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RESEARCH ARTICLE

Antioxidant capacities, ascorbic acid and total phenol contents of the plants sold as rose hip in Turkey

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Summary

The aim of this study is to examine the quality controls and determine antioxidant capacities, ascorbic acid and total phenol contents of the plants sold as Rose hip in Turkey. According to the European Pharmacopoeia 7.0, microscopic features and physicochemical parameters such as total ash value, loss on drying and foreign matter of the samples were determined. Antioxidant capacity of Rose hip decoctions was assessed by three in vitro methods (DPPH and ABTS radical scavenging assays, metal-chelation capacity). The total phenol content of the samples was measured by Folin-Ciocalteu assay. Thin Layer Chromatography (TLC) and Ultra Performance Liquid Chromatography- Time-of-Flight-Mass Spectrometer (UPLC-TOF-MS) analysis were used to determine the presence of ascorbic acid in the decoctions and ethanol extracts of the samples. The results of quality control analysis showed that the only one rosehip decoction (sample 1) contains a small amount of ascorbic acid and rose hips sold in akhtars are not suitable to Rose hip monograph of the European Pharmacopoeia 7.0. On the other hand, rose hip decoctions exhibited strong antioxidant activity. Based on the results of quality control analysis, rose hips sold in akhtars were found to be unsafe for human consumption. This study demonstrated that Rose hip decoctions show strong inhibitor activity on ABTS and DPPH radical formation and but does not contain ascorbic acid.

Key Words: *Rose hip; European Pharmacopoeia 7.0; antioxidant activity; ascorbic acid; total phenol content*

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Türkiye'de kuşburnu olarak satılan bitkilerin antioksidan kapasiteleri, askorbik asit ve total fenol içerikleri

Özet

Çalışmanın amacı, Türkiye'de aktarlarda kuşburnu olarak satılan bitkilerde kalite kontroller yapmak ve antioksidan kapasiteleri, askorbik asit ve total fenol içeriklerini tespit etmektir. Avrupa Farmakopesi 7.0'a göre, örneklerin mikroskobik özellikleri ve Bütün Kül değeri, bütün kül, kurutmada kayıp ve yabancı madde tayini gibi fizokimyasal parametreleri belirlenmiştir. Kuşburnu dekoksiyonlarının antioksidan kapasitesi üç in vitro yöntem (DPPH ve ABTS radikal süpürücü yöntemler, metal şelatlama kapasitesi) ile değerlendirilmiştir. Örneklerin total fenol içerikleri, Folin-Ciocalteu yöntemi ile ölçülmüştür. Örnek dekoksiyonları ve etanol ekstrelerindeki askorbik asit varlığını tespit etmek için, İnce Tabaka Kromatografisi (İTK) ve Ultra Performans Sıvı Kromatografisi-uçuş süresi-kütle Spektrometresi (UPLC-TOF-MS) analizleri kullanılmıştır. Kalite kontrol analizleri, sadece bir kuşburnu dekoksiyonunun (örnek 1) az miktarda askorbik asit içerdiğini ve aktarlarda satılan kuşburnu örneklerinin Avrupa Farmakopesi 7.0 monografına uygun olmadığını göstermiştir. Diğer yandan, Kuşburnu örnekleri güçlü antioksidan aktivite göstermiştir. Kalite kontrol analizleri sonuçları temel alındığında, aktarlarda satılan kuşburnu örneklerinin tüketiminin güvenli olmadığı bulunmuştur. Bu çalışma göstermiştir ki, kuşburnu dekoksiyonları ABTS ve DPPH radikal oluşumu üzerinde inhibitor aktiviteler göstermekte fakat askorbik asit içermemektedir.

