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ANTIOXIDANT POTENTIAL OF CHERRY STALK EXTRACTS

Dejan PRVULOVIĆ[a], Radenka KOLAROV[a], Mirjana LJUBOJEVIĆ[a], Goran BARAĆ[a], Marijana PEIĆ TUKULJAC[a,*]

ABSTRACT. Cherry stalks, as primary by-products of sour cherry (*Prunus cerasus* L.) and sweet cherry (*Prunus avium* L.) harvesting, are often considered agricultural and industrial waste. However, cherry stalks are recognized in traditional and ethno medicine for their therapeutic properties, due to their high content of natural antioxidants. In this study, the concentrations of total phenolic and total flavonoid compounds, as well as antioxidant activity, were evaluated in different cherry stalk extracts (aqueous, methanol, ethanol, and acetone) obtained from sweet cherry cultivars, sour cherry cultivars, and a wild cherry sample. The results depended on the cultivar and the solvent used for extraction. The highest concentrations of total phenolic and total flavonoid compounds were observed in acetone extracts. In various antioxidant tests, the antioxidant activity varied depending on the genotype.

Keywords: *antioxidant capacity, cherry, extraction solvent, Prunus avium L., Prunus cerasus L., phenolics, stalks*

INTRODUCTION

Reduction of carbon footprint in sustainable agriculture is one of the main goals of modern humanity. Action plan established by European Union involves reducing, recovering, reusing, and recycling materials and energy through circular economy. By-products originated from different agricultural and food processing operations are rich sources of bioactive and nutritional

[a] *University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000, Novi Sad, Serbia*
* *Corresponding author: marijana.peic@polj.uns.ac.rs*

compounds and could be used for development of new and innovative food products [1]. Most widespread compounds in agro-food by-products are dietary fibers, caroteonids, and different polyphenolic molecules produced from primary and secondary metabolism of plants [2]. By-products of fruits and vegetables are the most abundant waste, with a percentage of the residues around 40–50% of the total discards and it could be in form of pulp, seeds, pomace or in other forms [3]. Phytochemical valorisation of agro-food by-products is one of the first steps for identification of bioactive compounds with health-related properties.

Cherry stalks (or stems) are, together with cherry stones, one of the main by-products obtained after the harvesting and processing of sour cherries (*Prunus cerasus* L.) and sweet cherries (*Prunus avium* L.). Although stalks are generally recognised as a waste from agriculture and food industry, the traditional and ethno medicine use them as a herbal remedy for some disorders [4]. In Romania, Bosnia and Herzegovina, Turkey, Iran and other countries infusion (herbal tea) or decoction is used for treatment of different kidney disorders, to relief of renal stones, increase urine output, and mild urinary tract infections [4,5,6,7]. The cherry stalks are also used for its sedative properties, to reduce inflammation, treat obesity, positive effect on cardiovascular system and smooth muscle [4,8]. Cherry stalk's extracts posses strong antimicrobial activity and proved to be effective against some pathogenic bacteria that are resistant to the tested antibiotic [9]. Sour and sweet cherry stalks extracts also exhibited anticancer properties *in vitro* conditions [10].

It is assumed that health beneficial properties (anti-inflammatory and diuretic) are results of presence of high concentration of natural antioxidants (primarily flavonoids) presents in the salks [7,11]. Major phenolic compounds present in sweet cherry stems are mostly different hydroxycinnamic acids: *ρ*-coumaric, ferulic, caffeic, chlorogenic and neochlorogenic acid [8].

For many years, natural sources of antioxidants have attracted considerable attention in scientific research, with increasing focus on the potential use of various agricultural by-products, such as cherry stalks. Although previous research has examined the antioxidant properties of various parts of the cherry plant, it has mostly focused on the fruits, while the stalks have been significantly less studied. This study aims to provide a detailed evaluation of the total phenolic content and antioxidant potential of extracts from the stalks of thirteen sweet cherry cultivars, three sour cherry cultivars, and one wild cherry sample, using four different solvents. The novelty of this work lies in its systematic comparison of various cherry types and extraction solvents, which has not been previously addressed in such depth, highlighting the potential of cherry stalks as valuable bioactive by-products.

