PHENOLIC COMPOUNDS IN TRADITIONAL BULGARIAN MEDICAL PLANTS

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ABSTRACT

A number of plants and plant products have medicinal properties that have been validated by recent scientific developments throughout the world, owing to their potent pharmacological activity, low toxicity and economic viability. In recent years, the use of natural antioxidants present in traditional medicinal plants has become of special interest in the scientific world due to their presumed safety and nutritional and therapeutic value (Kishore Dubey et al., 2015; Ajila et al., 2007). The majority of the antioxidant activity of plants is due to the presence of phenolic compounds (flavonoids, phenolic acids and alcohols, stilbenes, tocopherols, tetratolens), acorbic acid and carotenoids (Kishore Dubey et al., 2015).

Bulgaria is situated in the Balkan Peninsula, South-East Europe, Mediterranean and continental climates. The relief of the country is quite diverse ranging from plains to low hills and high mountains. The climate is moderate continental to modified continental, but in southern regions reflects rather a strong Mediterranean influence. As a result of this climatic condition, the Bulgarian flora is remarkable for its diversity (3500 plant species including 600 known medicinal plants) (Ivancheva and Stancheva, 2000; Ivancheva et al., 2006). Traditional Bulgarian medicinal plants have been used to treat human diseases in Bulgaria for thousands of years, and people are becoming increasingly interested in them because of their good health effects and low toxicity. In recent years, studies on the antioxidant activities of Traditional Bulgarian medicinal plants have increased remarkably in light of the increased interest in their potential as a rich source of natural antioxidants. Several studies have indicated that Traditional Bulgarian medicinal plants possess more potent antioxidant activities than common dietary plants, and contain a wide variety of natural antioxidants, such as total phenolics, flavonoids and tannins (Atanasova et al., 2011). In recent years, interest in plant-derived food additives has grown. Furthermore plant extracts of Bulgarian white birch (Betula pendula L.) leaves have been shown to possess health-promoting properties. The white birch leaves extract was strong diuretic and have effect at nephrolithiasis and urinary bladder lithiasos, sedative effect on spasms of smooth muscle. It might be used in following conditions: kidney diseases, ischia nerve inflammation and podagra and atherosclerosis and also it has an antimicrobial effect (Christova-Bagdassarian et al., 2014; Harbone, 1993). The extracts from the leaves of the white birch significantly increase diuresis, and with this and the emission of sodium and chloride ions, ie act as diuretikum. Until recently it was assumed that the diuretic action is due to the presence of resinosin substances. Therefore birch buds were preferred because they are rich in resins. However, it is clear that flavonoids have a greater role in the diuretic effect. They are contained mainly in the leaves. In addition, the leaves contain potassium nitrate, which enhances the diuretic effect of the flavonoids. This effect was related to total flavonoids (Christova-Bagdassarian et al., 2014; Neoretal, 2006).

Cotinus coggygria one of two species constituting a minor genus of the family Anacardiaceae, viz., Cotinus coggygria Scop. (syn. Rhus cotinus L.) itself and Cotinusovobatus Raf., the American smoketree. Its wide distribution extends from southern Europe, the Mediterranean, Moldova and the Caucasus, to central China and the Himalayas (Christova-Bagdassarian et al., 2016; Novakovic et al., 2007; Mate et al., 2011). C. coggygria is a common medicinal plant (well known as ‘smradika’ or ‘tetra’) in the Bulgarian folk medicine for outer use predominantly (Christova-Bagdassarian et al., 2016; Landzhew Chemical L., 2010; Ivanova et al., 2013).

Plants of the family Anacardiaceae have a long history of use by various peoples for medicinal and other purposes. Rhus glabraus traditionally used in the treatment of bacterial diseases such as syphilis, gonorrhea, dysentery and gangrene, while R. coriaria, besides its common use as a spice consisting of ground dried fruits with salt, is also widely used as a medicinal herb, particularly for wound healing (Christova-Bagdassarian et al., 2016; Matej et al., 2011; Rayne and Mazz, 2007). In folk medicine, Cotinus coggygria routinely used as an antiseptic, anti-inflammatory, antimicrobial and antihypertensive agent in wound-healing (Christova-Bagdassarian et al., 2016; Rayne and Mazz, 2007; Demirci et al.,...
The leaves from chicory (Cichorium intybus L.), white birch (Betula pendula), zdravec (Geranium macrorrhizum L.), smriddka (Cotinus coggyria, syn.: Rhus cotinus L.) and rose hip fruits (Rosa canina L.) were harvested from different regions of Bulgaria. All sample data are stated in the sampling protocol. The dried leaves and rose hip fruit were kept in a dry place until further use.