Anahtar kelimeler: *Kuşburnu; Avrupa Farmakopesi 7.0; antioksidan aktivite; askorbik asit; total fenol içeriği*

INTRODUCTION

The genus *Rosa* (Rosaceae) naturally grows and is represented by twenty-four species in Anatolia (1). The most common species, *Rosa canina* L., is called as "Rose hip, dogrose" all over the world. The plant and its extracts have long been used in the food and cosmetics industries (2). In Anatolia, *R. canina* is commonly known by local names, such as kuşburnu, köpek gülü, şilan, yabani gül, itburnu and the branches, flowers, fruits and roots of the plant have been widely utilized for the treatment of cold, bronchitis, diarrhea, kidney diseases, hemorrhoids, diabetes mellitus and stomach problems in the various regions of Anatolia since ancient times (3,4). In Turkey, the rose hips sold in markets and akhtars (seller of herbs and spices) have been consumed as herbal tea by public. People usually prefer rose hip

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teas because of its ascorbic acid content and benefits for human health. Today, Rose hip is official in the European, Hungarian, Japanese, and British Herbal Pharmacopoeias, approved in the German Commission E monographs, and has been used to treat rheumatism, gout, febrile diseases, kidney and urinary tract diseases (5-9). Therefore, pharmacopoeia analysis is important and necessary to provide safety and quality of herbal products sold in markets and akthars.

Excessive free radical generation in organism can lead to serious health problems like bronchitis, rheumatism and diabetes mellilitus. Antioxidant rich plants play an effective role in inhibiting and scavenging these free radicals (10). Rose hip teas consumption is widespread in Turkey due to their uniquely beneficial effects on health. For the first time, the antioxidant activity of traditionally prepared decoctions from rose hip samples used as folk remedies in Anatolia was assessed in this work.

This study was designed to determine the quality standards of the plants sold as rose hips in akhtars. Firstly, microscopic features of the rose hip samples were investigated. Total ash, foreign matter and loss on drying analysis of the samples were achieved according to the European Pharmacopoeia 7.0 (5). Moreover, antioxidant capacities (DPPH and ABTS radical scavenging, ferrous-ion chelating assays) and total phenol contents of the akhtar samples were investigated. Additionally, ascorbic acid analysis in Rose hip samples was performed by using TLC method and UPLC-TOF-MS technique.

MATERIALS AND METHODS

Plant materials

The name of provinces and purchase date of 10 plant samples which were sold as rose hip used in this study are listed in Table 1. The obtained materials (already in dried form) were identified by Prof. Dr. Murat Ekici (Department of Biology, Faculty of Science, Gazi University, Ankara) and are kept at the Pharmacognosy Research Laboratory of the Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Microscopic analysis

Firstly, achenes of the plant were removed. Then remaining parts of rose hips were powdered and viewed under a microscope (Olympus CH2 model, Japan), using chloral hydrate and Sartur solutions. Photographies of the tissues were taken using Samsung S730 camera.

Preparation of the extracts

For the European Pharmacopoeia 7.0 analysis: 5 g of the powdered drug and 25 mL of ethanol (96%) were shaked for 30 min and filtered.

For the antioxidant activity assays: 1 g of the air-dried plant material was added to 100 mL distilled water and boiled on slow heat for 30 min. The decoctions were then filtered and the filtrates were pooled and liyophilized *in vacuo*.

Procedures given in European Pharmacopoeia 7.0 were used to determine total ash, foreign matter, loss on drying values and ascorbic acid content of the samples (5).

Foreign matter

Foreign matters from plant material (100 g) with the naked eye or by use of a lens (6x) were seperated and then this mass was weighed. Foreign matter content of rose hips was expressed in g of foreign matter/100 g plant.

Total Ash

1 g of rose hip weighed accurately in a silica dish was first heated over Bunsen burner flame to volatilize a part of organic matter and them transferred to a temperature controlled muffle furnace for 3 h at 600°C. Then the remaining part in silica dish was cooled in desiccator and weighted. Total ash analysis was carried out in triplicate for every plant sample. Total ash content was calculated as percentage by the formula given below.

Percentage of total ash = (weight of dry ash residue/ weight of dry plant)x100

Loss on drying

After 1 g of rose hip weighed accurately in a glass weighing bottles was dried in an oven at 100-105°C for 3 h, it was cooled in a desiccator and weighed repeat the procedure until constant weight come. The difference between the first and second weighings was considered as loss on drying.

