QUERCETIN CONTENT AND ANTIOXIDANT ACTIVITY OF ARMENIAN CRATAEGUS LAEVIGATA, PLANTAGO MAJOR AND ARTEMISIA ABSINTHIUM PLANTS EXTRACTS

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ABSTRACT

In present study in vitro antioxidant properties of ethanolic extracts of Armenian plants Crataegus laevigata, Plantago major and Artemisia absinthium was investigated by DPPH stable radical chemical model with simultaneous monitoring of the total flavonoids and selected polyphenolic compounds content. Experimental results indicates that ethanolic extract of Crataegus laevigata exhibit the highest radical scavenging activity in neutralization of DPPH with an IC₅₀ value of 12.5 ± 0.08 µl, while the lowest activity was reported in Plantago major extract (IC₅₀ = 45 ± 0.1 µl) and IC₅₀ of Artemisia absinthium extract was 35 ± 0.1 µl. Present study also demonstrates a possible relationship between quercetin content and antioxidant activity of extracts. Crataegus laevigata ethanolic extract showed highest antiradical potential as well as the highest concentration of quercetin (7.47 ± 0.2 µg/ml) and it can be proposed as a potential sources of natural antioxidants and bioactive phytopharmaceuticals.

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KEYWORDS
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Polyphenolic compounds
Quercetin
Crataegus laevigata
Plantago major
Artemisia absinthium
1 Introduction

Despite the widespread uses of *Crataegus laevigata*, *Plantago major* and *Artemisia absinthium* as medicines and spices, the literature contains few reports of antioxidant activity of these plants. The hawthorn (*Crataegus laevigata*) extracts was especially used to treat cardiovascular diseases (Chang et al., 2005). With this extract of tree has cardiotonic properties including vasodilatory (Brixius et al., 2006), chronotropic (Veveris et al., 2004) and high antioxidant effects (Zhang et al., 2014).

Similarly, leaves extracts of *Plantago major* (also known as ribwort) are also used as an antioxidant, anti-inflammatory, anti-fungal, anti-infective, anti-cancer agent and for wound healing purposes (Nunez-Guillen et al., 1997; Samuelsen, 2000; Park & Chang, 2004; Shim et al., 2007). Furthermore, traditional use of wormwood (*Artemisia absinthium*) was also reported by researchers (Park et al., 2000; Craciunescu et al., 2012). It used as a stomach medicine for gastric pain and as a cardiac stimulant. Wormwood extract has protective effect on hepatic damage (Park et al., 2000). Because of the presence of high flavonoids and phenolic compounds it has high antioxidant activity (Craciunescu et al., 2012).

The application of herbal extracts for medicinal purposes is determined by the presence and the complex action of various secondary metabolites among the most important from the standpoint of biological activity are flavonoids. Flavonoids seem to play an important role in human health and to possess beneficial effects in the prevention of human diseases (Kanti & Syed, 2009). The antioxidant capacity of flavonoids confers a therapeutic potential in cardiovascular diseases (Yao et al., 2004), gastric or duodenal ulcers (Moreira et al., 2004) and cancer (Yang et al. 2000). There were reports containing information about the antiviral and antiallergic actions of flavonoids, as well as their anti-thrombotic and anti-inflammatory properties (Nijveldt et al., 2001; Nair et al., 2002; Kwon et al., 2005).

In the present study we carried out a comparative analysis of phenolic compounds of selected Armenian plant species, total flavonoids content and the radical scavenging activity of its extracts.

2 Materials and Methods

2.1 Plant materials

Plants *C. laevigata*, *P. major* and *A. absinthium* were collected in September 2013 from various regions of the Republic of Armenia.

2.2 Extraction

The plant leaves and flowers were dried in air and ground in a mixer. Finely powdered material (10 g) was macerated in 100 ml 70% ethanol and incubated on magnetic stirrer at room temperature during 24 h period. The macerates were centrifuged 15 min at 8000 g to get rid of solids.

2.3 Determination of total flavonoids content

Aluminum chloride calorimetric method (Chang et al., 2002) modified by Vardapetyan et al. (2013) was used for flavonoids determination. The sample absorbance was measured at 430 NM on the spectrophotometer (JENWAY 6405) in a 1 cm cuvette. The flavonoid content was calculated by linear analysis. Values were expressed as mean ± SD.

2.4 Determination of free radical scavenging activities

The stable 2,2-diphenyl-picyrlydrazyl radical (DPPH) was dissolved in 96% ethanol and used for determination of free radical-scavenging activity of the extracts (Mensor et al., 2001; Vardapetyan et al., 2013) The IC_{50} value shows the amount of extract required for neutralization of the 50% of DPPH free radicals.

2.5 High-Performance Liquid Chromatography (HPLC) analysis

Analysis of the samples was performed by HPLC on an Agilent 1100 chromatograph (Agilent Technologies, USA) with UV - spectrophotometric detector and data processing software ChemStation. Separation was performed on a column Hypersil ODS 125/2.0 mm with particle diameter of 3 μm. Gradient elution of the samples and standards were performed using distilled water (eluent A) and acetonitrile (eluent B).

Table 1 Values of IC_{50}, total flavonoid content, and quercetin concentration in ethanolic extracts (mean ± SD, p < 0.05).

