PHENOLIC COMPOUNDS, ANTIOXIDANT AND ANTIMICROBIAL PROPERTIES OF THE WILD CHERRY (Prunus avium L.) STEM

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The aim of this study was to determine the total phenolic content, evaluate antioxidant properties and antimicrobial potential, and identify phenolic compounds in alcoholic and aqueous extracts of the wild cherry (Prunus avium L.) stems collected in Bosnia and Herzegovina. Alcoholic extracts had higher contents of phenolic and flavonoid components, as well as the antioxidant and ferric reducing antioxidant capacity in comparison to aqueous extracts. All extracts were characterized by HPLC analysis. Furthermore, for the first time, the antimicrobial properties of wild cherry stem extracts were evaluated. Quercetin and (+)-catechin were the main compounds identified in the alcoholic extract, followed by chlorogenic acid and rutin. Quercetin was also the major component detected in aqueous extracts. Besides, alcoholic extract showed better antibacterial properties against Staphylococcus aureus as a representative gram-positive bacteria than infusion, whereas none of the samples showed antibacterial activity against Escherichia coli and fungus Candida albicans.

KEY WORDS: cherry stems, bioactive components, antioxidant activity, antimicrobial properties

INTRODUCTION

Plants have been used as natural sources of medicinal agents from the beginning of human civilization. Medicinal usage of plants has increased in recent years because of their antioxidant, antiviral, antibacterial and antitumor activity. Fruits are considered a natural source of antioxidants, containing anthocyanins and polyphenols, compounds that can reduce the risk of diseases caused by oxidative stress, such as cancer and cardiovascular diseases (1, 2). The vernacular name "cherry" refers to the fruits of Prunus, an arborescent genus of the Rosaceae family native to Asia and Eastern Europe. The species Prunus avium L. (sweet cherry) is geographically distributed around the world, especially in areas with a moderate climate. Sweet cherries are important commercially as a table
For medicinal and therapeutic purposes all parts of the plant are used – fruit, stem and bark of the cherry tree. Consumption of sweet cherry has been associated with beneficial health effects (3,4). Cherry fruits exhibit relatively high antioxidant activity, low glycemic response, COX 1 and 2 enzyme inhibition and anti-carcinogenic effects in vitro and in animal experiments (5).

Previous studies suggested that the health benefits of cherries are due to their antioxidant and anti-inflammatory activities. It has been reported that the consumption of sweet cherry fruits alleviates arthritis and gout-related pain (6). Also, reduction of the proliferation of human colon cancer cells has been associated with the consumption of cherry fruits (7). It was reported that extracts of wild cherry bark exhibit anti-proliferative activity in human colorectal cancer cells (8). Polyphenolics, which are plant secondary metabolites, are believed to provide those benefits (9).

From a therapeutic perspective, the stem is an extremely valuable part of the cherry. In folk medicine, cherry stems have long been recognized as a natural diuretic when prepared as tea or dried and encapsulated (10). It is assumed that anti-inflammatory and diuretic properties result from natural antioxidants (flavonoids). In a cross-sectional questionnaire study conducted in Turkey, in the population which have taken up alternative treatments, 6.2% of hypertensive patients were consuming cherry stems in the form of tea as a part of their treatment for diuresis (11). The diuretic activity of powdered cherry stem in 13 healthy volunteers was also evaluated (12). The study found that mean levels of urine calcium, sodium, chloride, and urine volume increased, but the amount of urine potassium and urine osmolality did not change after administration of cherry stem. Although authors observed no adverse reaction, they stressed that because of rising calcium excretion, it should be used with caution in patients with urolithiasis.

Tea preparation of the wild cherry stems has been used since centuries in traditional medicine of Bosnia and Herzegovina as diuretic agent that helps promoting proper kidney function. It is also used for breaking down and clearing stones from the bladder and kidneys (13).