Thin-Layer Chromatography

Plate: TLC silica gel F_{254} plate (Aluminium Sheets, 20 cm x 20 cm), Mobile phase: Acetic acid:acetone:methanol:toluene (5:5:20:20 v/v/v/v), revelator: 0.2 g/L solution of dichlorophenolindophenol, sodium salt in ethanol (96%), Reference solution: 10 mg of ascorbic acid was dissolved in 5 mL of ethanol.

Phytochemical analysis by UPLC-TOF-MS

Preparation of sample and references

A few crystals of ascorbic acid (AppliChem, A3604), 20 mg dried ethanol extracts and decoctions of Rose hips were dissolved in methanol.

Chromatographic system

Chromatographic separations were performed on a 2.1 mm x 100 mm Acquity UPLC BEH, 1.7 µm C_{18} column using an Acquity UltraPerformance Liquid Chromatography system (Waters Corp, Milford, MA). All solvents were filtered through a 0.45 mm filter and were degassed by sonication in an ultrasonic bath before use.

The mobile phase was composed of aqueous formic acid (A; 0.1%, v/v) and acetonitrile-formic acid (B; 0.1%, v/v); A:B was as follows: 0 min, 8:2; 5 min, 5:95; 6.5 min, 5:95; 7.5 min, 8:2; the flow rate was 0.25 mL/min and the column temperature was maintained at 40 °C. The total runtime was 7.5 min.

TOF instrumentation

Mass spectrometry was performed on a Micromass LCT Premier XE (Waters MS Technologies, Manchester, UK) orthogonal acceleration Time-of-Flight mass spectrometer operation in both positive and negative ion mode with electrospray ionization (Z-spray). The desolvation gas flow was set to 700 L/h at a temperature of 300 °C. The cone gas flow was set to 11 L/h and the source temperature was set to100 °C. The cone and the aperture 1 voltages were set to 15 V and 5 V, respectively. The aperture 1 voltage was set to 5 V. The LCT Premier XE was operated in Woptics mode with 412.500 resolutions.

Data processing

The mass spectrometric data were collected in full scan mode the *m/z* were from 100 to 1000 in both negative and positive ion. The data were collected and analyzed by MassLynx V 4.1 software (Micromass, Manchester, UK) to search for expected compounds with accurate mass and fragmentions information.

Total Phenol Content

The extracts or gallic acid (Sigma G7384), 2.5 mL of Folin-Ciocalteau's reagent (Sigma-Aldrich F9252) and sodium carbonate solution were mixed into test tubes. The tubes were vortexed and incubated at room temperature for 15 min. The absorbance was measured at 765 nm. The total phenol values are expressed in terms of gallic acid equivalent (GAE) (10).