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>IC50 (µl)</th>
<th>Total flavonoid content (mg/ml)</th>
<th>Quercetin concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Crataegus laevigata</em></td>
<td>12.5 ± 0.08</td>
<td>8.09 ± 0.07</td>
<td>7.47 ± 0.2</td>
</tr>
<tr>
<td><em>Plantago major</em></td>
<td>45 ± 0.1</td>
<td>3.97 ± 0.04</td>
<td>2.77 ± 0.8</td>
</tr>
<tr>
<td><em>Artemisia absinthium</em></td>
<td>35 ± 0.1</td>
<td>2.05 ± 0.04</td>
<td>4.03 ± 0.12</td>
</tr>
</tbody>
</table>
The gradient elution initial conditions were 0% of eluent B with linear gradient to 10% from 0 to 10 min, followed by linear gradient to 50% of eluent B in 20 min, and then to 80% of eluent B in 30 min. The flow rate was 1 ml/min. The sample injection volume was 20 μl. Rutin, quercetin, morin, quercetin, apigenin, kaempferol (Roth, Germany) were used as markers. Stock solutions of these compounds were prepared by dissolving them in precise batches in 96% ethanol. Before use, all samples were filtered through a membrane filter with a pore diameter of 0.45 microns. Detection was performed at 370nm. Relative percentage amounts of the separated compounds were calculated automatically from peak areas of the total chromatograms.

2.6 Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated three times (Vardapetyan et al., 2013). The data were analyzed by a one-way analysis of variance (ANOVA) and the values of p < 0.05 were considered as significant.

3 Results and Discussion

Results of determination of flavones from C. laevigata, P. major and A. absinthium plant leaves extracts are presented in the Table 1. It was reported that the most abundant in flavonoids proved to be the ethanolic extract of C. laevigata (8.09 ± 0.07 mg/ml) while the extract of P. major and A. absinthium contain 3.97 ± 0.04 and 2.05 ± 0.04 mg/ml flavonoids, respectively.

The obtained antiradical activities of all investigated extracts against DPPH radicals were dose-dependent. C. laevigata extract showed the highest free radical scavenging activity in the DPPH assay (IC₅₀=12.5± 0.08 μl). While the lowest activity was found in extract of P. major (IC₅₀ = 45 ± 0.1 μl) and IC₅₀ of A. absinthium extract was 35 ± 0.1 μl.
Figure 3 HPLC of ethanolic extract of *C. laevigata*.

Figure 4 Total flavonoids content (left) and IC₅₀ values (right) of tested plants ethanolic extracts (mean ± SD of five independent experiments, p<0.05).

Figure 5 IC₅₀ values and quercetin content of tested plants ethanolic extracts (mean ± SD of five independent experiments, p<0.05).
Table 2 Polyphenolic compounds content (in %) in ethanolic extracts of Artemisia absinthium, Plantago major and Crataegus laevigata plants extracts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ret. Time</th>
<th>Content %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A. absinthium</td>
</tr>
<tr>
<td>Rutin</td>
<td>3.2</td>
<td>-</td>
</tr>
<tr>
<td>Quercetin</td>
<td>6.1</td>
<td>0.8527</td>
</tr>
<tr>
<td>Morin</td>
<td>13.4</td>
<td>0.4463</td>
</tr>
<tr>
<td>Quercetin</td>
<td>15.1</td>
<td>2.3736</td>
</tr>
<tr>
<td>Apigenin</td>
<td>17.5</td>
<td>2.3392</td>
</tr>
<tr>
<td>Kaempferol</td>
<td>18.3</td>
<td>1.0318</td>
</tr>
</tbody>
</table>

The standard quercetin shows an IC₅₀ at 0.01 mg/ml. During the term of number of DPPH scavenged per molecule of the quercetin the results showed that at 15 min 1 molecule of quercetin gives protons to 7.6 molecules of DPPH, which is in accordance with literature data (Milackova et al., 2013). As the decrease of the IC₅₀ value shows the scavenging activity increase, the antioxidant activities of testing extracts relative to DPPH stable radical scavenging could be arranged in a descending row: C. laevigata > A. absinthium > P. major.

HPLC analyses of ethanolic extracts are presented in figures 1, 2 and 3. Among the selected phenolic compounds in C. laevigata extract were identified: rutin, quercetin, quercitin, in Plantago major extract: rutin, quercetin, morin, quercetin. Similarly, in A. absinthium all selected phenolic compounds were identified except rutin. As envisioned from the data in the table 2 only two major components such as quercetin and quercitin (quercetin-3-O- α- L ramno - pyranosyl) are exemplified in all plant extracts. The percentages of the compounds of the extracts are shown in Table 2. There are many reports suggesting that the DPPH scavenging activity of the extracts is directly related with the total phenolic content (Saeed et al., 2012). However, it is noteworthy that in investigated extracts was no correlation between total flavonoids and antioxidant activity (figure 4). Moreover, in our study was shown the direct relationship only between the antiradical activity and quercetin concentration in selected plant extracts (figure 5). Based on the obtained data, it can be conclude that the antiradical activity largely depends on the qualitative composition of flavonoids and does not always depend on their total content in extracts. Due to the high content of quercetin and total flavonoids in ethanolic extract of C. laevigata, it appears that has many potential beneficial effects on health (Gregory, 2011). Use of studied tree for treating of cardiovascular diseases (Carlstrom et al., 2007; Augustyniak et al., 2010), gastric ulcerations (Alarcon et al., 1994) and wide range of oxidative stress induced diseases (Traka & Mithen 2011) are in accordance with the findings of the previous researchers.

4 Conclusions

It was revealed a direct relationship between the quercetin content and the antiradical activity of selected plant ethanolic extracts. Such correlation indicates an important role of quercetin in extracts antiradical activity. At the same time contribution of other phytochemical compounds into antiradical activity does not exclude. The results of the study revealed the importance of Crataegus laevigata as a source of natural antioxidants and bioactive phytopharmaceuticals. Subsequently, certain plant extracts or their active components with high antioxidant activity may be very helpful in treatment novel strategies for free radical mediated disorders.

References