In this study, the name "wild cherry" is applied to species of Prunus avium growing in wildness. It is widely found in various regions of Bosnia and Herzegovina. It occurs only as a single tree, endangered and protected by law. The fruits of wild cherry are clearly smaller and fewer in number than by the cultivated varieties, red to black purple in color. The fruit is slightly acidic and bitter compared to the domestic varieties.

Most of the previous research has been conducted on cherry fruits, whereas cherry stems, which have been traditionally used in folk medicine as sedatives, diuretics and draining, have not been fully tested and characterized in respect to their bioactive ingredients and bioactive properties (12). Chemical composition and antioxidant and antitumor effects of the sweet cherry (P. avium L.) fruits and stems were recently compared (14). The authors reported that the stem extract revealed higher antioxidant potential than the tested extract from fruits, whereas the fruits extract was the only one showing antitumor potential.

The aim of this study, in addition to the quantification of the total phenolic content, was also aimed determine the flavonoid content and characterize phenolic composition of alcoholic and aqueous extracts from the wild cherry stem. Additionally, antioxidant pro-

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Properties were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and Ferric Reducing Antioxidant Power (FRAP) assay. *In vitro* antimicrobial activities against gram-positive bacteria *Staphylococcus aureus*, gram-negative bacteria *Escherichia coli* and fungus *Candida albicans* were evaluated. To our best knowledge, this is the first study on the evaluation of antimicrobial activity of the *Prunus avium* stem.

Based on the results and knowledge gained from this project the possibility of further testing of bioactive ingredients of wild cherry stems, isolation and characterization of individual components and testing their effects on certain physiological processes will be provided.

**MATERIALS AND METHODS**

**Plant material and preparation of extracts**

Wild cherry stems were collected from trees growing in natural habitat from three locations in Northern Bosnia in June 2015 and the biological authentication was carried out by Prof. dr. Senida Osmanović, biologist at the Faculty of Science, University Tuzla. Voucher specimens were deposited at the Herbarium of the Department of Pharmacognosy, University of Tuzla. The samples were air dried and stored in desiccator protected from light. Before extraction, stems were ground to a powder and mixed to obtain a homogenous sample. For preparation of the alcoholic extract, 5 g of the stem powder was extracted in an ultrasonic bath with 50 mL of ethanol at 25 °C. After 30 min the solution was filtered and the residue on the filter paper was extracted again in the same way. The combined extracts were evaporated at 40 °C using rotary evaporator (Büchi R-210, Switzerland) under reduced pressure to remove ethanol. The extraction yield was 19±2.5%. For the aqueous extract, 5 g of cherry stems were added to 50 mL of boiling distilled water and left to stand at room temperature for 10 min. The solution was filtered and evaporated. The extraction yield was 8.8±1.4%. Extraction was repeated at least three times, and the results are given as mean ± standard deviation (SD).

**Standards and reagents**

Phenolic compound reference standards, (+)-catechin and rutin trihydrate, were purchased from Sigma-Aldrich (Germany), quercetin dihydrate from HWI Analytik GmbH (Germany) and chlorogenic acid from Aeros Organic (USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu reagent were obtained from Sigma-Aldrich (Germany). 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (≥99.9%) was obtained from Himedia Laboratories (India) and Methanol HPLC grade (≥99.9%) from Roth (Germany).

**Microbial strains**

The antimicrobial activity of alcoholic and aqueous extracts was evaluated using laboratory control strains obtained from the American Type Culture Collection: gram-positive bacteria *Staphylococcus aureus* ATCC 6538 P, gram-negative bacteria *Escherichia coli*
ATCC 10536 and one fungus *Candida albicans* ATCC 10231. Positive controls were Kanamycin for *S. aureus* and Chloramphenicol for *E. coli*. Nystatin was positive control for *C. albicans*.

