CHEMICAL COMPOSITION, BIOLOGICAL POTENTIALS AND ANTIMICROBIAL ACTIVITY OF WILD AND CULTIVATED BLACKBERRIES

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The aim of this study was to compare the chemical compositions and biological potentials (antioxidant potentials, α-glucosidase test and antimicrobial activity) of wild and cultivated blackberry varieties from two different locations. The chemical compositions were evaluated in fresh samples, while the biological potentials were measured in dry blackberry extracts. The highest dry matter (15.73 g/100 g fw) was obtained for wild blackberry from Verići (Bw2). The significantly higher content of sugars (6.07 g/100 g fw) and ascorbic acid (21.36 g/100 g dw) was found in Chester Thornless blackberry (Bc1) comparing to other samples. The wild blackberry from Javorani (Bw1) showed the highest amount of polyphenols, as well as antioxidant potentials on DPPH (184.26 mmol Trolox/kg dw), ABTS (340.26 mmol Trolox/kg dw) and OH (944.03 mmol BHT/kg dw) radicals. The antihyperglycemic potentials of the extracts were determined by the α-glucosidase test. The cultivated blackberry Čačanka Bestrna (Bc2) showed the stronger inhibition of α-glucosidase enzyme (α-GIP = 50.69 %) than other varieties (p<0.05). Wild blackberry extracts showed higher antibacterial and antifungal activity towards Staphylococcus aureus, Escherichia coli, and Aspergillus niger. The results presented in this study indicated the differences between wild and cultivated blackberry varieties, as well as mutual differences in chemical composition, polyphenol contents, biological potentials, along with the antimicrobial activity of wild and cultivated blackberry varieties from two different locations in Bosnia and Herzegovina.

KEY WORDS: blackberry, chemical composition, antioxidative capacity, α-glucosidase, antimicrobial activity

INTRODUCTION

Blackberries (Rubus fruticosus) are complex species in genetic background, growth characteristics, and number of varieties (1, 2). Fresh blackberry is nutrient rich food - due to chemical composition and excellent organoleptic properties (3). Blackberries are traditionally used to make jams, juices, syrups and wines (4, 5). The fruits of wild blackber-
ries are smaller and with a higher content of dry matter, in comparison with cultivated varieties (6). Based on previous research findings, cultivated blackberry as a raw material showed excellent quality in production, while wild blackberry had higher nutritional quality and significantly stronger biological potential (6, 7). Synthetic fertilizers are regularly used in conventional cultivation, which guarantees higher nitrogen sources, accelerating the development of plants and the synthesis of secondary metabolites. The absence of synthetic pesticides leads to the exposure of plants to stressful situations due to insect attack, which leads to development of natural responses, such as producing polyphenolic compounds (8, 9).

Many studies showed conclusively that the most of the antioxidant activity may be due to the compounds such as flavones, isoflavones, flavonoids, and anthocyanins more than the traditionally considered vitamins C (10). Due to the great potential of anthocyanins, as natural and harmless pigments, there is increasing number of studies in various areas such as the development of analytical methods for their isolation, identification, and usage in food and pharmaceutical products (11-13). It was reported that the extracts of blackberry contained 71% of the dominant anthocyanin, cyanidin 3-glucoside and possessed strong chemopreventive and chemotherapeutic activities (14, 15). Blackberries represent good sources of bioactive compounds with certain antiradical, and antidiabetic effects, which correlated with their antioxidant activities and total polyphenolic contents (16, 17). The polyphenolic components have a positive effect on the regulation of type II diabetes, by inhibiting enzyme α-glucosidase and controlling glucose absorption in the blood (18, 19). Kaume et al. (20) also reported protective effects of anthocyanins on the vascular system in diabetic patients.

