liquid chromatography coupled to electrospray ionisation tandem mass spectrometry and diode-array detection. *Rapid Communications in Mass Spectrometry* **16**(7): 655–662.

Kerasioti E, Apostolou A, Kafantaris I, Chronis K, Kokka E, *et al.* (2019). Polyphenolic composition of rosa canina, rosa sempervivens and pyrocantha coccinea extracts and assessment of their antioxidant activity in human endothelial cells. *Antioxidants* **8**(4).

Lattanzio F, Greco E, Carretta D, Cervellati R, Govoni P, *et al.* (2011). In vivo anti-inflammatory effect of Rosa canina L. extract. *J Ethnopharmacol* **137**(1): 880–885.

Montazeri N, Baher E, Mirzajani F, Barami Z, Yousefian S (2011). Phytochemical contents and biological activities of rosa canina fruit from Iran. *J Med Plant Res* **5**(18): 4584–4589.

Nilsson O (1997). *Rosa*. In P. H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands* **4**:106-128 Edinburgh University Press.

Orhan N, Aslan M, Hosbas S, Deliorman Orhan D (2009). Antidiabetic effect and antioxidant potential of *Rosa canina* fruits. *Pharmacogn Mag* **5**(20): 309–315.

Patel S (2013). Rose hips as complementary and alternative medicine: Overview of the present status and prospects. *Med J Nutrition Metab* **6**(2): 89–97.

Pavek PLS (2012). *Plant guide for dog rose (Rosa canina* L.*).*

Ph. Eur. (2013). No Title. *European Pharmacopeia 8th Edition*, 1263.

Re R, Pellegrinni N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1998). Antioxidant activity applying an improved ABTS radical cation decolorisation assay. *Free Radicals in Biologt and Medicine* **26**: 1231–1237.

Rein E, Kharazmi A, Winther K (2004). A herbal remedy, Hyben Vital (stand. powder of a subspecies of *Rosa canina* fruits), reduces pain and improves general wellbeing in patients with osteoarthritis - A double-blind, placebo-controlled, randomised trial. *Phytomedicine* **11**(5): 383–391.

Singleton VL, Orthofer R, Lamuela-Ravento´s RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In L. Packer (Ed.). *Methods in Enzymology* **299**: 152-178. Academic Press.

Taneva I, Petkova N, Dimov I, Ivanov I, Denev P (2016). Characterization of Rose Hip (*Rosa canina* L.) Fruits Extracts and Evaluation of Their in vitro Antioxidant Activity. *Journal of Pharmacognosy and Phytochemistry* **5**(2): 35–38.

Winther K, Apel K, Thamsborg G (2005). A powder made from seeds and shells of a rose-hip subspecies (*Rosa canina*) reduces symptoms of knee and hip osteoarthritis: A randomized, double-blind, placebo-controlled clinical trial. *Scand J Rheumatol* **34**(4): 302–308.

Yildiz O, Alpaslan M (2012). Properties of rose hip marmalades. *Food Technol Biotechnol* **50**(1): 98–106.