**Evaluation of bioactive properties and phenolic compounds**

*Total phenolic content (TPC)* in the alcoholic and aqueous extracts was determined spectrophotometrically after reaction with the Folin-Ciocalteu phenol reagent (15). The extracts were dissolved in methanol to a final concentration of 0.25 mg/mL. 50 μl of extracts, 450 μl deionized water and 2.5 mL of Folin-Ciocalteu reagent are mixed and incubated for 5 min. 2 mL of 7% sodium bicarbonate solution was added, filled with water up to 100 mL and incubated for 1.5 hours at 30 °C. Absorbance of the resulting blue colored liquids was measured at 765 nm using a Shimadzu UV-mini-1240 UV-Vis Spectrophotometer. Quantitative analysis was performed based on the standard calibration curve of gallic acid in methanol. The concentrations of gallic acid in the solution from which the curve was prepared were 50, 100, 150, 250 and 500 mg/L (y=0.0014x+0.0244, R²=0.9912). The result was expressed as mg of gallic acid equivalent per gram of dry weight of sample (mg GAE/g).

*Total flavonoids content (TFC)* was determined by 24 h precipitation reaction with formaldehyde (16). In a 50 mL flask 5 mL of the extract (1 mg/mL) in methanol, 5 mL 1:4 HCl and 2.5 mL of formaldehyde solution were added, incubated for 24 hours at room temperature and filtered. The remaining phenolic compounds, evaluated as non-flavonoid content (TNFC), were determined according to the previously mentioned procedure for TPC determination with the Folin-Ciocalteu method. TFC was calculated as subtraction of TPC and TNFC.

**Determination of antioxidant capacity using DPPH method.** 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was performed according to the method described earlier (17). Samples were dissolved in methanol at a final concentration of 1 mg/mL, filtered and kept in the dark. The reaction solution was prepared by mixing 10-150 μL of the alcoholic extract and 50-1000 μL aqueous extracts with 3 mL of 0.135 mM solution of DPPH, and filled by methanol to 2 mL. The mixture was incubated for 30 min in the dark at room temperature. The absorbance is measured against the blank (methanol) at 517 nm. For the DPPH control solution, 3 mL of 0.5 mM DPPH solution was dissolved in 1 mL of methanol. The radical scavenging effect (%) or percent inhibition of DPPH radical was calculated according to the equation: [(Asample-Acontrol)/Acontrol] x100, where Asample is the absorbance of the solution containing the sample at 515 nm and Acontrol is the absorbance of the DPPH solution. The results are expressed as the IC 50 value (mg/mL) or the concentration of extract that caused 50% neutralization of DPPH radicals.

**The Ferric Reducing Antioxidant Power (FRAP) assay.** The method is based on the ability of the extract to reduce Fe³⁺ ions to Fe²⁺ ions. The resulting Fe²⁺ ions form with the reagent 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) a blue colored complex, which reaches the absorption maximum at 593 nm. The reaction is performed in an acidic medium in order to maintain good solubility of iron. The determination of ferric reducing antioxidant power or ferric reducing ability (FRAP assay) was performed as described earlier.
Briefly, the working FRAP reagent was prepared freshly by mixing 10 volumes of 300 mM acetate buffer pH 3.6, with 1 volume of 10 mM TPTZ (2,4,6-tri(2-pyridyl)-s-triazine) in 40 mM HCl and with 1 volume of 20 mM ferric chloride. A volume of 0.1 mL of the sample of the concentration 1 mg/mL and 3 mL of fresh prepared FRAP reagent were added, and after 30 min the absorbance was measured at 593 nm compared to the blank (3 mL of FRAP reagent + 0.1 mL of solvent that was used to dissolve the samples). Aqueous solutions of FeSO₄·7H₂O (200–1000 μM) were used for the calibration (y=0.0007x+0.117, R²=0.9947), and the results were expressed as FRAP value mg Fe²⁺/g of sample.

