Abstract

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ANTIOXIDANT AND HEMOLYTIC PROPERTIES OF DIFFERENT EXTRACTS FROM PRUNELLA VULGARIS L. LEAVES

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The plant Prunella vulgaris L. has long been used in traditional European and Asian medicine. Its extracts reveal immunoprotective, antioxidant, antinflammatory, and antietrogenic features. We obtained aqueous and ethanol extracts from P. vulgaris L. leaves; carried out HPLC analysis; detected the total flavonoids content and the antiradical activity. The
effect of different extracts of Prunella vulgaris L. on the activity of superoxide dismutase of healthy donors’ erythrocytes was studied. It was shown that the major component of ethanol extracts is rosmarinic acid. 50 % ethanol extract of P. vulgaris features high antiradical, antioxidant (SOD) and low hemolytic activity.

**Keywords:** Prunella vulgaris L. extracts, total flavonoid content, HPLC analysis; antiradical activity, erythrocytolysis, SOD

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The total flavonoid content (TFC) was determined based on the flavonoids ability to chelate Al 3+ atoms at 430 nm [11]. The antiradical activity of the studied extracts was based on the bleaching of the stable DPPH radical (Fluka) at 517 nm measured on a UV/Vis spectrophotometer (JENWAY 6405) [12]. The antiradical activity (ARA, %) was calculated following the formula below, taking into account the value of the residual absorption (correction factor – CF).

\[
\text{ARA} = \frac{(RA_{\text{control}} - RA_{\text{sample}})}{(RA_{\text{control}} - \text{CF})} \times 100
\]

The antiradical activity (ARA, %) was calculated following the formula below, taking into account the value of the residual absorption (correction factor – CF). The extract activity was determined by the IC50 value, which indicated the amount of extract required to quench 50 % of DPPH radical in 1 ml of the solution, was determined based on dose-dependent ARA curves. To determine the major components of the flavonoid class, a high-performance liquid chromatography (HPLC) method was employed using a Waters Alliance 2695 chromatograph with a spectrophotometric and diode array detector, as well as MassLynx data processing software. Separation was done on a C-18 column (250×4 mm, particle size – 4.5 nm) at an elution rate of 1 ml/min with the following gradient elution system: deionized water with 0.1 ml/l of 90 % orthophosphoric acid (solution A) – acetonitrile (solution B); 0–5 min linear solution A was adjusted from 10 % to 40 %, then to 50 % over the next 3 minutes and for the last 12 minutes, this ratio of solutions was maintained isocratically. Ethanol solutions of quercetin, rutin, apigenin, kaempferol at 365 nm, naringenin – 290 nm, genistein – 261 nm (all by Sigma company) were used as standards.

Erythrocytes were obtained from the blood of healthy people as well as the blood of patients affected with periodic disease. The optical density of the erythrocyte suspension was adjusted to 0.7 optical units (6.2×10^5 cells/ml) [9]. The erythrocyte resistance was evaluated by changing the optical absorption of the erythrocyte suspension at 680 nm for 10–15 minutes, the readings being taken every 30 seconds, on the Spexcer M400 spectrophotograph (Carlzeiss, Germany). The effect of Prunella vulgaris L. extracts (standardized by...
Rosmarinic acid, on erythrocyte resistance, was determined by the following formula:

\[ P = \frac{A_x}{(A_0 - Ah)} \times 100 \% \]

where \( A_x \) is the optical density (OD) of the sample after irradiation for \( x \) time; \( A_0 \) – OD of unhemolyzed erythrocyte suspension; \( Ah \) – OD of the sample after full lysis, which does not reach the zero value because of hemichrome development.

The SOD activity was determined by the ability of the enzyme to inhibit adrenaline autooxidation detected by the adrenaline oxidation product with absorption at around 347 nm. The optical density of the solutions was recorded on a SF 26 spectrophotometer (Germany) at room temperature. SOD was obtained from erythrocytes hemolysate, and the activity was determined [13] subject to the formula

\[ \text{SOD activity (CU)} = \frac{(100 \% - \text{inhibition} \%) \times (\text{act. unit})}{\Delta D_{\text{control}}} \]

The statistical analysis was carried out using the Microsoft Office Excel (\( p < 0.05 \)).

