THE POTENTIAL BIOACTIVITY OF THE WILD GROWN ROSEHIP (Rosa canina L.) AND POMEGRANATE (Punica granatum L.)

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The aim of this study was the determination of the antioxidant and antihyperglycemic effect of wild grown rosehip and pomegranate fruit extracts, and the determination of the polyphenolic content (total phenols, flavonoids, flavonols, flavan-3-ols and total and monomeric anthocyanins). The antioxidant activity of rosehip fruit in view of stable DPPH and ABTS radicals was higher comparing to pomegranate fruit, while according to the OH radical these two samples showed a similar effect (94.17 and 92.03 μg/mL). A dry rosehip fruit extract was found to have a 1.6 times more pronounced antihyperglycemic activity (1.42 mg/mL) compared to the dried pomegranate fruit extract (2.26 mg/mL). The content of total phenols of rosehip fruit was 8.75 mg GAE/gFPM and 22.01 mg GAE/gPPM, higher than the content of total phenols of pomegranate fruit (3.44 mg GAE/gFPM and 15.43 mg GAE/gPPM). The content of total phenols in rosehip and pomegranate fruits was higher than the content of total flavonoids, flavonols and flavan-3-ol and total and monomeric anthocyanins, which indicates that these components had the greatest impact on bioactivity of wiled grown fruits. Due to their bioactivity, the fruits of wild grown rosehip and pomegranate can be considered as potential functional food and food suitable for diabetics.

Introduction

Daily consumption of fruits and vegetables provides for body essential nutrients, such as fibers and bioactive compounds, and reduces the risk of developing some chronic diseases [1]. Wild grown edible fruits may have the potential to confer beneficial health effects due to their bioactivity and polyphenolic compounds [2]. Rosehip (Rosa canina L.) belongs to the rose family (Rosaceae) and is a good source of bioactive compounds such as: vitamins, carotenoids, amino acids, organic acids, minerals, as well as phenolic compounds that show antioxidant, anticancer and antimutagenic effects [3-6]. Rosehip fruits are less often used fresh, but mainly during the industrial processing for marmalade, jelly, syrup, herbal tea and the wine production [7]. Wild grown pomegranate (Punica granatum L.) is considered to be one of the oldest edible fruit and it is classified in the family Punicaceae. The edible part of the pomegranate fruit are the arils (52% of the fruit weight) comprising 78% juice and 22% seeds [8]). Fruits of wild grown pomegranate are good sources of many, for health beneficial compounds, such as tannins, anthocyanins, phenolic and organic acids [9].

α-glucosidase is an enzyme found in the small intestine of the human digestive tract and is involved in the final step of digestion of carbohydrates to glucose. People with the increased activity of this enzyme suffer from postprandial hyperglycemia that occurs after eating, when the blood glucose level is above 180 mg/dL. Carbohydrates digestion and absorption slow down with the inhibition of α-glucosidase and the increase in the glucose levels after the meal decreases [10-12]. Studies have shown that polyphenolic compounds from fruit are effective α-glucosidase inhibitors and able to cause an antihyperglycemic effect. In addition, polyphenolic compounds flavonoids, flavones, isoflavones and antocyanins show other bioactivities such as the antioxidant effect [13].

The aim of this study was the determination of the antioxidant and antihyperglycemic effect of wild grown rosehip and pomegranate fruit extracts, and the determination of the polyphenolic content (total phenols, flavonoids, flavonols, flavan-3-ols and total and monomeric anthocyanins).

Experimental

Plant material

Fully ripe, wiled grown fruits were picked manually in October 2019, rosehips (Rosa canina L.) at the locality of Ro-vine, the municipality of Gradiška, and pomegranates (Punica granatum L.) at the locality of Žegulja, the municipality of Stolac. The fruit samples were cleaned from inedible parts, homogenized in an electric mill and stored at -18 °C until analysis. The dry matter content of the fruits was determined according to the standard method [19].