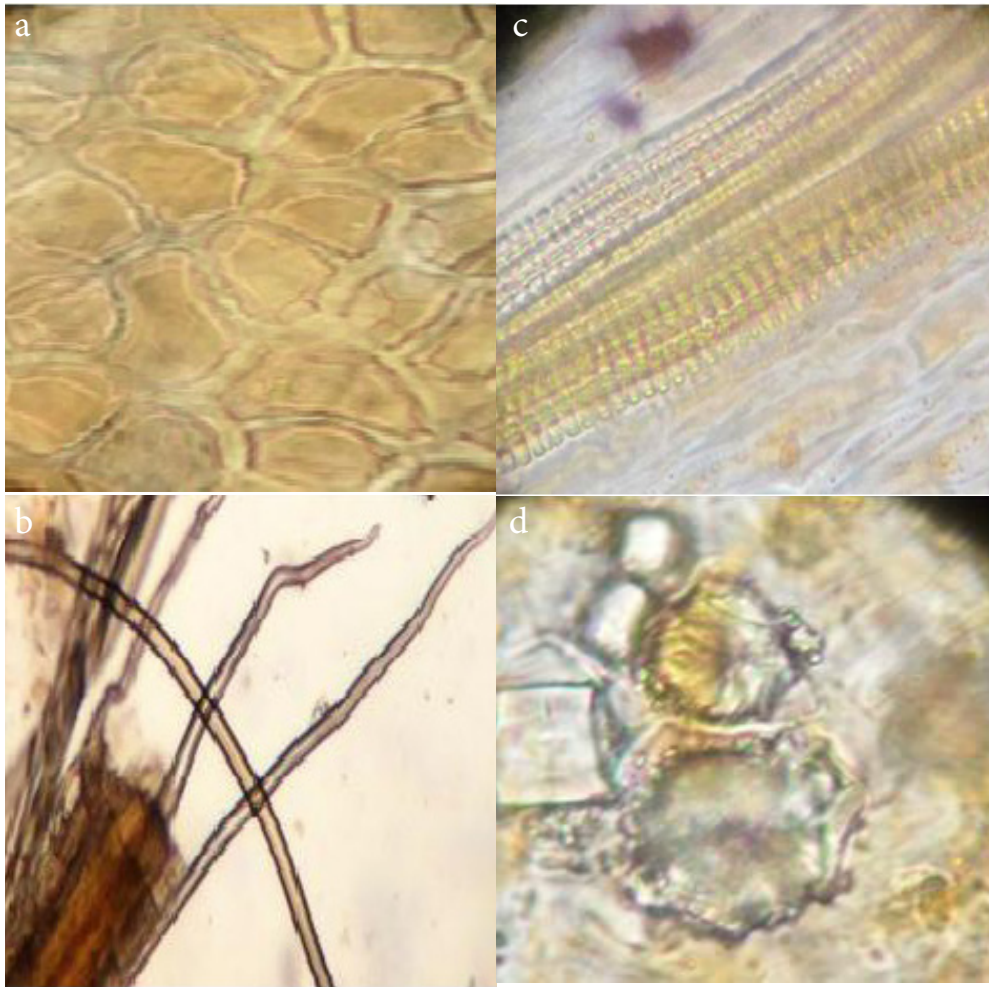

Figure 1. Microscopic view of diagnostic tissue elements of Rose hip samples
(a) The outer epidermis with orange-yellow contents (10x40) (b) Vascular bundles (10x10) (c) Covering hair (10x10) (d) Calcium oxalate crystals (druses and simple crystals, 10x40)

Antioxidant activity assays

DPPH Radical Scavenging Assay

The DPPH (2,2-Diphenyl-1-picryl-hydrazyl, Sigma D9132) in methanol (4×10^{-4} M) solution was prepared daily, before the UV measurements (10). The decoctions were mixed with DPPH• solution. The samples were kept in the dark for 30 min at room temperature and then the decrease in absorption was measured at 515 nm on a 96-well microplate reader (VersaMax Molecular Devices, USA). Butylated hydroxytoluen (BHT) was used as a reference compound. The experiment was carried out in triplicate.

ABTS Radical Scavenging Assay

The 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$) was formed by reacting ABTS aqueous solution (7 mM) with 2.45 mM of ammonium persulfate (11). The $ABTS^{\bullet+}$ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. 10 µL of six different concentrations (2000-250 µg/mL) of each extract was added to 0.990 µL of diluted $ABTS^{\bullet+}$ solution. After 6 min., the absorbance reading was taken at 734 nm. All analyses were done at least in triplicate. Trolox was used as positive control.

Ferrous-ion chelating Assay

100 µl of four different concentrations 2000, 1000, 500, and 250 µg/mL of each extract, which dissolved in methanol, was mixed with 10 µL of aqueous $FeCl_2$. After 5 min incubation at room temperature, the reaction was initiated by 20 µl ferrozine. After 10 min, the absorbance at 562 nm was measured against blank solution by using the Eliza reader. Disodium EDTA was used as positive control (10).

Statistical analysis

All values are expressed as the mean±the standard error of the mean (SEM); linear regression analyses and correlation coefficients to determine the relationship between two variables were calculated using MS-DOS software (GraphPad InStat statistical program).

RESULTS AND DISCUSSION

According to European Pharmacopoeia 7.0., Rose hip samples should be consisted the diagnostic tissue elements such as the outer epidermis with orange-yellow contents, covering hair, vascular bundles (long and thick lignified walled 30-45 µm) and calcium oxalate crystals. The results of microscopic examination revealed the presence of all diagnostic tissue elements in the purchased Rose hip samples from akhtars. The microscopic features of the samples were found to be suitable to the European Pharmacopoeia 7.0 (Figure 1). During the microscopic study, the presence of some fungal contaminants in samples 3-6 was identified.