RESULTS AND DISCUSSION

The total phenolic content in cherry stalk extracts is presented in Table 1. The total phenolic content ranged from 9.98 to 30.91 mg GAE/g DW in aqueous extracts, from 13.13 to 39 mg GAE/g DW in methanolic extracts, from 13.54 to 34.89 mg GAE/g DW in ethanolic extracts, and from 18.58 to 52.29 mg GAE/g DW in acetone extracts. The highest content of phenolic compounds was found in the acetone extract of sour cherry cultivar Érdi bőtermő.

Table 1. Content of total phenolics in cherry stalk extracts

	Cultivar	Extraction solvent			
		Water	70% methanol	70% ethanol	70% acetone
Sweet cherry	Alex	9.98 ^{bB} ± 0.16	13.67 ^{bcB} ± 1.02	16.63 ^{cdB} ± 1.27	18.58 ^{bA} ± 0.66
	Bigarreau Burlat	17.73 ^{eD} ± 2.43	27.28 ^{bcB} ± 0.37	23.75 ^{deC} ± 0.18	35.85 ^{eA} ± 0.05
	Carmen	14.15 ^{cdD} ± 1.09	19.83 ^{dC} ± 0.66	21.11 ^{bB} ± 0.94	30.04 ^{dA} ± 0.54
	Germersdorfer	12.11 ^{cC} ± 0.79	16.21 ^{bB} ± 1.71	17.01 ^{cB} ± 1.11	23.01 ^{cA} ± 0.65
	Hedelfinger	21.68 ^{eD} ± 1.03	24.73 ^{efC} ± 0.88	29.04 ^{eB} ± 1.14	37.95 ^{fgA} ± 1.00
	Katalin	17.54 ^{iD} ± 0.42	20.54 ^{jC} ± 0.54	23.76 ^{ijkB} ± 1.26	33.07 ^{lA} ± 2.59
	Linda	15.66 ^{hD} ± 0.37	17.36 ^{iC} ± 1.45	17.14 ^{hiB} ± 1.43	27.99 ^{kA} ± 1.26
	New Star	10.57 ^{jD} ± 3.86	18.09 ^{ghB} ± 0.29	16.70 ^{ijC} ± 1.19	23.86 ^{iA} ± 2.24
	Peter	13.88 ^{ghC} ± 0.43	20.17 ^{fgB} ± 1.05	19.61 ^{fghB} ± 0.77	29.83 ^{fgA} ± 1.83
	Sandor	19.98 ^{dD} ± 0.35	26.86 ^{cdB} ± 1.22	23.99 ^{deC} ± 0.49	38.25 ^{dA} ± 0.67
	Solomary gomboly	27.85 ^{eB} ± 1.28	26.45 ^{ijC} ± 0.80	25.60 ^{kC} ± 2.77	42.98 ^{kA} ± 1.53
	Summit	13.20 ^{fghC} ± 1.07	17.64 ^{kghB} ± 1.63	15.73 ^{ijBC} ± 0.70	27.93 ^{hA} ± 1.07
	Valery Chkalov	22.48 ^{fgC} ± 0.41	27.78 ^{efB} ± 0.58	28.56 ^{fgB} ± 2.67	40.84 ^{gA} ± 1.08
Sour cherry	Érdi bőtermő	30.91 ^{aD} ± 0.44	39.00 ^{aB} ± 1.93	34.89 ^{aC} ± 2.23	52.29 ^{aA} ± 0.91
	Kantor-jánosi	12.52 ^{hC} ± 0.34	13.13 ^{ijB} ± 0.82	14.32 ^{jkB} ± 1.42	24.04 ^{jA} ± 0.47
	Oblačinska	17.67 ^{eD} ± 0.40	21.67 ^{eB} ± 0.43	20.82 ^{fC} ± 1.46	31.93 ^{fA} ± 1.26
Wild cherry		21.50 ^{fC} ± 2.27	14.11 ^{hBC} ± 1.10	13.54 ^{ghB} ± 0.81	21.61 ^{hA} ± 1.02

Expressed as mg gallic acid equivalents (GAE)/g dry weight; Values are means ± SD; values preceded by the same lowercase letter in the column do not differ significantly according to the Duncan's multiple range test (p<0.05)

Moreover, the total phenolic content in all extracts of the Érdi bőtermő cultivar was significantly higher than that of the other tested cultivars. In the aqueous extracts of the stalks cultivar Alex (sweet cherry), the lowest concentration of phenolic compounds was observed.