Preparation of the extracts

The samples (5 g) were treated in an ultrasonic bath for 15 minutes and were extracted with 80% ethanol for 10 minutes...
each, with solvomodule 1:10 m/v. The extraction procedure was performed in triplicate.

The solvent from the liquid extracts was removed by evaporation on a rotary evaporator. The resulting extracts were dried in a vacuum desiccator to constant weight and stored in the refrigerator at +4 °C until the antihyperglycemic activity analysis.

The determination of total phenolic, flavonoid, flavonol, flavan-3-ol and total and monomeric anthocyanins contents.

Liquid ethanolic extracts were used for the determination of the content of total phenols, flavonoids, flavonols and flavan-3-ol and the antioxidant activity. For the antihyperglycemic effect determination, the obtained extracts were evaporated until dry. Total phenolics content was determined spectrophotometrically (λ=765 nm) by a modified Folin-Ciocalteu method [14]. The results were expressed as mg of gallic acid equivalent per gram of the fresh plant material (mg GAE/g FPM). Total flavonoids content was measured at λ=420 nm as described by Ordon et al. [15]. The results were expressed as mg of quercetin equivalent per gram of the fresh plant material (mg Qc/g FPM). Total flavanols content was measured at λ=510 nm by Kumaran and Karunakaran [16]. The results were expressed as mg of quercetin equivalent per gram of the fresh plant material (mg Qc/g FPM). Total flavan-3-ols content was measured at λ=500 nm [17]. The results were expressed as µg of catechin equivalent per gram of the fresh plant material (µg CAT/g FPM). To determine total and monomeric anthocyanins, 20 grams of the sample was extracted with the solution of 1.5M HCl and 96% ethanol (85:15, v/v) for 24 hours at 0 °C. The spectrophotometric pH differential method was used to determine monomeric and total (monomeric plus polymerized) anthocyanins [18]. Two dilutions of the sample were prepared in the 0.025M KCl solution and in the 0.4M sodium acetate solution adjusted respectively to pH 1.0 and 4.5 with HCl. The absorbance of each dilution was measured at 520 and 700 nm against a distilled water blank. The contents of total and monomeric anthocyanins were expressed as mg of cyanidin-3-glucoside per gram of the fresh plant material (mg CyG/g FPM).

Spectrophotometric measurements were performed on a UV-VIS spectrophotometer (PerkinElmer Lambda 25). In order to facilitate the comparison of our results with the literature data, the content of total phenols, flavonoids, flavonols, flavan-3-ols and total and monomeric anthocyanins of rosehip and pomegranate fruit samples were also calculated and expressed on the dry plant material (DPM).

Antioxidative activity

The antioxidant activity of the samples considering the stable DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical was determined by the method of Liyana-Pathiranan and Shaihid [20]. Trolox solutions in the range of 0.25-4 µg/mL were used to make the calibration curve and the measuring wavelength was 515 nm. The antioxidant activity of the samples considering the stable ABTS (2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonyl)-diammonium salt) radical was determined by the method of Re et al. [21]. Trolox solutions in the range of 1.25-10 µg/mL were used to make the calibration curve, and the measuring wavelength was 734 nm. The ability of ethanol extracts to neutralize the hydroxyl radical was determined according to the method described in the literature [22]. The results of the antioxidant activity are presented as EC50 (the concentration of the extract or standard that has the ability to inhibit 50% DPPH, ABTS and OH radicals) and TEAC value (Trolox Equivalent Antioxidant Activity for DPPH and ABTS radicals).

Antihyperglycemic activity

The influence of dry extracts of rosehips and wild grown pomegranate on the inhibition of the enzyme α-glucosidase activity (antihyperglycemic effect) was determined by a modified method according to Chan et al. [23]. The method is based on measuring the increase in absorbance at the wavelength of 405 nm, which originates from 4-nitrophenol released from 4-nitrophenyl-α-D-glucopyranoside under the action of the enzyme α-glucosidase after 20 minutes of incubation with and without the sample (control) on 37 °C. The results are presented as EC50.