Different types and varieties of blackberry showed in vitro antibacterial activity against a wide range of bacteria (21-27). The intensity of the antimicrobial effect depends on the type of extract (water, ethanol, methanol, ethyl acetate, etc.) and extract concentrations. In most experiments, Gram-positive bacteria are more susceptible to the effect of extracts than Gram-negative bacteria, which was explained by the differences in cell wall structure (28). The antimicrobial activity of blackberry extracts is correlated to the content of various polyphenolic compounds, especially anthocyanins and ellagitannins (24, 29). The content of antifungal components in different parts of plants is largely dependent on external influences and pathogen attacks, which induce the synthesis and activation of phytosterol deficient substances (30, 31). Defensive substances are synthesized from the precursor de novo in response to abiotic or biotic stress, so there may be variability in their content between the different populations and different individuals.

Blackberries are very important berries in Bosnia and Herzegovina, but the differences in chemical and polyphenol compositions in varieties on different locations have not been investigated yet. The aim of this study was to evaluate differences in chemical components, biological potentials, and antimicrobial activity between two wild and cultivated blackberry varieties from two different locations in Bosnia and Herzegovina.
EXPERIMENTAL

Chemicals and instruments

Chemicals and solvents used in this study were of analytical grade, supplied by Sigma-Aldrich (St. Louis, MO, USA), Lach Ner s.r.o. (Neratovice, Czech Republic), and Zorka Pharma, Šabac (Serbia). Nutrient broth, Nutrient agar, Mueller Hinton broth, Mueller Hinton agar and Sabouraud dextrose agar were products of Liofilchem S.r.l. (Italy) and Ampicillin (10 µg), while Gentamicin (10 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg) antibiotic susceptibility discs, were produced by Mast Group Ltd. (UK). All spectrophotometric measurements were conducted with Lambda 25 UV/VIS spectrophotometer, Perkin Elmer.

Plant material

In this study, four varieties of blackberries from two locations in the north-west part of Bosnia and Herzegovina were used. The study was conducted on four blackberry varieties: two cultivated (Chester Thornless (Bc1) and Čačanska Bestrna (Bc2)) and two wild varieties from Javorani (44.6110° N, 17.2605° E) (Bw1) and from Verići (44.9244° N, 17.0026° E) (Bw2). The blackberries were picked manually in the stage of full ripeness. For chemical analyses, fresh samples were used, and dry extracts for the other analyses. The extracts were prepared using a commercial homogenizer (Coral PJ 500, Fagor, Spain) and were extracted by applying Soxlets method with 80 % ethanol (v/v). The samples were dried at temperatures up to 50 °C in a vacuum rotary evaporator (Devarot, Slovenia). Dry extracts were kept in dark vials and refrigerated until the use.

Determination of chemical components

Chemical components: dry matter, crude cellulose, acidity, ascorbic acid, and ash in fresh blackberry samples were determined according to Official Methods of Analysis of AOAC (AOAC 2000): AOAC 925.09, AOAC 991.42, AOAC 942.15, AOAC 967.21, and AOAC 923.03, respectively. Acidity (volatile) of fruit titrable acidity was expressed conveniently in g of citric acid monohydrate per 100 ml.

Total sugars were determined by the Luff-Schoorl method, which is based on the iodometric determination of the unreduced Cu(II) ions (32).

Total polyphenolics

Total polyphenolics (TPh) in blackberries extracts were determined spectrophotometrically according to the Folin-Ciocalteau method, as described by Wolfe et al. (33). Gallic acid standard in different concentrations (0-500 µg/ml) was used to obtain a standard calibration curve. The results were expressed in mg of gallic acid equivalent per gram of dry weight of extract (mg GAE/g dw).
Total flavonoids

The total flavonoids (TF) in the extracts were determined spectrophotometrically using the method as described of Woisky and Salatino (34). Quercetin standard in different concentrations (0-50 µg/ml) was used for the calibration curve. The results were expressed as mg of quercetin per gram of dry weight of extract (mg Qv/g dw).

Total flavonols

The total flavonols (TFl) were determined spectrophotometrically using the method as described of Yermakov et al. (35). Quercetin standard in different concentrations (0-50 µg/ml) was used for calibration of a standard curve. The results were expressed as mg of the quercetin equivalent per gram of dry weight of extract (mg Qv/g dw).