Phenolic compounds are important secondary metabolites in plants due to their significant antioxidant activity, which includes free radical-scavenging, hydrogen donation, singlet oxygen quenching, metal ion chelation, and also acting as a substrate for radicals such as hydroxyl and superoxide [12]. The phenolic content depends on cultivar, growing conditions as well as the solvent used for extraction [13,14]. The content of total phenols compounds in methanolic extracts of sweet cherry stalks was in accordance with the study of Afonso et al. (from 23.59 to 32.49 mg GAE/g) [15]. On the other hand, total phenolic content in ethanolic and aqueous extracts of wild cherry was lower than those reported by Ademović et al. (121.3 and 74.1 mg GAE/g, respectively) [16].

According to earlier research, acetone is a suitable solvent for extracting polyphenols, especially those with higher molecular weights [14]. In this study, it has been observed that acetone extracts contain the highest amount of total phenolic compounds compared to extracts obtained using other solvents. The total phenol content of acetone extracts differed significantly among all cultivars when compared to other extracts. The amounts of total phenols in the acetone extracts were comparable to the results in our previous study [17].

In Table 2 are presented the results of total flavonoids content in cherry stalk extracts.

Flavonoids are the most widespread class of phenolic compounds in plants where play a role in various cellular activities like as signalling, pigmentation and plant protection against different stressors [18]. The total flavonoids content was ranged from 8.31 to 23.17 mg QE/g DW in water extracts, from 9.71 to 21.33 mg QE/ g DW in methanolic extracts, from 11.74 to 28.07 mg QE/ g DW in ethanolic extracts, as well as from 18.24 to 33.90 mg QE/ g DW in acetone extracts. The acetone extract of the sour cherry stalks cultivar Érdi bőtermő contained the highest amount of flavonoids, while the lowest content was found in the aqueous extracts of the sweet cherry cultivar Alex.

In *P. avium* by-products, such as stalk, pulp, seed, and leaf the highest concentration of total flavonoids was found in the extracts obtained using a solvent of moderate polarity [19]. The highest concentration of total flavonoids was found in acetone extracts. The studies of Do et al. and Xiong et al., also showed higher amount of total phenolic compounds as well as total flavonoids compounds in acetone extracts of *Osmanthus fragrans*' seed and *Limnophila aromatica* than ethanol and methanol extracts. In recent researches, it has

been observed that acetone extracts (50-80%) contained higher amount of total phenols and total flavonoids in compared to their corresponding alcoholic-water mixtures [20].

