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...
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 akthars. 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 lyophilized 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 separated 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 then 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.

$$\text{Percentage of total ash} = \left(\frac{\text{weight of dry ash residue}}{\text{weight of dry plant}}\right) \times 100$$

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<sub>254</sub> plate (Aluminium Sheets, 20 cm x 20 cm), Mobile phase: Acetic acid:acetone:methanol:toluene (5:5:20:20 v/v/v/v), re- elator: 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<sub>18</sub> 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 to 100 °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 fragmentations 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](image)

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

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

SEM: Standard Error of the Mean
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⁺ radical scavenging activities of the decoctions of rose hip-called species

<table>
<thead>
<tr>
<th>Sample no</th>
<th>DPPH Radical Scavenging Capacity (Inhibition %±SEM)</th>
<th>Inhibition of ABTS⁺ radical formation (%)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2000 µg/mL</td>
<td>1000 µg/mL</td>
</tr>
<tr>
<td>1</td>
<td>71.2±0.8</td>
<td>58.0±1.5</td>
</tr>
<tr>
<td>2</td>
<td>62.6±1.6</td>
<td>31.5±2.4</td>
</tr>
<tr>
<td>3</td>
<td>89.1±1.6</td>
<td>70.7±1.5</td>
</tr>
<tr>
<td>4</td>
<td>77.2±3.6</td>
<td>52.3±3.8</td>
</tr>
<tr>
<td>5</td>
<td>83.3±1.4</td>
<td>57.4±0.9</td>
</tr>
<tr>
<td>6</td>
<td>84.4±2.2</td>
<td>62.6±1.9</td>
</tr>
<tr>
<td>7</td>
<td>84.5±1.8</td>
<td>65.5±3.4</td>
</tr>
<tr>
<td>8</td>
<td>87.8±0.5</td>
<td>81.7±0.7</td>
</tr>
<tr>
<td>9</td>
<td>93.4±0.3</td>
<td>87.7±0.7</td>
</tr>
<tr>
<td>10</td>
<td>90.2±0.4</td>
<td>74.9±1.1</td>
</tr>
<tr>
<td>BHT</td>
<td>89.8±0.3</td>
<td>87.8±0.5</td>
</tr>
</tbody>
</table>

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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REFERENCES