**High Performance Liquid Chromatography (HPLC).** Phenolic compounds were determined by High-Performance Liquid Chromatography (HPLC, Shimadzu LC-20AT, Japan) gradient controlled system consisting of the solvent delivery system, autosampler, pump, and UV-VIS detector. The separation method for the analysis was described previously (19). The phenolic components were detected at 280 nm. The separation was carried out using a Selectra C18 column (4.6x250 mm, 5 μm) at room temperature. The elution solvent A was 0.01 M phosphoric acid and solvent B was 100% methanol. The following binary gradient was applied: the gradient was started with 5% B in order to reach 50% B at 10 min, 70% B at 15 min, 80% B at 20 min and 100% B at 25 min. The injection volume was 20 μL and flow rate 1 mL/min. Samples for HPLC analysis were filtered through membrane filter prior to injection. The phenolic compounds were identified by comparing their retention times with those obtained for the standard compounds.

**Antimicrobial testing.** The antibacterial activity of the cherry stems alcoholic and aqueous extract was tested by the agar diffusion method according to the American Clinical Laboratory Standards Institute (CLSI) (20) using 100 μL of suspension of the tested microorganisms containing 2.0 × 10⁶ colony forming units (cfu/mL). Mueller-Hinton agar (15 mL), sterilized in a flask and cooled to 45–50 °C, was distributed to sterilized Petri dishes with a diameter of 9 cm. The sterile swab was used to transfer the bacterial suspension and inoculate the bacteria on the surface of Mueller-Hinton agar. The wells with a diameter of 10 mm were cut with the sterile stainless steel borer down to the plastic into Mueller-Hinton agar plates and then filled with a volume of 25 and 50 μL of the extracts samples dissolved in water at a concentration of 50 mg/mL. Two wells were prepared per plate. The Petri dishes were kept incubated at 37 °C for 24 h. The antimicrobial activity was measured on the basis of the diameter of the growth inhibition as follows: (<10 mm) – no antimicrobial activity; (10-15 mm) – weak antimicrobial activity; (16-20 mm) – moderate antimicrobial activity; (>20 mm) – certain antimicrobial activity (21). Kanamycin, chloramphenicol and nystatin (10.0 mg/mL) were used as reference.

**Statistical analysis**

All experiments were carried out in triplicates. The results are presented as mean ± standard deviation and analyzed by SPSS v. 20.0 program.
RESULTS AND DISCUSSION

The total phenolic content of the wild cherry stem using the Folin-Ciocalteu assay is expressed in terms of gallic acid equivalent (GAE). The determination was based on the standard calibration curve of different concentrations of gallic acid and expressed as mg of GAE/g of extract. The results in Table 1 show that the alcoholic extract had a higher total phenolic content (121.3 mg GAE/g) than the aqueous extract (74.1 mg GAE/g). The content of flavonoid was also higher for the alcoholic extract, 26.9 mg GAE/g, compared to 5.9 mg GAE/g for the aqueous extract. The value for total phenolic content of the alcoholic extract was in accordance with the result reported by Bursal et al. (22) (146 mg GAE/g), whereas the value for the aqueous extract was much lower than those reported by these authors (118 mg GAE/g).

Table 1. Total phenolic content, flavonoid content and antioxidant activity of the alcoholic and aqueous extract of wild cherry stems

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total phenolics (mg GAE/g)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total flavonoid (mg GAE/g)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>DPPH IC50% (mg/mL)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>FRAP (mg Fe&lt;sub&gt;2+&lt;/sub&gt;/g)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcoholic</td>
<td>121.3±9.7</td>
<td>26.9±1.4</td>
<td>29.75±0.21</td>
<td>194.05±8.3</td>
</tr>
<tr>
<td>Aqueous</td>
<td>74.1±7.4</td>
<td>5.9±2.2</td>
<td>227.96±3.15</td>
<td>63.66±5.7</td>
</tr>
</tbody>
</table>

Each value in the table represents the mean ± SD (n = 3)

<sup>a</sup> GAE - gallic acid equivalent

<sup>b</sup> IC50% - concentration of the sample that causes 50% neutralization of DPPH radicals, higher values correspond to lower antioxidant potential