Total and monomeric anthocyanins

Total anthocyanins (TAc) were determined using the "pH single" method at pH 1.0 and monomeric anthocyanin (TMAc) using the "pH-differential" method at pH 1.0 and pH 4.5, according to Giusti and Wrolstad (36). The results were expressed as cyanidin-3-glucoside equivalent per gram of dry weight of extract (CyG/g dw), using the molar extinction coefficient of 26.900 l/cm mol and molecular mass of 449.2 g/mol of cyanidin 3-glucoside.

Determination of antioxidant capacity

DPPH radical test

The radical scavenging activity of the blackberry extracts against stable DPPH radicals was determined according to the method Liyana-Pathiranana and Shahid (37). The antioxidative activity of the samples towards DPPH radicals was expressed as mmol Trolox/kg dw. The Trolox standard in different concentrations (0-20 µg/ml) was used for the calibration curve. The results are expressed as mmol of Trolox equivalents per kg of dry weight of extract (mmol Trolox/kg dw).

ABTS radical test

The radical scavenging activity of the blackberry extracts against stable ABTS radicals was determined according to the method of Re et al. (38). The antioxidative activity of the samples towards ABTS radicals was expressed as mmol Trolox/ kg dw. The Trolox standard in different concentrations (0-5 µg/ml) was used for the calibration curve. The results are expressed as mmol of Trolox equivalents per kg of dry weight of extract (mmol Trolox/kg dw).
Hydroxyl (•OH) radical neutralization test

The degradation of the level of 2-deoxy-D-ribose under the influence of a hydroxyl radical generated in Fenton's reagent was determined by a method modified by Nađpal (39). The capacity of the blackberry extracts for catching hydroxyl radicals was tested after the •OH was generated by Fenton's reagent in the system Fe³⁺-ascorbate-EDTA-H₂O₂. The BHT standards in different concentrations (0–50 µg/ml) were used for the calibration curve. The antioxidative activity of the samples on hydroxyl radicals was expressed as mmol of BHT equivalents per kg of dry weight of extract (mmol BHT/kg dw).

α-Glucosidase inhibitory potential

α-Glucosidase inhibitory potential (α-GIP) was assessed using the method reported by Tumbas Saponjac et al. (40), where each well contained 100 µl of 2 mmol/l 4-nitrophenyl α-D-glucopyranoside in 10 mmol/l potassium phosphate buffer (pH 7.0) and 20 µl of the samples diluted in buffer. The reaction was initiated by the addition of 100 µl of the enzyme solution (56.66 mU/ml). The plates were incubated at 37 °C for 10 min. The absorbance of 4-nitrophenol released from 4 nitrophenyl α-D-glucopyranoside at 405 nm was measured and α-GIP (%) was calculated.

Antimicrobial potentials

The antimicrobial activity of the blackberry extracts was tested by using agar wells method on Mueller-Hinton agar (MHA) as described by Balouiri et al. (41). The inhibition zones were reported in millimeter (mm). Staphylococcus aureus (ATCC 25923) and Escherichia coli (ATCC 25922) were used as references for the antibacterial assay. The plant dry extracts were dissolved in ethanol at a concentration of 50 mg/ml. The MHA agar plates were swabbed (with sterile cotton swabs) with broth culture (10⁷ CFU/ml) under aseptic conditions and the wells (diameter = 6 mm) were filled with 30 µl of the tested samples and incubated at 37 °C for 24 hours. The antibiotic susceptibility discs Ampicillin (10µg), Gentamicin (10µg), Erythomycin (15µg) and Ciprofloxacin (5 µg) were used as positive, and 80% ethanol (v/v) as negative control.

The antifungal activity of the blackberry extracts was tested on Aspergillus niger mycelium growth by the agar dilution method according to EUCAST (42) and Balouiri et al. (41). Aspergillus niger was isolated from apple samples and maintained on Sabouraud Dextrose Agar (SDA). The experiments were conducted in Petri plates of SDA, to which various concentrations (0.625 to 2.5 mg/ml) of the extracts had been added, inoculated at the centre with a mycelial disc (5 mm diameter). The control plates with appropriate concentration of ethanol (4 %, 2 % and 1%) and without any sample were inoculated the same way. The fungal colony diameter was recorded in millimeters after the incubation at 28 °C for 7 days. The percentages of growth inhibition (FI %) were calculated as: FI % = (DC-DT)x100/DC, where is DC – the colony diameter for control; DT – the colony diameter for treated mycelium.
The antimicrobial activity of the extracts was performed in eight replicates, and the results were expressed as the mean ± SD.