According to the European Pharmacopoeia 7.0, foreign matter values for rose hips should not exceed 1% (w/w) (Table 1). All rose hip samples (0.00±0.73% w/w) were found to contain less than 1% foreign matter except sample 5 (3.10% w/w).

Total ash method determines the total amount of non-volatile inorganic compounds of the drug. Total ash contents of rose hip samples ranged between 3.85±0.85% and 6.75±0.65% (w/w). These values were all below the European Pharmacopoeia maximum acceptable limit of 7% (w/w) (Table 1).

Table 1. Total ash, Loss on drying, Foreign matter contents of the rosehip-called species sold in akhtars

Sample no	Purchase date	Name of the province	Total Phenol Content (%)±SEM	Loss on drying %±SEM	Total ash %±SEM	Foreign matter %±SEM
1	June 2010	Mersin/Namrun	7.32±0.73	13.83±0.44	5.53±024	0.01±0.00
2	June 2010	Mersin/Gülnar	5.77±0.63	7.90±0.34	6.32±0.16	0.01±0.00
3	July 2010	Mersin	8.80±0.10	8.21±1.94	4.42±0.23	0.19±0.02
4	July 2010	Konya/Hadim	7.40±0.15	1.62±0.36	5.19±0.13	0.48±0.05
5	June 2010	Mersin	8.77±0.49	6.13±0.10	5.64±0.25	3.10±0.24
6	July 2010	Konya	9.77±0.19	7.89±0.24	3.85±0.85	0.73±0.01
7	June 2010	Mersin	7.73±0.24	7.28±0.12	6.75±0.65	-0-
8	July 2010	Konya/Höyük	11.03±0.33	11.64±0.27	5.10±1.03	0.11±0.02
9	July 2010	Malatya	10.30±0.25	8.74±0.35	5.42±0.34	0.02±0.00
10	July 2010	Sivas	8.10±0.50	9.06±0.40	4.58±0.54	-0-