Sample preparation
A dry simple of 0.5 g was weighted and phenolic and flavonoid compounds were extracted with 50 mL 80% aqueous methanol on an ultrasonic bath for 20 min. An aliquot (2 mL) of the extracts was ultracentrifuged for 5 min at 14 000 rpm. The extract prepared in this way was used for further spectrophotometric determination of polyphenols.

Determination of total phenolics assay
The total phenolic contents of medicinal plants were determined by using the Folin-Ciocalteau assay. An aliquot (1 mL) of extracts or standard solution of gallic acid (10, 20, 40, 60, 80, 100 and 120 mg/L) was added to 25 mL volumetric flask, containing 9 mL of distilled deionised water (dd H2O). A reagent blank using dd H2O was prepared. One milliliter of Folin-Ciocalteau’s phenol reagent was added to the mixture and shaken. After 5 min, 10 mL of 7% Na2CO3 solution was added to the mixture. To the solution the dd H2O was added up to volume of 25 mL and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (Marinova et al., 2005).

Determination of total flavonoids assay
The total flavonoid contents were determined by aluminum chloride colorimetric assay. An aliquot (1 mL) of extracts or standard solution of catechin (10, 20, 40, 60, 80, 100 and 120 mg/L) was added to 25 mL volumetric flask, containing 9 mL of distilled deionised water (dd H2O). To the solution the dd H2O was added up to volume of 25 mL and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (Marinova et al., 2005).

Rutin assay
The analyses of rutin content in Bulgarian medicinal plants were performed according to The International Pharmacopoeia and AOAC method, after modified methods with using 80% aqueous methanol. Pippet 2 mL aliquots solution into 50 mL volumetric flask was added to 2 mL deionized water (dd H2O) and 5 mL ammoniummolybdat. The solution was added volume (50 mL) with dd H2O and mixed. Was prepared standard solution of rutin (0.0200 g dissolved into 2 mL methanol) was added volume (50 mL) with 80% aqueous methanol. An aliquot (1 mL) of standard solution into 50 mL volumetric flask and dilute to volume with distilled deionized water (dd H2O). A reagent blank using dd H2O was prepared. The absorbance against prepared reagent blank was determined at 360 nm with an UV-Vis Spectrophotometer BOECO – Germany. All samples were analyzed in duplicates (Atanassova et al., 2009a).

Calculations
Calculations are based on averaging results from analyses of duplicate samples. Calculate content (C) of rutin (R) in sample as follows:

\[
R(%) = \frac{A_{sample} \times C_{X} \times 50 \times 100}{A_{reagent} \times W \times 2}
\]

Where:
- \(A_{sample}\) - Absorbance of sample was determined at 360 nm;
- \(A_{reagent}\) - Absorbance of standard solution was determined at 360 nm;
- \(C\) – Concentration of standard solution of rutin (g/mL);
- \(W\) – weight (g) of sample for analyses;
- 2 – Volume (mL) of sample for analyses;
- 100 – Percent, %.

Tannins assay
The analyses of tannin content in traditional Bulgarian medicinal plants were performed according to The International Pharmacopoeia and AOAC method, after modified methods. Measured 25 mL of this infusion into 1 L conical flask and add 25 mL indigo solution and 750 mL distilled deionized water (dd H2O). Titrated with 0.1 N water solution of KMnO4 until blue solution changes to green, then add a few drops at time until solution becomes golden yellow. Was prepared standard solution of Indigo carmine (dissolve 6 g indigo carmine in 500 mL distilled deionized water (dd H2O) by heating, cool add 50 mL 96% - 98% H2SO4, diluted to 1 L and then filtered. For the blank similarly titrated mixture of
25 mL indigocarmine solution and 750mL ddH₂O. All samples were analyzed in duplicates (Atanassova et al., 2009b).