Statistical data processing was performed in OriginPro 8.0, and the results were presented as mean ± standard deviation (SD) of three parallel measurements.

Results and discussion

The polyphenolic composition of wild grown rosehip and pomegranate samples is shown in Table 1. The content of total phenols in the rosehip sample was 8.75 mg GAE/g FPM, which is higher compared to the results of other authors (from 2.57 to 8.13 mg GAE/g FPM) [2, 24-26]. Murathan et al. [27] reported higher values of total phenols in different rosehip species, ranging from 10.81 to 62.98 mg GAE/g FPM. The content of total flavonoids was 1.08 mg Qc/g FPM, about 3 times higher than the content of total flavonoids that Cosmulescu et al. [2] found in Romanian wild grown rosehip. The content of total flavonols and flavan-3-ols was 0.057 mg Qc/g FPM and 1.30 µg CAT/g FPM, respectively. According to the obtained results (Table 1), the content of total phenols, flavonoids, flavonols and flavan-3-ols in rosehip are higher than the content of this compounds found in the pomegranate sample extracts. According to Cosmulescu et al. [2] these differences can be explained due to genetic factors and a different ability to synthesize the secondary metabolites of species. That ability of the plants was selected in their phylogenetic development process, and there are relevant differences with regard to the synthesis and accumulation of secondary metabolites within species.

The content of total phenols in the pomegranate sample was 3.44 mg GAE/g FPM. The content of total flavonoids, flavonols and flavan-3-ol was significantly lower than the content of total phenols (Table 1). The content of total anthocyanins in the pomegranate sample was 0.07 mg CyG/g FPM, and the same content was found in cultivated pomegranate juice (cultivar ‘Ruby’) and wild grown pomegranate fruit in the literature data [28, 29]. The dry matter content of
the rosehip sample was 39.76%. According to the literature data, the dry matter content in fruits of wild grown rosehip ranges from 20.5 to 56.7% [27, 30]. The pomegranate sample had 22.30% of the dry matter, slightly higher than the value (19.60%) reported by Calin-Sánchez et al. [9].

Table 1. The content of total phenols (TP), flavonoids (TF), flavonols (TFl), flavan-3-ols (TFl3ol) and total (TA) and monomeric anthocyanins (MA) in wild grown rosehip and pomegranate samples expressed per gram of the fresh plant material.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TP mgGAE/g</th>
<th>TF mgGAE/g</th>
<th>TFl mgGAE/g</th>
<th>TFl3ol µgCAT/g</th>
<th>TA mgCyG/g</th>
<th>MA mgCyG/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosehip</td>
<td>0.75±0.07</td>
<td>1.28±0.04</td>
<td>0.05±0.02</td>
<td>1.30±0.08</td>
<td>0.19±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>3.46±0.23</td>
<td>0.81±0.01</td>
<td>0.19±0.01</td>
<td>0.41±0.14</td>
<td>0.21±0.01</td>
<td>0.08±0.01</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard deviation SD (n=3). GAE - gallic acid, Qc - quercetin hydrate, CAT - catechin, CyG - cyanidin-3-glucoside.

The polyphenolic composition of the wild grown rosehip and pomegranate samples expressed on the dry plant material is shown in Table 2. Expressed on the dry plant material, the content of total phenols of rosehip sample was lower in relation to the literature data [5, 6].

Table 2. The content of total phenols (TP), flavonoids (TF), flavonols (TFl), flavan-3-ols (TFl3ol) and total (TA) and monomeric anthocyanins (MA) in wild grown rosehip and pomegranate samples expressed per gram of the dry plant material.