SEM: Standard Error of the Mean

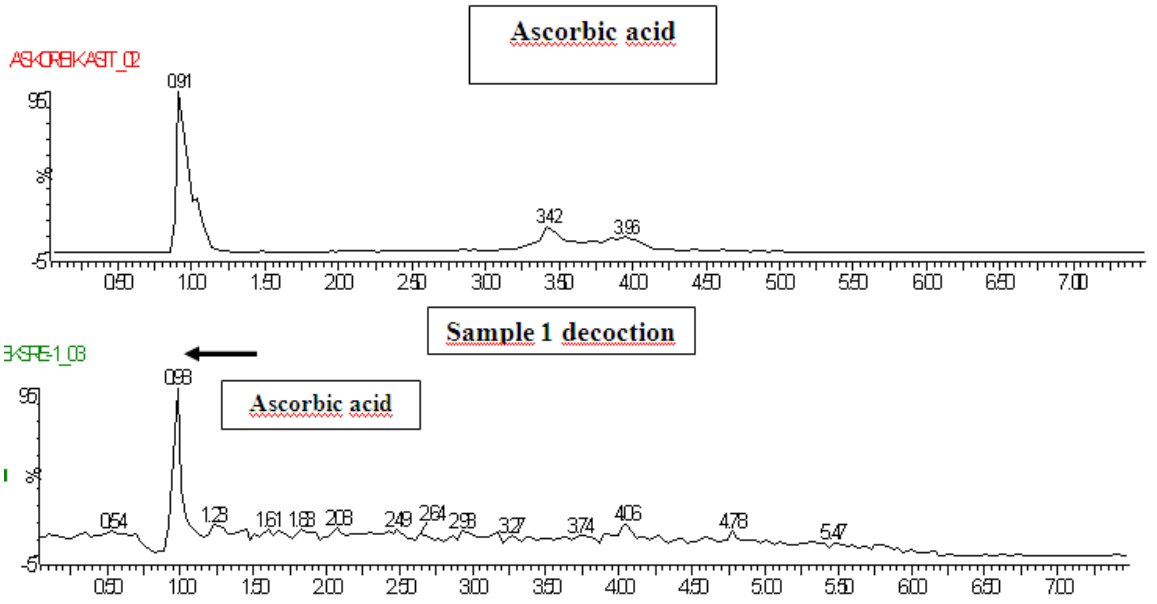

Figure 2. UPLC-TOF-MS chromatogram of Ascorbic acid and Sample 1 decoction

According to the European Pharmacopoeia, the loss on drying values for the rose hips should not exceed 10% (w/w) (Table 1). Except for samples 1 (13.83±0.44%) and 8 (11.64±0.27 %), loss on drying values determined in all rose hips were in agreement with the prescribed values for rose hip in European Pharmacopoeia. Loss on drying is the most frequently used method to measure moisture content of plants. High moisture content in plants can lead to fungal and bacterial proliferation.

In European Pharmacopoeia 7.0, TLC is recommended for qualitative evaluation of ascorbic acid in the dried hypanthias of the fruit. But, when the TLC plate was sprayed with dichlorophenolindophenol solution R., no spots were seen on the plate. In fact, the dried plant samples are not suitable to analysis of ascorbic acid. The process of drying may cause decrease of the content of ascorbic acid in plants. In addition to TLC technique, the more accurate UPLC-TOF-MS technique was used for qualitative analysis of ascorbic acid. UPLC-TOF-MS analysis demonstrated that only sample 1 decoction contains a small amount of ascorbic acid (Figure 2)

As seen in Table 2, DPPH radical scavenging activity of all samples increased with an increasing concentration. At 2000 µg/mL concentration, samples 9 (93.4±0.3%) and 10 (90.2±0.4%) exhibited higher activity than BHT (89.8±0.3%), but DPPH radical scavenging activity of samples 3 (89.1±1.6%) and 8 (87.8±0.5%) were similar to that of BHT.

Table 2. DPPH free radical and $ABTS^{\cdot+}$ radical scavenging activities of the decoctions of rose hip-called species

Sample no	DPPH Radical Scavenging Capacity (Inhibition %±SEM)				Inhibition of $ABTS^{\cdot+}$ radical formation (%)±SEM			
	2000 µg/mL	1000 µg/mL	500 µg/mL	250 µg/mL	2000 µg/mL	1000 µg/mL	500 µg/mL	250 µg/mL
1	71.2±0.8	58.0±1.5	36.6±0.9	33.7±5.4	46.9±0.1	27.7±0.7	10.7±0.4	5.4±0.9
2	62.6±1.6	31.5±2.4	25.1±1.7	15.4±1.6	60.7±0.4	44.0±0.8	20.5±2.0	15.1±1.0
3	89.1±1.6	70.7±1.5	56.2±3.1	38.2±1.5	69.3±0.3	15.5±0.6	-0-	-0-
4	77.2±3.6	52.3±3.8	45.1±4.5	28.0±3.9	72.3±0.5	32.2±0.5	14.9±0.7	3.1±0.3
5	83.3±1.4	57.4±0.9	51.0±0.8	25.5±1.7	69.7±2.1	35.9±1.4	17.1±0.4	11.7±1.1
6	84.4±2.2	62.6±1.9	47.9±3.8	42.1±2.3	56.7±0.7	34.8±0.9	16.7±1.8	7.6±1.2
7	84.5±1.8	65.5±3.4	64.9±0.1	41.0±2.9	60.5±0.2	29.5±0.2	10.1±1.0	4.3±0.0
8	87.8±0.5	81.7±0.7	63.8±1.7	45.7±2.0	88.2±0.6	45.4±0.4	14.8±0.4	5.2±1.2
9	93.4±0.3	87.7±0.7	68.2±1.0	47.9±0.0	52.8±0.4	33.5±1.7	5.9±0.2	-0-
10	90.2±0.4	74.9±1.1	52.3±2.6	29.7±1.9	61.2±1.1	35.1±2.2	30.2±0.2	4.8±0.3
BHT	89.8±0.3	87.8±0.5	73.2±0.7	38.6±2.0	99.6±0.1	99.9±0.1	82.7±0.8	44.4±0.2

SEM: Standard Error of the Mean

Trolox significantly showed the highest inhibitions on ABTS radical formation at 2000, 1000 and 500 µg/mL concentrations. The lowest inhibition was detected in sample 1 decoction with 46.9±0.1% at 2000 µg/mL concentration. Sample 8 decoction (88.2±0.6%) had the highest antioxidant activity among the decoctions tested. Except for samples 1 and 8, ABTS radical scavenging activities of all samples were found to be ranged from 52.8±0.4% to 72.3±0.5% (Table 2).