Table 2. Content of total flavonoids

	Cultivar	Extraction solvent			
		Water	70% methanol	70% ethanol	70% acetone
Sweet cherry	Alex	$8.31^{gC} \pm 0.63$	$10.17^{iC} \pm 0.74$	$11.74^{gB} \pm 2.43$	$19.60^{ghA} \pm 0.71$
	Bigarreau Burlat	$9.45^{efgC} \pm 0.52$	$14.44^{fgB} \pm 0.23$	$20.76^{fgB} \pm 3.81$	$23.84^{bcdA} \pm 0.52$
	Carmen	$10.24^{defC} \pm 0.61$	$15.41^{efB} \pm 0.76$	$17.26^{cdeA} \pm 3.50$	$21.81^{deA} \pm 1.00$
	Germersdorfer	$10.28^{deC} \pm 1.50$	$14.85^{fB} \pm 0.83$	$14.38^{fgB} \pm 0.84$	$18.24^{fA} \pm 1.07$
	Hedelfinger	$11.70^{cdB} \pm 0.66$	$14.90^{fgB} \pm 1.11$	$19.73^{bcA} \pm 4.80$	$24.14^{bcA} \pm 2.01$
	Katalin	$12.74^{cD} \pm 1.04$	$17.34^{cdC} \pm 1.10$	$21.09^{cdB} \pm 2.54$	$24.92^{bcA} \pm 1.43$
	Linda	$12.56^{cdB} \pm 1.91$	$12.86^{ghB} \pm 1.43$	$16.56^{defA} \pm 2.22$	$18.26^{fA} \pm 0.95$
	New Star	$8.83^{efgD} \pm 0.90$	$12.3^{hiC}5 \pm 0.10$	$15.85^{fgB} \pm 1.13$	$19.4^{efA}2 \pm 1.91$
	Peter	$7.74^{hD} \pm 1.96$	$17.54^{bcdAB} \pm 1.01$	$14.62^{fgB} \pm 2.34$	$20.82^{efA} \pm 1.34$
	Sandor	$12.61^{cC} \pm 1.00$	$18.94^{bB} \pm 1.62$	$16.31^{efgB} \pm 1.29$	$23.76^{cdA} \pm 1.53$
	Solomary gomboly	$23.17^{aB} \pm 1.56$	$16.33^{deC} \pm 0.10$	$20.94^{bcdB} \pm 0.62$	$25.84^{bA} \pm 2.11$
	Summit	$8.42^{fghB} \pm 1.53$	$11.62^{iB} \pm 0.48$	$14.11^{fgB} \pm 4.37$	$22.30^{cdA} \pm 2.34$
	Valery Chkalov	$16.57^{bB} \pm 1.11$	$17.87^{bcB} \pm 1.62$	$24.55^{abA} \pm 3.52$	$25.27^{bcA} \pm 0.36$
Sour cherry	Érdi Bőtermő	$22.39^{aC} \pm 0.75$	$21.33^{aC} \pm 0.72$	$28.07^{aB} \pm 1.14$	$33.90^{aA} \pm 2.44$
	Kántor-jánosi	$9.64^{efgB} \pm 0.70$	$11.67^{iB} \pm 0.55$	$17.07^{defA} \pm 1.73$	$18.87^{fA} \pm 0.54$
	Oblačinska	$12.08^{cdD} \pm 0.33$	$18.26^{bB} \pm 0.74$	$20.38^{cdeB} \pm 1.32$	$25.33^{bA} \pm 1.90$
Wild cherry		$10.40^{defC} \pm 1.92$	$9.71^{iC} \pm 0.58$	$14.16^{fgB} \pm 1.45$	$19.00^{efA} \pm 0.68$

Expressed as mg quercetin equivalents (QE)/g dry weight; Values are means ± SD; values preceded by the same lowercase letter in the column do not differ significantly according to the t-test (p<0.05); Values preceded by the same capital letters in a row do not differ significantly according to Duncan's multiple range test (p<0.05)

In addition, the total phenolic and total flavonoid content in cherry stalk extracts are presented in the heatmap (Figure 1). The variation in the content of these compounds is represented by a color gradient, ranging