<table>
<thead>
<tr>
<th>Samples</th>
<th>TP mgGAE/g</th>
<th>TF mgGAE/g</th>
<th>TFl mgGAE/g</th>
<th>TFl3ol µgCAT/g</th>
<th>TA mgCyG/g</th>
<th>MA mgCyG/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosehip</td>
<td>22.51±1.19</td>
<td>2.71±0.10</td>
<td>0.14±0.05</td>
<td>3.27±0.21</td>
<td>0.18±0.03</td>
<td>0.08±0.05</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>15.43±1.05</td>
<td>3.65±0.10</td>
<td>0.09±0.01</td>
<td>3.16±0.63</td>
<td>0.21±0.01</td>
<td>0.27±0.02</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard deviation SD (n=3). GAE - gallic acid, Qc - quercetin hydrate, CAT - catechin, CyG - cyanidin-3-glucoside.

The total anthocyanins content of the rosehip sample, expressed on the dry plant material, was higher in relation to the value obtained by Murathan et al. [27]. According to the results of other authors, the content of total phenols of pomegranate fruit ranges from 4.92 to 7.57 mg GAE/DPM [9, 31], which is lower than the content of total phenols in pomegranate presented in Table 2.

Table 3. Antioxidative activity of ethanolic extracts of wild grown rosehip and pomegranate samples.

<table>
<thead>
<tr>
<th>Method</th>
<th>DPPH test</th>
<th>ABTS test</th>
<th>Neutralization of OH radicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Samples</td>
<td>EC50 µg/mL</td>
<td>TEAC µmol/L</td>
<td>EC50 µg/mL</td>
</tr>
<tr>
<td>Rosehip</td>
<td>956.20±1.0</td>
<td>25.2±1.33</td>
<td>137.8±0.29</td>
</tr>
<tr>
<td>Pomegranate</td>
<td>291.2±2.13</td>
<td>19.2±1.21</td>
<td>152.6±2.94</td>
</tr>
</tbody>
</table>

Results are presented as mean ± standard deviation SD (n=3). EC50 – extract concentration that inhibits 50% of the radical. TEAC - Trolox Equivalent Antioxidant Activity.

The antioxidant activity of rosehip and pomegranate fruit extracts, considering stable DPPH, ABTS and OH radicals is shown in Table 3. Other authors found a higher antioxidant activity (DPPH) of rosehip expressed as the EC50 value [5, 24]. On the other hand, the antioxidant activity (DPPH and ABTS) expressed as TEAC value (Table 3) was higher than the antioxidant activity of rosehip reported by Demir et al. [5] and Cosmulescu et al. [2].

The antioxidative activity of pomegranate present in Table 3 was lower than the antioxidative activity of pomegranate researched by other authors [9, 31]. According to the results of DPPH and ABTS tests presented as EC50 and as TEAC values, it can be concluded that the rosehip extract had a higher antioxidant activity than the pomegranate extract. At the same time, the rosehip sample had a higher content of polyphenolic components compared to the pomegranate sample (Table 1).

The effect of rosehip and pomegranate extracts on the neutralization of OH radicals was approximately equal (94.17 and 92.03 µg/mL). Nadjpal [22] found higher values of methanol extracts of rosehip on the neutralization of OH radicals (475 µg/mL), indicating a lower antioxidant activity compared to the antioxidant activity of the samples tested in this study.

The number of people suffering from hyperglycemia and diabetes is increasing worldwide. The inhibition of carbohydrate digestive enzymes by dietary phenolics may represent a mechanism for delivering some of the health benefits attributed to a diet rich in fruits [32].

The dry extract of the rosehip fruit sample showed about the 1.6 times better antihyperglycemic activity compared to the dry extract of the pomegranate fruit sample (Figure 1). Also, the content of total phenols in the rosehip fruit is about 1.4 times higher than the content of total phenols in the pomegranate fruit (expressed on the dry plant material of the sample, Table 2), based on which it can be assumed that the concentration of total phenols directly affects the inhibition of the α-glucosidase enzyme activity. Kunyanga et al. [33] found that food ingredients (cereals, legumes and oil seeds) with a high phenolic content exhibited relatively higher antioxidant and antihyperglycemic activities.