On the other hand, rose hip samples did not exert any ferrous ion chelating effect as compared to the reference, EDTA.

Total phenol contents of the samples were calculated using the standard curve equation: y = 0.9095x + 0.047, r^2=0.9991. Samples 8 and 9 had the highest phenolic content 11.03±0.33 and 10.30±0.25 g GAE per 100 g, while total phenolic content of sample 2 had the lowest (5.77±0.63%) (Table 1). No correlation was observed between total phenol contents and ABTS radical scavenging activities of rose hip samples. But, DPPH radical scavenging activity of the samples was correlated with their phenolic content.

The plant part officinal in the European Pharmacopoeia 7.0 is recorded as the rose hip without achenes. When we examined the morphological characters of the samples, all of them were found to have achenes. Primarily for this reason, the macroscopic features of purchased samples from akhtars were not suitable to Rose hip monograph of the European Pharmacopoeia 7.0. Ahmed and Güvenç (2009) determined the morphological and anatomical characters of the rose hip samples that are sold by herbalists in Ankara province (Turkey) and also compared their characteristics with defined morphological and anatomical features in European Pharmacopoeia 6.0 (12). Taking into consideration the European Pharmacopoeia standards, there have been so far no detailed anatomical and physicochemical studies on rose hips sold in akhtars.

The purchased rose hips were analyzed for the physicochemical properties in terms of foreign matter and total ash contents, loss on drying. Samples 2, 7, 9, and 10 were found to have suitable characteristics. Among rose hip samples analyzed, remarkable high total phenolic content was found in samples 8 and 9. In this work, the antioxidant capacity of decoctions prepared from samples was tested. Because, the decoctions prepared from rose hips have been consumed for their medicinal purposes in Turkish traditional medicine. Results of antioxidant activity tests showed that Rose hip decoctions had strong inhibitor activity on ABTS and DPPH radical formation. Our literature survey found that the antioxidant activity of various parts of plant, such as seed, fruit and leaves was determined by different *in vitro* methods (reducing power, ferrous ion chelating and DPPH free radical, superoxide radical, hydroxyl radical, peroxyl radical, nitric oxide radical, ABTS radical, hydrogen peroxide scavenging assays). To determine the antioxidant activity of plant, the extracts (ethyl acetate, *n*-hexane, chloroform, methanol, acetone extracts and infusions) obtained with the various solvents used were assessed in studies (13-20). As far as is known, this is the first report on antioxidant activities of traditionally prepared decoctions of rose hips used as folk remedy in Anatolia. The results concluded that rosehip decoctions have considerably radical (DPPH and ABTS) scavenging activity. The TLC and UPLC-TOF-MS analysis show that the decoctions prepared from dried rosehips boiled in cold water over an open fire for thirty minutes do not contain ascorbic acid. These findings suggest that strong antioxidant effects of rose hips could mostly be due to their phenolic and carotenoid contents.

CONCLUSIONS

The rose hip tea is considered to have many health benefits and also one of the best seller herbal teas in the markets, which can be consumed by public in Turkey. The present study demonstrated that Rose hip decoctions show strong inhibitor activity on ABTS and DPPH radical formation and does not contain ascorbic acid. Therefore, the decoction method should not be utilized in order to benefit from high ascorbic acid content of rosehips. Based on the results of quality control analysis, rosehips sold in akhtars were found to be unsafe for human consumption. As conclusion, in terms of public health protection, the Ministry of Health and Ministry of Agriculture have to make serious audits on herbal teas sold in akhtars.

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