Statistic analysis

The experiments were performed in at least three repetitions. The results were expressed as mean value ± SD. The statistical data were processed using the programme IBM SPSS Statistics 20, while ANOVA variance analysis was used to determine the significance of differences among the arithmetical means by Duncan's test (p≤0.05).

RESULT AND DISCUSSION

Chemical composition

The obtained results showed that the highest content of dry matter and ash was achieved in the wild blackberries: Bw1 (14.84 g/100 g fw) and Bw2 (15.73 g/100 g fw) (Table 1), with the statistical significant difference (p≤0.05). The fresh blackberry samples, Bw1 and Bw2 had a higher value of total dry matter and ash comparing to the results published by the National Nutrient Database for Standard Reference (43).

Table 1. The chemical composition of fresh blackberry samples

<table>
<thead>
<tr>
<th>Fresh sample</th>
<th>Dry matter g/100g fw</th>
<th>Ash g/100g fw</th>
<th>Total sugars g/100g fw</th>
<th>Crude cellulose g/100g fw</th>
<th>Acidity ml/100g fw</th>
<th>% L-ascorbic acid mg/100g fw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc1</td>
<td>10.69±0.17☎</td>
<td>0.32±0.00a</td>
<td>6.07±0.22a</td>
<td>1.05±0.11a</td>
<td>1.40±0.01a</td>
<td>21.36±0.48a</td>
</tr>
<tr>
<td>Bc2</td>
<td>11.93±0.19b</td>
<td>0.36±0.02b</td>
<td>2.28±0.17b</td>
<td>1.42±0.06b</td>
<td>1.55±0.02b</td>
<td>6.45±0.24b</td>
</tr>
<tr>
<td>Bw1</td>
<td>14.84±0.12c</td>
<td>0.45±0.01c</td>
<td>4.19±0.29c</td>
<td>2.44±0.11c</td>
<td>0.73±0.02c</td>
<td>7.11±0.20c</td>
</tr>
<tr>
<td>Bw2</td>
<td>15.73±0.31d</td>
<td>0.45±0.00c</td>
<td>5.38±0.18d</td>
<td>1.36±0.10d</td>
<td>1.20±0.02d</td>
<td>8.53±0.34d</td>
</tr>
</tbody>
</table>

The results are presented as mean value ± standard deviation (n=3).

a,b,c,d Different letters above each number indicate a significant difference at the 5 % level (Duncan’s tests).

The highest values of total sugars and ascorbic acid were obtained in Bc1 sample, with a significant difference in relation to the contents of other tested samples (p≤0.05). A high content of total sugars was also present in the sample Bw2 (5.38 g/100g fw), while Bc1 sample showed the highest content of ascorbic acid, whose content in the other samples was even more than three times lower (Table 1). The relationship between total sugars and total acidity is very important in determining the fruit quality (44). The samples of Čačanska bestrna showed slightly higher ash value, similar total sugars and acidity values, and lower values of cellulose comparing to the results reported in the study by Stajčić et al. (45). The same author in Thornfree variety samples obtained significantly higher values, which were similar to the values obtained in the wild variety samples (Bw1 and Bw2). The ascorbic acid contents (6.45 – 21.36 mg/100 g) are in accordance
with the results reported by Zia-Ul-Haq et al. (44), ranging from 6 to 20.4 mg/100 g. Guedes et al. (46) reported that tropical varieties of the Brazos and Ebano contained ascorbic acid at a significantly higher levels (42.69 mg/100g and 55.78 mg/100g, respectively). The lowest value of sugars and the highest values of acidity was found in the sample Bc2. The reason for this was probably the microbiological and enzymatic degradation of sugars because this variety achieved maturity stage before the others.