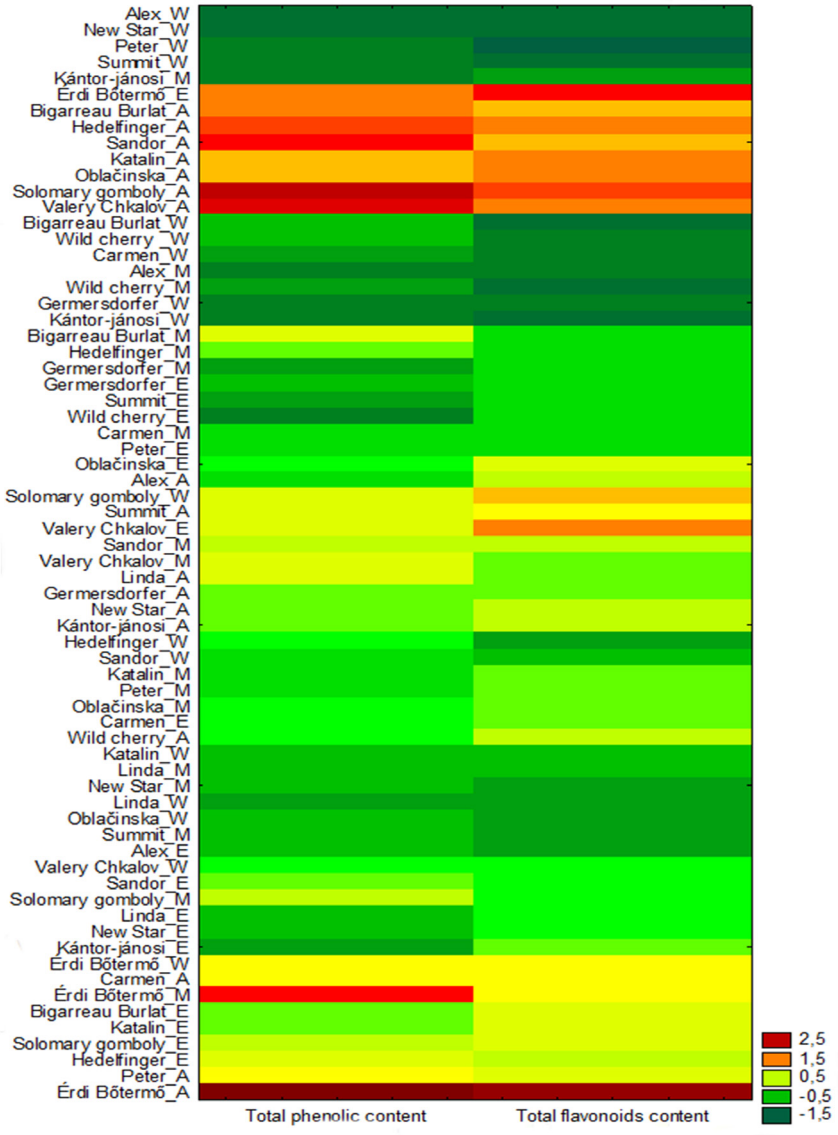

Figure 1. Heat map representation of the total phenolic and total flavonoid contents in different cultivars of cherry stalk extracts prepared using different extraction solutions (W-water, M-methanol, E-ethanol, A- acetone)

from dark green (indicating the lowest concentration) to dark red (indicating the highest concentration). The heatmap clearly confirmed the effect of the extraction solvent on the total phenolic and flavonoid contents. However, differences between varieties (sweet, sour, and wild) are also noticeable, as some cultivars consistently exhibit high phenolic content regardless of the solvent used, highlighting their abundant phenolic composition. Comparing cherry varieties (sweet, sour, and wild), sour cherry cultivars generally exhibited higher levels of total phenolics and flavonoids than sweet and wild cherry samples. Wild cherry showed relatively low phenolic and flavonoid content regardless of the solvent used. Additionally, differences are observed among cultivars within each cherry variety. The cultivars Érdi bőtermő, Valery Chkalov, Sandor and Solomary Gomboly exhibited a color range corresponding to high levels of total phenolics and flavonoids, especially in acetone extracts. On the other side, cultivars New Star and Kántor-jánosi, even in acetone, showed lower content, which may indicate varietal variability in phenolic composition.

In order to evaluate antioxidant activity, five antioxidant tests with different reaction principles were applied. The values of DPPH (2,2-diphenyl-1-picryhydrazyl) assay were ranged from 3.68 to 25.10 mg Trolox/g DW in aqueous extracts, from 11.39 to 42.93 mg Trolox/g DW, from 7.19 to 27.45 mg Trolox/g DW in ethanolic extracts and from 14.04 to 52.02 mg Trolox/g DW in acetone extracts (Figure 2).

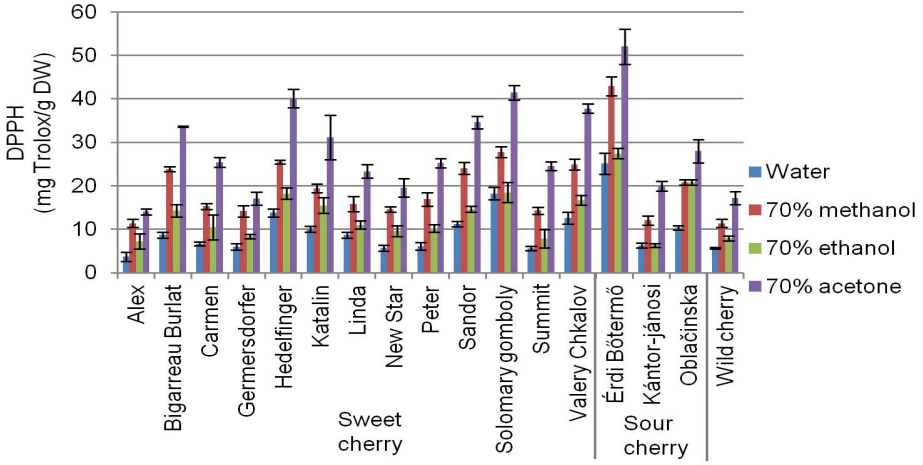

Figure 2. Antioxidant activity measured by DPPH assay

The results of FRAP (Ferric Reducing Antioxidant Power) assay are presented in Figure 3. In aqueous extracts FRAP values varied between 4.12

and 16.73 mg Trolox/g DW, in methanolic extracts between 7.93 and 31.44 mg Trolox/g DW, in ethanolic extracts between 4.13 and 18.07 mg Trolox/g DW and in acetone extracts between 14.92 and 46.62 mg Trolox/g DW.