**Results and Discussion.** A TFC analysis revealed that in most *P. vulgaris* extracts the indicator is quite high. The maximum TFC was found in 25 % of the *P. vulgaris* ethanol extract (24.75±0.7494 μg/g); it was lower in 50 % of the ethanol extract. Relatively low TFC was found in 96 % of ethanol, aqueous and 70 % of ethanol *P. vulgaris* extracts (Table). A HPLC analysis of extracts showed that the major component of ethanol extracts is rosmarinic acid (RA) (Fig. 1). The top high content of RA was identified in 50 % ethanol extract, which was 1.6 times as high as in the 96 % extract, and 1.1 times as high as in the 70 % extract. From the ARA point of view, the 70 % extract was the most active, while the least active was the 96 % ethanol extract.

**Table**

<table>
<thead>
<tr>
<th>Samples</th>
<th>RA content (mg/g)</th>
<th>TFC (μg/g)</th>
<th>IC50 (%)</th>
<th>SOD activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract</td>
<td>-</td>
<td>12.68±0.02472</td>
<td>74.0±0.05</td>
<td>50.7±1.1</td>
</tr>
<tr>
<td>25 % ethanol extract</td>
<td>-</td>
<td>24.74±0.7494</td>
<td>10.5±0.04</td>
<td>52.4±1.2</td>
</tr>
<tr>
<td>50 % ethanol extract</td>
<td>1.063±0.03</td>
<td>16.17±0.0826</td>
<td>0.36±0.002</td>
<td>18.7±0.9</td>
</tr>
<tr>
<td>70 % ethanol extract</td>
<td>0.982±0.01</td>
<td>12.28±0.31752</td>
<td>0.45±0.02</td>
<td>80.4±1.6</td>
</tr>
<tr>
<td>96 % ethanol extract</td>
<td>0.669±0.02</td>
<td>13.13±0.0192</td>
<td>0.65±0.01</td>
<td>22.2±0.6</td>
</tr>
</tbody>
</table>

Significant difference, \( p < 0.05 \).

The results showed that the stable DPPH radical-neutralizing activity of all the extracts was of dose-dependent nature. However, there is no correlation observed between the TFC and the ARA of extracts, which may be due to the different qualitative composition of their flavonoids or to the presence of other compounds groups with antioxidant properties.

The hemolytic effect of extracts on erythrocytes was investigated straight after the addition of 0.1 ml of extract to 1 ml of the erythrocyte suspension in dark conditions, in the light without/with incubation. At dark incubation (60 min) the *P. vulgaris* extracts revealed no hemolytic activity. After 60 min incubation, the 50 % and 70 % of the *P. vulgaris* ethanol extracts led to partial hemolysis. The aqueous extract of *P. vulgaris* after 24 hours basically showed no hemolysis. The 25 % extract had insignificant hemolytic activity (erythrocyte resistance – 81 %), and the 70 % and 96 % *P. vulgaris* ethanol extracts after 24 hours resulted in complete hemolysis. In the light, the erythrocyte resistance remained 100 % for *P. vulgaris* aqueous extracts, whereas all the ethanol extracts resulted in destroyed red blood cells at different points (Fig. 2). The outcomes of the experiment suggest that *P. vulgaris* contains photosensitizers that get extracted with ethanol.

Fig. 1. HPLC analysis of *Prunella vulgaris* L. ethanol extracts

Fig. 2. Photohemolysis induced by *P. vulgaris* extracts

Fig. 3. Erythrocyte SOD activity in the presence of *P. vulgaris* extracts
With incubation for one hour, the endo- and exogenous systems of ROS neutralization revealed an almost similar low inhibitory activity, which may depend on the neutralization reactions through the incubation.

**Conclusions.** Therefore, the highest ARA was observed in the 50 % *P. vulgaris* ethanol extract, where the content of RA was maximal. The TFC in this extract is lower, which is indicative of the major contribution that RA – and not flavonoids – has in ARA. The high ARA level in the 25 % extract is manifested not through RA only, yet also through other active components. Low dark-period hemolytic activity was observed in the aqueous, in the 25 %, as well as in the 50 % extracts of *P. vulgaris* leaves. The 50 % and 96 % ethanol extracts have high SOD activity.

The outcomes obtained from the experiments described above could be used to summarize that high antiradical, antioxidant (SOD), and low hemolytic activity resides in the 50 % extract of *P. vulgaris*.

**Disclosures:**
The authors declare no conflict of interest.

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