Figure 1. Antihyperglycemic activity of dry ethanolic extracts of wild grown rosehips and pomegranate fruits. EC50 values were obtained from the diagram: antihyperglycemic activity in relation to the concentration (wild rosehip/pomegranate).

Both samples showed a higher antihyperglycemic activity compared to aqueous extracts of edible parts of mandarin orange, apple, watermelon and grapefruit, but
lower compared to the mulberry fruit ethanol extract [11, 34].

Conclusion

The results of this study indicate a significant antioxidant and antihyperglycemic activity of ethanolic extracts of wild grown rosehip and pomegranate. Among the investigated fruits, the rosehip with a higher content of phenolic components exhibited a relatively higher antioxidant as well as antihyperglycemic activity. The content of total phenols in the rosehip fruit was about 1.4 times higher than the content of total phenols in the pomegranate fruit, and it can be assumed that the concentration of total phenols directly affects the inhibition of the α-glucosidase enzyme activity. The content of total phenols in rosehip and pomegranate fruits was significantly higher than the content of total flavonoids, flavonols and flavan-3-ol and total and monomeric anthocyanins, which indicates that these components had the greatest impact on bioactivity of wild grown fruits. Due to their bioactivity, the fruits of wild grown rosehip and pomegranate can be considered as potential functional food and food suitable for diabetics.

Abbreviations and symbols

FPM - Fresh Plant Material  
DPM - Dry Plant Material  
GAE - Gallic Acid Equivalent  
Qc - quer cet in hydrate  
CAT - catechin  
CyG - cyanidin-3-glucoside  
DPHH - 2,2-diphenyl-1-picryl-hydrazyl  
ABTS - 2,2-Azinobis-(3-ethylbenzothiazoline-6-sulfonyl)-di ammonium salt  
TROLOX - 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid  
TEAC - Trolox Equivalent Antioxidative Activity

References

[22] J. Nadpal, Fitochemijski skrining i biološka aktivnost ekstrakata i tradicionalnih proizvoda od plodova divljih ruža (Rosa L.; Rosaceae). Thesis, Faculty of Technology,
University of Novi Sad, 2017.


Izvod

**POTENCIJALNA BIOAKTIVNOST PLODOVA SAMONIKLOG ŠIPKA (Rosa canina L.) I NARA (Punica granatum L.)**

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Cilj rada bio je određivanje antioksidativnog i antihiperlinskih efekta etanolnih ekstrakata ploda samoniklog šipka i nara, kao i određivanje sadržaja ukupnih fenolnih komponenti u ispitivanim plodovima. Antioksidativna aktivnost ploda šipka na stabilne DPPH i ABTS radikale bila je veća u odnosu na plod nara, dok su prema OH radikalu ova dva uzorka pokazala sličan efekat (94,17 i 92,03 µg/mL). Ustanovljeno je da suvi ekstrakt ploda šipka ima 1,6 puta izraženije antihiperlinski delovanje (1,42 mg/mL) u odnosu na suvi ekstrakt ploda nara (2,26 mg/mL). Sadržaj ukupnih fenolnih komponenti u plodovima šipka i nara izražen na masu svežeg biljnog materijala iznosio je 8,75 mg GAE/g i 3,44 mg GAE/g, odnosno 22,01 mg GAE/g i 15,43 mg GAE/g, na suvu materiju biljnog materijala. Sadržaj ukupnih fenolnih komponenti u plodovima šipka i nara bio je veći od sadržaja ukupnih flavonoida, flavonola i flavan-3-ola kao i ukupnih i monomernih antocijanata, što ukazuje da su ove komponente imale najveći uticaj na bioaktivnost samoniklih plodova. Zbog utvrđene bioaktivnosti, plodovi samoniklog šipka i nara mogu se smatrati potencijalnom funkcionalnom hranom i hranom pogodnom za dijabetičare.

**Ključne reči:** polifenolne komponente, antioksidativnost, antihiperlinski dejetstvo