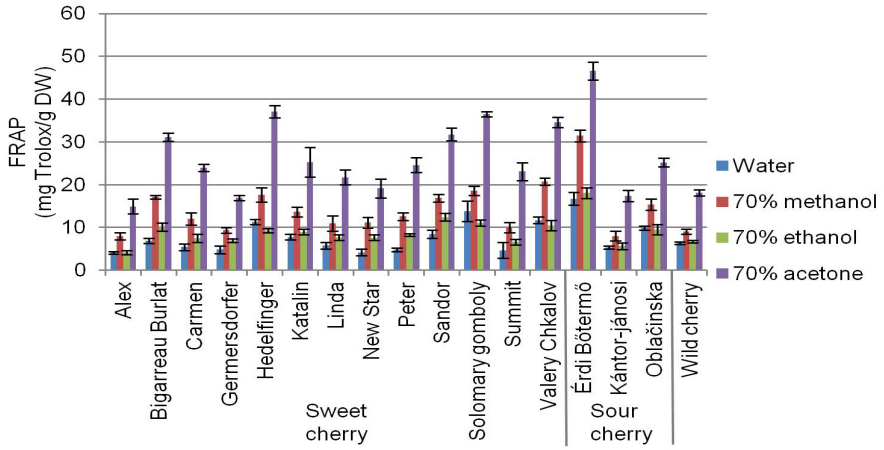

Figure 3. Antioxidant activity in cherry stalk extracts measured by FRAP assay

In aqueous extracts ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) values ranged from 12.73 to 68.34 mg Trolox/g DW, in methanolic extracts from 9.41 to 52.86 mg Trolox/g DW, in ethanolic extracts from 18.67 to 69.33 mg Trolox/g DW and in acetone extracts from 19.12 to 62.03 mg Trolox/g DW (Figure 4).

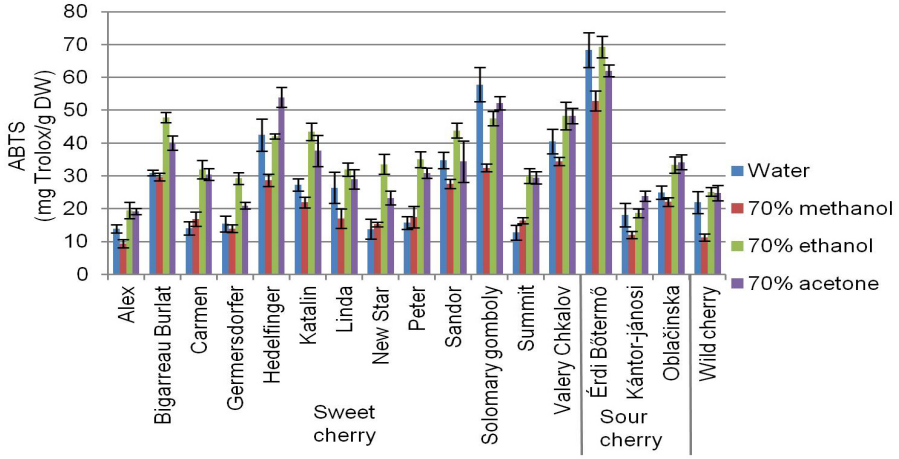

Figure 4. Antioxidant activity in cherry stalk extracts measured by ABTS assay

The TAA (total antioxidant activity) in cherry stalks are shown in Figure 5. The value of TAA ranged from 7.33 to 26.84 mgAA/g DW in aqueous extracts, from 13.46 to 37.81 mgAA/g DW in methanolic extracts, from 13.57 to 32.15 mgAA/g DW in ethanolic extracts, from 16.22 to 38.67 mgAA/g DW.