Calculations
Calculations are based on averaging results from analyses of duplicate samples. Calculate content (%) of tannins (T) in sample as follows:

$$\text{Tr} (%) = \left( \frac{V - V_0}{4.755} \times \frac{0.004157 \times 250 \times 100}{g \times 25} \right) \times 100$$

Where:
- V – Volume of 0.1 N water solution of KMnO₄ for titration of sample, mL;
- V₀ – Volume of 0.1 N water solution of KMnO₄ for titration of blank sample, mL;
- 0.004157 – Tannins equivalent in 1 mL of 0.1 N water solution of KMnO₄;
- g – Mass of the sample for analyses, g;
- 100 – Percent, %.

Statistical analysis
All experiments were performed in triplicates. Analysis at every time point from each experiment was carried out induplicate or triplicate. The statistical parameters are calculated in terms of the reproducibility of the experimental data using a statistical package universal ANOVA.

RESULTS AND DISCUSSION
Different phytochemicals have various protective and therapeutic effects which are essential to prevent diseases and maintain a state of well being. Methanolic extract of rose hip fruits (Rosa canina L.), chicory (Cichorium intybus L.), zdravec (Geraemia macrocarpa L.), smradlika (Cotinus coggygria, syn.: Rhus cotinus L.) and white birch (Betula pendula L.) were analyzed for phytoconstituents. The quantitative estimation of phenolic compounds of white birch (Betula pendula L.), smradlika (Cotinus coggygria, syn.: Rhus cotinus L.), zdravec (Geraemia macrocarpa L.), chicory (Cichorium intybus L.) and rose hip fruits (Rosa canina L.) show that the traditional Bulgarian medical plants are rich in total phenolics, total flavonoids according to the data shown in the Table 1 and Figure 1. It is well that plant flavonoids and phenols in general, are highly effective free radical scavenging and antioxidants. The phenolic constituents found in herbs have attracted considerable attention as being the main agents of antioxidant activity, although they are not the only ones. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential. Hence, the antioxidant activity of phenolics plays an important role in the adsorption or neutralization of free radicals (Dutra et al., 2008; Laguerre et al., 2007).

The major compounds that are related to the antioxidant potential of a plant (or plant part) are the total phenolics and, more specifically, the flavonoids (Dutra et al., 2008; Kumar Ashok et al., 2012), though there is no consensus as to whether these are the sole substances in plants that act in scavenging free radicals. It is well known that plant phenolics, in general are highly effective in free radical scavenging and they are antioxidants. The presence of these phytochemicals in medical plants is thus a significant finding of the present study. The content of total phenolics and total flavonoids in white birch (Betula pendula L.), varying between 5256.30 mg GAE/100g dw to 2245.70 mg CE/100g dw, was found to be much higher than in rose hip fruits (Rosa canina L.) - 406.79 mg GAE/100g dw to 290.13 mg CE/100g dw, respectively, as shown in Table 1 and Figure 1 with gallic acid and catechin as standards. These results indicate that the higher antioxidant activity of the white birch (Betula pendula L.) methanol extract, compared to the rose hip fruits (Rosa canina L.) methanol extract, may be correlated to the phenolic and flavonoid content of respective medical plant extract.

<table>
<thead>
<tr>
<th>Bulgarian medical plants</th>
<th>Total phenolics (mg GAE/100g dw)</th>
<th>Total flavonoids (mg CE/100g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rose hip fruits (Rosa canina)</td>
<td>406.79±0.02</td>
<td>290.13±0.01</td>
</tr>
<tr>
<td>Chicory (Cichorium intybus)</td>
<td>635.87±0.05</td>
<td>315.15±0.04</td>
</tr>
<tr>
<td>Zdravec (Geraemia macrocarpa)</td>
<td>1530.70±0.02</td>
<td>110.20±0.06</td>
</tr>
<tr>
<td>Smradlika (Cotinus coggygria)</td>
<td>2581.60±0.02</td>
<td>810.40±0.04</td>
</tr>
<tr>
<td>White birch (Betula pendula)</td>
<td>5256.30±0.04</td>
<td>2245.70±0.04</td>
</tr>
</tbody>
</table>

Figure 1 Total phenolics and total flavonoids in Traditional Bulgarian medical plants.