<sup>c</sup> FRAP - ferric reducing antioxidant power

The DPPH radical scavenging ability is one of the best-known and frequently employed methods for evaluating antioxidant activity. DPPH is a stable free radical because the spare electron is delocalized over the whole molecule. The donation of H<sup>+</sup> to the DPPH radicals changes color of the solution from violet to pale yellow. This assay determines the scavenging of the stable DPPH radicals by antioxidants compounds present in the extracts. The IC50 value was determined from the plotted graph of scavenging activity against various concentrations of extracts and infusion, and it is defined as the concentration of antioxidant necessary to decrease the initial DPPH radicals concentration by 50%. The results presented in Table 1 show the higher rate of DPPH scavenging activity of the alcoholic stem extract as compared to the aqueous stem extract. This may be explained by the presence of a higher content of phenolic compounds in the alcoholic extract. Both samples were able to reduce the stable violet DPPH radical to the yellow DPPH-H, reaching 50% of reduction with the IC 50 value of 39.58 µg/mL for the alcoholic extract and 227.96 µg/mL for the aqueous extract. Bursal et al (22) reported that both extracts (ethanolic and aqueous) of the cherry stem, showed significant DPPH radical scavenging activity with similar IC 50 values of 17.36 and 23.38 µg/mL, respectively. Contrary to that, Bastos et al. (14) reported much higher DPPH values for both extracts
with the values of 360 µg/mL 630 µg/mL for the ethanolic and aqueous extract, respectively. These differences could be explained by the different extraction methodologies applied in the studies.

![Graph](image)

**Figure 1.** DPPH scavenging activity expressed as the inhibition of DPPH radicals (%) of the alcoholic (a) and aqueous extract (b)

The ferric reducing ability of the two extracts was also tested. The presence of antioxidants in the sample would reduce the ferric ion (Fe³⁺) to the corresponding ferrous ion (Fe²⁺) by donating an electron. The reducing power of alcoholic and aqueous extracts are expressed as mg Fe²⁺ per gram of dry extract, and the results are shown in Table 1. The measured reducing power of alcoholic extract was 194.05 mg Fe²⁺/g dry sample, which is
significantly higher than that of the aqueous extract (63.66 mg Fe²⁺/g). The alcoholic stems extract exhibited higher antioxidant and ferric reducing power in comparison to the aqueous extract. This is related to the higher phenolic compounds concentration found in the alcoholic extract.

A composition of the contents of phenolic compounds are of great interest to scientists, manufacturers and consumers due to their influence on product quality and their protective and preventive roles in the pathogenesis of certain types of cancer and several other chronic diseases. In the literature, there are several reports on the identification and quantification of phenolic compounds in *P. avium* L. fruits. The most commonly found compounds are phenolic acids (neochlorogenic, chlorogenic and *p*-coumaroylquinic acids), anthocyanins, flavonols (rutin) and flavan-3-ols (catechin, epicatechin) (23, 24).

To our knowledge, only two reports have been published on chemical composition of cherry stem. Bursal et al. (22) studied phenolic acids in ethanol and aqueous extracts of cherry stems by LC-MS/MS and found pyrogallol, ferulic acid, *p*-coumaric acid, gallic acid, ascorbic acid and *p*-hydroxybenzoic acid. Bastos et al. (14) compared the HPLC phenolic profile of *P. avium* L. fruits and stems. They detected more phenolic compounds in the stem than in fruit.

In this study, the analysis of phenolic compounds was performed using liquid gradient method developed by Escrapa and Gonzales (19). The HPLC phenolic profiles of the alcoholic and aqueous extracts of cherry stems were recorded at 280 nm. Figure 2 shows the chromatograms obtained for the mixture of four commercially available phenolic compounds standards (a), alcoholic extract (b) and aqueous extract (c). The retention times (RT) for the phenolic standard shown in the chromatogram in Figure 2a were as follows: (+)-catechin RT 11.5 min, chlorogenic acid RT 12.0 min, rutin RT 16.0 min, and quercetin RT 19.6 min.