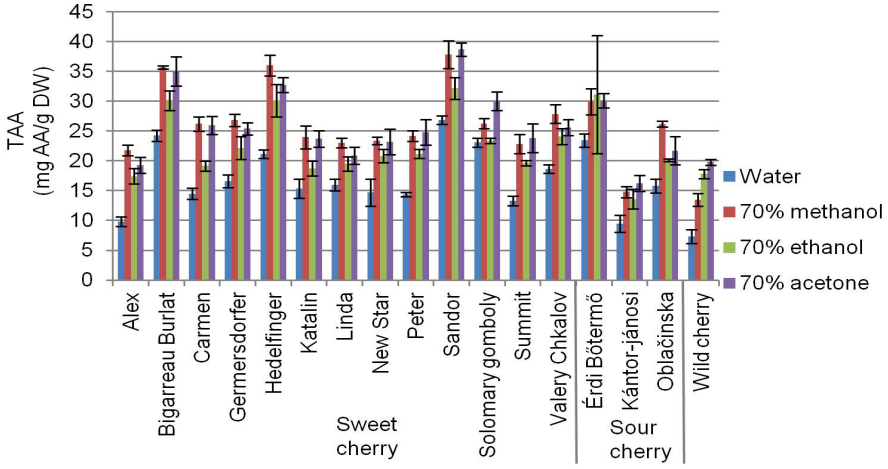

Figure 5. Total antioxidant activity (TAA) in cherry stalk extracts

The values of NBT (Nitroblue tetrazolium) assay were ranged from 0.65 to 1.42% in the arqueous extracts, from 0.84 to 2.17 % in methanolic extracts, from 1.58 to 2.74% in ethanolic extracts and from 1.41 to 2.60 % in acetone extracts (Figure 6).

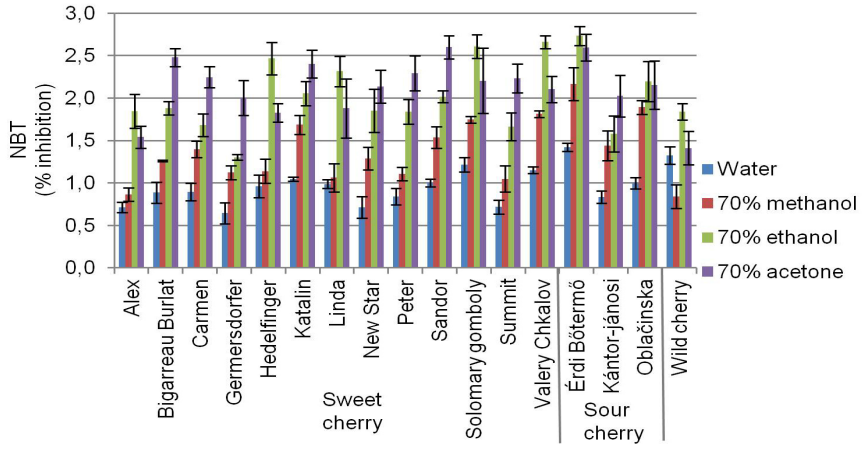

Figure 6. NBT test in cherry stalk extracts

Based on the obtained results of the antioxidant tests, the antioxidant activity is influenced by the extraction solvents. Beyond solvent effects, antioxidant activity also varied significantly among cherry types (sweet, sour, and wild) and also among cultivars within each type. Sour cherries consistently demonstrated stronger activity, likely due to their higher content of phenolic compounds. The variation in the results of antioxidant tests can be explained by the fact that the same antioxidant compounds can act differently when scavenging various types of radicals [21]. The extracts of all tested genotypes increased the capability of scavenging DPPH$^{\bullet}$ radicals in the following order: water < methanol < ethanol < acetone, which is in line with the research of Cisowska et al. in mulberry extracts [22]. Cisowska et al. [22] reported the same results for ABTS as for DPPH, which is not in agreement with the current study. In accordance with our findings, Dailey and Vuong [20] observed differences in antioxidant activity measured by DPPH, ABTS, and the FRAP test, explaining this variation by the different chemical properties of phenolic compounds. The correlation analyses between total phenols, total flavonoids and antioxidant activity is presented in Table 3. The strong positive correlations were observed among total phenols, DPPH and FRAP tests ($r>0,9$; $p<0.05$). These results indicate that phenolic compounds are the primary antioxidants in cherry stalks extracts.