The presence of rutin and tannins in chicory (Cichorium intybus L.), rose hip fruits (Rosa canina L.), zdravec (Geraemia macrocarpa L.), white birch (Betula pendula L.) and smradlika (Cotinus coggygria, syn.: Rhus cotinus L.) show that the traditional Bulgarian medical herbs are rich in tannins, rutin according to the data shown in the Table 2 and Figure 2. The phytochemical screening and quantitative estimation of the percentage of chemical constituents of the plants studied showed that the dry herbs were rich in rutin and tannins. Tannins are distributed all over the plant kingdom (Bate-Smith, 1962). The term tannin refers to the use of tannins in tanning animal hides into leather; however, the term is widely applied to any large polyphenolic compound containing sufficient hydroxyl and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules. Tannins have molecular weights ranging from 500 to over 3000 (Bate-Smith, 1962; Cheng et al., 2003). Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorrhoidal compounds (Bate-Smith, 1962; Lin et al., 2004). Tannins can also be effective in protecting the kidneys (52.55). Tannins are also beneficial when applied to the mucosal lining of the mouth (Bate-Smith, 1962; Habtemariam and Varghese, 2015). Rutin is a common dietary flavonoid widely distributed in the plant kingdom. It is also present in plant-derived beverages and foods as well as numerous medicinal and nutritional preparations (Sando and Lloyd, 1924; Habtemariam and Varghese, 2015). Today, rutin is among the most popular natural flavonoids known for its multifunctional nutritional and therapeutic uses (Sando and Lloyd, 1924; Habtemariam and Lenti, 2015). As far as commercial exploitation of rutin is concerned, however, very few plants store it in large amounts to merit the cost of its extraction from natural sources (Sando and Lloyd, 1924). They were known to show medicinal activity as well as exhibiting physiological activity. The presence of these phenolic compounds in traditional Bulgarian medical herbs is a significant finding in this present study. The content for rutin of white birch (Betula pendula L.) varied between 6.24 % was found to be much higher than and chicory (Cichorium intybus L.) - 2.09 %, respectively as shown in Table 2 and Figure 2 with rutin as standards. The content for tannins of smradlika (Cotinus coggygria, syn.: Rhus cotinus L.) varied between 11.15 % was found to be much higher than and chicory (Cichorium intybus L.) - 2.26 %, respectively as shown in Table 2 and Figure 2 and KMnO₄ as titrate. It is important to notice that the comparison of
the results for rutin and tannin contents in the medical herbs will be not correct because of the different methods of analysis.

**Table 2 Tannins and rutin in studied Traditional Bulgarian medical plants**

<table>
<thead>
<tr>
<th>Bulgarian medical plants</th>
<th>Tannins, (%)</th>
<th>Rutin, (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicory (Cichorium intybus L.)</td>
<td>2.26 ± 0.04</td>
<td>2.09 ± 0.01</td>
</tr>
<tr>
<td>Rose hip fruits (Rosa canina L.)</td>
<td>4.11 ± 0.09</td>
<td>2.16 ± 0.04</td>
</tr>
<tr>
<td>Zdravec (Geranium macrorrhizum L.)</td>
<td>6.08 ± 0.07</td>
<td>3.20 ± 0.08</td>
</tr>
<tr>
<td>White birch (Betula pendula L.)</td>
<td>9.04 ± 0.03</td>
<td>6.24 ± 0.02</td>
</tr>
<tr>
<td>Smrdalika (Cotinus coggyria, syn.: Rhus cotinus L.)</td>
<td>11.15 ± 0.06</td>
<td>3.06 ± 0.06</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In conclusion, on the basis of the results of this research showed that total phenolic, total flavonoid, rutin and tannin contents are important components in traditional Bulgarian medical plants grown in the country. The use of medical plants as the first choice in self-treatment of minor conditions continues to expand rapidly across the world. This makes the safety of medical plants an important public health issue. The results can be used in public health campaigns to stimulate the consumption of traditional Bulgarian plants as chicory (Cichorium intybus L.), rose hip fruits (Rosa canina L.), zdravec (Geranium macrorrhizum L.), white birch (Betula pendula L.) and smrdalika (Cotinus coggyria, syn.: Rhus cotinus L.) which are able to provide significant health protection in order to prevent chronic diseases.

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