**Polyphenol contents**

The significantly higher content of total polyphenols, flavonoids and anthocyanins were determined in the wild blackberries samples Bw1 and Bw2 (Table 2). The total polyphenolic were about four to eight times higher than in the study by Huang et al. (47), whereas those in Bc1 and Bc2 were similar to the values reported by Dai et al. (14). The very low differences in the values of total flavonols were found for the samples Bc1 and Bw1, without statistically significant differences (p≥0.05). The presence of total anthocyanin in all extracts ranged between 33 % and 45 % in relation to the total polyphenolic contents.

**Table 2.** Total polyphenol contents in four blackberry extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>TPh mg Gal/g dw</th>
<th>TF mg Qv/g dw</th>
<th>TF1 mg Qv/g dw</th>
<th>TAe mg Cy/g dw</th>
<th>TMAe mg Cy/g dw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc1</td>
<td>26.94±2.33a</td>
<td>3.61±0.05a</td>
<td>4.81±0.18a</td>
<td>8.96±0.06a</td>
<td>6.96±0.13a</td>
</tr>
<tr>
<td>Bc2</td>
<td>21.59±1.58b</td>
<td>3.10±0.18b</td>
<td>2.24±0.09b</td>
<td>7.80±0.04b</td>
<td>6.10±0.15b</td>
</tr>
<tr>
<td>Bw1</td>
<td>40.18±1.26c</td>
<td>6.91±0.18c</td>
<td>5.24±0.09c</td>
<td>15.32±0.06c</td>
<td>12.59±0.17c</td>
</tr>
<tr>
<td>Bw2</td>
<td>31.83±1.14d</td>
<td>5.27±0.04d</td>
<td>4.80±0.07a</td>
<td>14.32±0.05d</td>
<td>12.30±0.14d</td>
</tr>
</tbody>
</table>

The results are presented as mean value ± standard deviation (n=3).

a,b,c,d The letters above each number indicate a significant difference at the 5 % level (Duncan’s tests).

The results presented in Table 2 are in accordance with the results of Reyes-Charmon (48), where wild blackberry showed higher contents of total polyphenol and anthocyanins compared to the other cultivated varieties. Significantly lower values for total polyphenols (12.23 mg GAE/g) were obtained by Oancea and Falin (49) in fresh blackberries comparing to the results in Table 2.

**Antioxidant capacity**

The antioxidant capacity of the wild and cultivated blackberries was evaluated using three methods: DPPH, ABTS and OH radicals tests. The highest potentials were obtained for the wild blackberries Bw1 and Bw2, while the cultivated blackberry Bw2 showed higher potential to OH radicals comparing to Bc1 and Bw2.
Table 3. Antioxidant capacity of blackberry extracts

<table>
<thead>
<tr>
<th>Extract</th>
<th>DPPH mmol Trolox/kg dw</th>
<th>ABTS mmol Trolox/kg dw</th>
<th>'OH' mmol BHT/kg dw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc1</td>
<td>109.07±7.35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>216.28±15.98&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>725.88±3.07&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bc2</td>
<td>92.01±3.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>179.49±14.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>891.14±28.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bw1</td>
<td>184.26±5.25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>340.26±22.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>944.03±16.06&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bw2</td>
<td>122.43±6.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>235.45±17.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>639.32±1.68&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

The results are presented as mean value ± standard deviation (n=3).

<sup>a,b,c,d</sup> - Different letters above each number indicate a significant difference at the 5 % level (Duncan’s tests).

BHT - butylated hydroxytoluene, Trolox-6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid.

In the study by Huang et al. (47), the DPPH radical-scavenging activity of all blackberry powder samples (114.8 mmol Trolox/kg dw) were very similar to that in Bc1 (109.07 mmol Trolox/kg dw) and Bw2 (122.43 mmol Trolox/kg dw). A higher antioxidative potential was found for the wild blackberry varieties, which is in accordance with the results of Reyes-Charmona et al. (48). The Bw1 showed the highest level of ability in the neutralization of hydroxyl radicals (Table 3). Basu et al. (50) reported a higher polyphenolic content in the blackberry extracts (269.5 mg GAE/g) compared to the present study, where the polyphenolic content in the extract was in the range from 21.59 to 40.18 mg GAE/g.