The peaks in the chromatograms of alcoholic and aqueous extracts could be tentatively assigned by applying the optimised chromatographic gradient elution, consisting of four elution steps. In the first gradient step (0-10 min) arbutin and gallic acid are eluted, in the second (10-15 min) catechins, chlorogenics and caffeic acids, in the third one (15-20 min) flavonoids that included flavan-3-ols, flavonols, flavones and isoflavones, whereas in the last one (20-25 min) are aglycones. In chromatograms of alcoholic extracts (Figure 2b) no peaks that could be related to hydroxybenzoic acids were recorded. In the second gradient, (+)-catechin and chlorogenic acid were identified based on their RT by comparison with the standards. In the third gradient, diverse flavonoids were eluted, where quercetin, as most intense peak, and rutin were identified. Also, the peak at the RT of 20.5 could be compared to the Escrapa and Gonzales (13) finding, and could belong to kaempferol. The chromatogram of the aqueous extract (Figure 2c) showed only one high-intensity peak, which based on the RT of standards was assigned to quercetin. Also, some peaks with very low intensity were detected. Bastos et al. (14) detected twenty three compounds in alcoholic extract of stems. By comparison with commercial standards they identified catechin, quercetin, rutin and kaempferol, but no chlorogenic acid. In aqueous stem extract they recorded fifteen peaks, where large amount of catechin, some quercetin, but no rutin was identified. The remaining compounds were tentatively assigned.
Figure 2. HPLC chromatograms at 280 nm obtained for phenolic compounds of: (a) polyphenolic compounds standards available commercially, (b) alcoholic extract and (c) aqueous extract. Peaks: (1) (+)-catechin, (2) chlorogenic acid, (3) rutin, (4) quercetin

The difference in the chromatograms of alcoholic and aqueous extracts is due to the solvent and techniques used for the extract preparation. Ethanol as an organic solvent can better dissolve active organic components required for biological activity of the sample. Also, ultrasound extraction technique leads to the disruption of the cell wall matrix and releases components such as phenolic compounds into the extraction solvent.

Many phytochemical preparations with high flavonoid content have been reported to exhibit antimicrobial activity (25). The results of this study suggest that cherry stems have strong antioxidant potential due to the presence of large quantities of phenols and flavonoids. Therefore, preliminary study on antimicrobial activity of alcoholic and aqueous extracts obtained from the wild cherry stems was performed against gram-positive bacteria *S. aureus*, gram-negative bacteria *E. coli* and one fungus *C. albicans*. Positive controls were kanamycin for *S. aureus*, chloramphenicol for *E. coli* and nystatin for *C. albicans*. *E. coli* is a common pathogenic bacteria for urinary tract infection and *S. aureus* is the cause of pneumonia and several infections in the gut or urinary tract.

The alcoholic and aqueous extracts were prepared by dissolving extracts in water in concentration of 50 mg/mL. As can be seen from Table 2, a volume of 25 \( \mu \)L (1.25 mg)
of the alcoholic extract showed a weak inhibitory effect on the gram-positive *S. aureus* strain, whereas 50 μL (2.5 mg) showed a moderate inhibitory effect on this strain. However, only a higher volume of the aqueous extract of 50 μL (2.5 mg) showed a weak inhibitory effect on the *S. aureus* strain. None of the tested samples showed inhibitory activity on the gram-negative bacteria *E. coli* and fungus *C. albicans*.

### Table 2. Antimicrobial effects of the alcoholic and aqueous extracts obtained from the wild cherry stems (*P. avium*)

<table>
<thead>
<tr>
<th>Samples (concentration 50 mg/mL)</th>
<th>Volume applied (μL)</th>
<th><em>Staphylococcus aureus</em></th>
<th><em>Escherichia coli</em></th>
<th><em>Candida albicans</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcoholic extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>&gt;20</td>
<td>12±0.70</td>
<td>&lt;3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>50</td>
<td>&gt;20</td>
<td>16±0.30</td>
<td>&lt;5</td>
<td>&lt;2</td>
</tr>
<tr>
<td><strong>Aqueous extract</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>&gt;20</td>
<td>&lt;6</td>
<td>&lt;3</td>
<td>&lt;2</td>
</tr>
<tr>
<td>50</td>
<td>&gt;20</td>
<td>11±0.40</td>
<td>&lt;5</td>
<td>&lt;2</td>
</tr>
<tr>
<td><strong>Positive controls (10 mg/mL)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kanamycin</td>
<td>25</td>
<td>&gt;20</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>25</td>
<td>-</td>
<td>&gt;20</td>
<td>-</td>
</tr>
<tr>
<td>Nystatin</td>
<td>25</td>
<td>-</td>
<td>-</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

(-) – no antimicrobial activity detected (<10 mm)

(+) – weak antimicrobial activity (inhibition zone 10-15 mm)

(++) – moderate antimicrobial activity (inhibition zone 16-20 mm)

(+++) – certain antimicrobial activity (inhibition zone >20 mm)

From this study it comes clear that the solvent used for extraction influenced the degree of antibacterial activity of the extracts. Ethanol as solvent and ultrasound extraction showed a higher extraction efficiency than infusion with water, which is evident from the total phenolic content and HPLC. Therefore, the alcoholic extract showed a higher antimicrobial activity than the aqueous one. The study conducted by Rauha et al. (26) on antimicrobial effects of different phenolics as pure substances showed that the growth of *S. aureus* was inhibited very effectively by quercetin and kaempferol, whereas these components showed only slight antibacterial activity against *E. coli*. Catechin and rutin failed to show any activity against *S. aureus* and *E. coli*. The yeast *C. albicans* was resistant to all of these compounds. It is evident that the stem extracts did not show as marked inhibition as the pure flavonoid compounds. It should be also noted that the assays relying on diffusion of tested extract may not give a reliable quantitative measure of antibacterial activity because a potent antibacterial flavonoid may have a low rate of diffusion (27). More studies are needed to define the antimicrobial properties of stems.

### CONCLUSION

In traditional medicine in Bosnia and Herzegovina, tea preparation of wild cherry stems is used because of its assumed diuretic properties. The aim of this study was to evaluate antioxidant and antimicrobial properties and identify phenolic compounds in
stems of wild cherry, *Prunus avium*. The analysis of total phenolic and flavonoid content and free radical scavenging activity showed that the extract from the stems of wild cherry fruits can be a potent source of natural antioxidants. The antioxidant and antiradical activities of the aqueous extract were found to be lower than that of the alcoholic extract. The results of *in vivo* studies suggest that the alcoholic extract was slightly active against gram-positive bacteria, whereas the aqueous extract showed weak antibacterial properties. None of the samples showed antibacterial properties against gram-negative bacteria.

**Acknowledgement**

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**REFERENCES**


Циљ овог рада био је да се одреди укупни садржај фенола, процене антиоксидативне особине и антимикробног потенцијала, као и да се идентификују фенолна једињења у алкохолним и воденим екстрактима петељке дивље трешње (*Prunus avium* L.) сакупљеним у Босни и Херцеговини. Алкохолни екстракти су садржавали више фенолних и flavonoидних компоненти у поређењу са воденим екстрактима. Такође, утврђено је да су имали виши антиоксидативни капацитет и способност редукције фери-јона. Сви екстракти су окарактерисани HPLC анализом. Поред кверцетина и (+)-катехина, као доминантних једињења, у алкохолном екстракту идентификовани су хлорогенска киселина и рутин. Кверцентин је, као доминантна компонента, идентификован и у воденом екстракту. По први пут испитане су и антимикробне особине екстраката петељки дивље трешње. Алкохолни екстракт је у поређењу са воденим екстрактом, показао боље антимикробне особине на *Staphylococcus aureus*, као представника грам-позитивних бактерија, док ниједан од испитиваних узорака није испољио активност на бактерију *Escherichia coli* и гљивицу *Candida albicans*.

Кључне речи: Петељка трешње, биоактивне компоненте, антиоксидантна активност, антимикробне особине

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