**Antihyperglycemic potentials**

α-Glucosidase is the enzyme located in the small intestine tract of human which is involved in the final step of carbohydrate digestion – the breakdown of starch and disaccharides to glucose (51, 52). The antihyperglycemic potentials of four blackberry extracts at concentrations of 0.5 mg/ml are Bc1 (41.54±2.45 %), Bc2 (50.69±3.05 %), Bw1 (43.10±1.17 %) and Bw2 (46.91±1.44 %), and under the given experimental conditions they all inhibited about one half of the alpha-glucosidase activity. Among the all extracts, the Bc2 showed the highest α-glucosidases inhibition activity (50.69 %). In the study by Tumbas Šaponjac et al. (53) the Clery and Marmolada strawberry pomace showed 50% of α-glucosidase inhibitory potential at the concentrations of 1.90 and 0.60 mg/ml, respectively. Girones-Vilaplana et al. (54) reported that the EC<sub>50</sub> values (inhibitory concentration of the extract that decreases 50% of α-glucosidase inhibition) for açai and maqui berries were in the range 0.33 – 2.14 mg/ml. In the study by Grace et al. (55) it was demonstrated that anthocyanins from blueberry had the potency to alleviate symptoms of hyperglycemia in diabetic mice. Also, Abidov et al. (56) performed a study where the adults with type 2 diabetes consumed blueberry extract, and the results showed that blueberry supplement reduced plasma glucose, thus possessing a pharmacologically relevant anti-inflammatory characteristic. Therefore, people with diabetes should be encouraged to consume berries, because they contain phytochemicals that could act in a similar ways to diabetes medications.
Antimicrobial activity

The wild blackberry extracts (Bw1 and Bw2) showed a higher inhibition of the growth of tested bacterial cultures compared to the cultivated varieties (Bc1 and Bc2). The lowest inhibitory effect on *E. coli* (6.65 mm) and *S. aureus* (8.10 mm) showed the extract from Bc1 cultivars. Compared to *E. coli*, the higher inhibition zone was obtained for the wild variety Bw1 (9.40 mm), in comparison with *S. aureus* of variety Bw2 (8.35 mm). Ethanol 80 % (v/v) did not affect bacterial growth because it quickly evaporated after placing the samples on incubation.

Table 4. Effect of blackberries extracts (50 mg/ml) on bacterial cultures growth

<table>
<thead>
<tr>
<th>Sample</th>
<th><em>E. coli</em></th>
<th><em>S. aureus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bc1</td>
<td>6.65±0.34*</td>
<td>8.10±0.32</td>
</tr>
<tr>
<td>Bc2</td>
<td>8.30±0.67</td>
<td>8.60±0.57</td>
</tr>
<tr>
<td>Bw1</td>
<td>9.40±0.81</td>
<td>8.35±0.47</td>
</tr>
<tr>
<td>Bw2</td>
<td>8.25±1.25</td>
<td>8.75±0.47</td>
</tr>
<tr>
<td>Ethanol 80 % (v/v)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ampicillin 10 mg</td>
<td>16.00±4.39</td>
<td>3.00±2.16</td>
</tr>
<tr>
<td>Ciprofloxacin 5 mg</td>
<td>37.00±3.37</td>
<td>29.25±2.99</td>
</tr>
<tr>
<td>Erytromycin 15 mg</td>
<td>10.75±1.50</td>
<td>27.75±2.99</td>
</tr>
<tr>
<td>Gentamicin 10 mg</td>
<td>25.50±1.29</td>
<td>29.75±1.90</td>
</tr>
</tbody>
</table>

*Diameter of inhibition zone in mm.

The inhibition zones of the Gram-negative bacterium *E. coli* showed the trend: Bw1 > Bw2 > Bc2 > Bc1, an exception being Bc1, which corresponds to the content of total phenols, flavonoids, flavonols, and anthocyanins. The inhibition zones for *S. aureus* are less varied and had the trend: Bw2 > Bc2 > Bw1 > Bc1, which was not proportional to the content of total phenols or any phenolic component. González et al. (24) found that some other (non-phenolic) components contained in the blackberry extract can produce selective antibacterial activity.

The extracts of blackberry fruits inhibited the growth of mycelium of *Aspergillus niger* depending on the concentration (Figure 1). The highest inhibition percentage the extract in the concentration of 2.5 mg/ml, then at 1.25 mg/ml, while the lowest concentration of extract (0.63 mg/ml) inhibited only slightly the growth of mycelium of *Aspergillus niger*. Ethanol at the concentrations of 4 %, 2%, and 1%, did not inhibit the micellar growth (results not shown). The extracts of cultured cultivars of blackberry (Bc1 and Bc2) had similar inhibition, while wild varieties (Bw1 and Bw2) showed very different antifungal effects.
According to Riaz et al. (22), extract of blackberries fruits showed no significant antifungal activity against *Aspergillus niger*, but extracts of cultivated blackberry Bc1, Bc2 and wild variety Bw2 at the concentration of 2.5 mg/ml inhibited the growth of mycelium *Aspergillus niger* by more than 25%. The inhibition of the growth of mycelium *A. niger* had the trend: Bw2 > Bc2 > Bc1 > Bw1.

**CONCLUSION**

The present study showed similar chemical compositions of two wild blackberry varieties, while some differences were observed in two cultivated varieties (Chester Thornless and Čačanska Bestrna) from two different locations in Bosnia and Herzegovina. This could be due to the influences of the climate, subgroup, and location. Also, the results indicated that the investigated four blackberry varieties present potential sources of natural antioxidant compounds and showed good biological potentials, where generally wild varieties showed better results. While medications can have side effects and drug interactions, consumption of fruits like blackberries, is a natural and easy way for people to improve glycemic control and overall health. Also, it was shown that the blackberry extracts possessed a good antimicrobial activity, where the wild blackberries exhibited higher growth inhibition of tested bacterial cultures compared to the cultivated varieties. The presented study confirmed that blackberry fruits are a good choice for human
nutrition, as a value-added ingredient for functional foods and for usage in the food and pharmaceutical industries.

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ХЕМИЈСКИ САСТАВ, БИОЛОШКИ ПОТЕНЦИЈАЛ И АНТИМИКРОБНА АКТИВНОСТ ДИВЉИХ И КУЛТИВИСАНИХ КУПИНА

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Циљ овог истраживања био је да се одреди хемијски састав и биолошки потенцијал (антиоксидативни потенцијал, α-глукозидаза тест и антимикробна активност) дивљих и култивисаних купина са две различите локације. Хемијски састав одређен је у свежим узорцима, док је биолошки потенцијал одређен у сувим екстрактима купине. Највећи садржај суве материје (15,73 g/100 g fw) био је у дивљој купини из Верића (Bw2). Значајно већи садржај шећера (6,07 g/100 g fw) и аскорбинске киселине (21,36 g/100 g dw) у односу на остале узорке, одређен је у Chester Thornless купини (Bc1). Дивља купина из Јаворана (Bw1) показала је највећи садржај полифенола, као и антиоксидативни потенцијал на DPPH (184,26 mmol Trolox/kg dw), ABTS (340,26 mmol Trolox/kg dw) и OH (944,03 mmol BHT/kg dw) радикале. Антихипергликемијски потенцијал екстраката одређен је α-глукозидаза тестом. Култивисана купина Čačanka Bestrna (Bc2) показала је најјачу инхибицију α-глукозидаза ензима (α-GIP = 50,69 %) у односу на остале узорке (p<0,05). Екстракти дивљих купина имали су бољу антибактеријску и антигљивичну активност на Staphylococcus aureus, Escherichia coli и Aspergillus niger. Приказани резултати указују на разлике између дивљих и култивисаних купина, као и на разлике у хемијском саставу, садржају полифенола, биолошком потенцијалу и антимикробној активности дивљих и култивисаних купина са две различите локације у Босни и Херцеговини.

Кључне речи: купина; хемијски састав; антиоксидативни капацитет; α-глукозида-за; антимикробна активност

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