Table 3. The correlation between total phenolics, total flavonoids and antioxidant activity

	DPPH	FRAP	ABTS	TAA	NBT
Total phenolics	0.956	0.921	0.769	0.677	0.705
Total flavonoids	0.782	0.751	0.742	0.539	0.795

Correlation at $p<0.05$

CONCLUSIONS

Based on the results, it can be concluded that the cherry stalks extracts contain a significant amounts of total phenolics and total flavonoids, as well as exhibit high antioxidant activity. Statistical analysis confirmed that wild cherry samples had significantly lower total phenolic and flavonoid contents, as well as antioxidant activity, compared to cultivated sweet and sour cherry cultivars. Although sour cherry cultivars generally exhibited higher levels of total phenolics, flavonoids, and antioxidant activity than sweet and wild cherry samples, notable differences were also observed among cultivars within each cherry type. These findings emphasize that both cherry type and individual cultivar, along with the extraction solvent, significantly influence

the antioxidant potential of cherry stalk extracts. When comparing extracts prepared with the same solvent, the highest values of total phenolic and total flavonoids were found in sour cherry cultivar Érdi Bőtermő. In the aqueous extracts of the sour cherry stalks from the cultivar Alex, the lowest amount of total phenols and total flavonoids was determined. Among the tested solvents, acetone extracts consistently showed the highest concentrations of total phenolics and flavonoids across all cultivars. Antioxidant activity measured by FRAP and DPPH assays showed the highest values for acetone extracts, regardless of cultivar. In contrast, the results of the NBT, ABTS, and TAA assays showed variation depending on genotype and solvent extraction.

EXPERIMENTAL SECTION

Plant material and extracts preparation

Fruits and stalks of sweet and sour cherry from different cultivars were harvested at commercial maturity from the productive orchard "Sloga" in Kać in vicinity of Novi Sad, Serbia. Wild cherry fruits and stalks were collected in the vicinity of Fruška Gora, in the village Rivica. The stalks were subsequently air-dried at ambient temperature until they reached a constant weight.

After drying, the plant material was ground into a fine powder, and extracts of cherry stem from each tested cultivar were prepared using four different extraction solutions: distilled water, 70% aqueous methanol solution, 70% aqueous ethanol solution, and 70% aqueous acetone solution. The extraction was carried out by mixing 1 g of plant material with 50 mL of the respective solvent (1:50, m/v), based on the ratio used in our previous study [23]. Extraction was performed using sonication for 20 minutes in an ultrasonic bath at room temperature. Then, the extracts were rapidly vacuum-filtered through a sintered glass funnel and stored in cold conditions until further analysis.

Determination of total phenols and total flavonoids

The total phenolic content in all examined extracts of cherry stalk was determined spectrophotometrically with Folin-Ciocalteu reagent as described by Kroyer [24]. The extracts (50 μL) were mixed with 2.5 mL of Folin-Ciocalteu reagent and incubated for 5 minutes. Then, 2 mL of saturated sodium carbonate solution was added, and the absorbance of the solutions were measured at 730 nm after 45 min. Garlic acid was used as standard and the results was expressed as mg gallic acid equivalents per gram of dry weight (mg GAE/g DW).

The total flavonoids content was determined following the method described by Saha et al [25]. The extracts (200 µL) were mixed with 2% $AlCl_3$ solution (3ml), and the solutions were incubated for 15min at room temperature. After that, the absorbance was read at 430 nm. The results were expressed as quercetin equivalents in mg per gram of dry weight (mg QE/g DW).

Determination of antioxidant activity

Antioxidant activity in cherry stalk extracts was determined using DPPH (2,2-diphenyl-1-picryhydrazyl), FRAP (ferric reducing antioxidant power), ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid), TAA (total antioxidant activity) and NBT (nitroblue tetrazolium) assays.

The DPPH test in cherry stalk extracts was assayed according to procedure reported by Lai and Lim [26] with slight modifications. DPPH reagent was dissolved in methanol until the absorbance of working solution approached ~0.7. DPPH reagent solution (1.5 ml) was mixed with 20 µl of extracts. After 30 min incubation, absorbance was read at 517nm.

The FRAP test was determined by the method reported by Valentão at. [27] with modifications. FRAP reagent was prepared by mixing acetate buffer pH 3.6 (300 mmol/dm^3) with a solution of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ; 10 mmol/dm^3) in HCl (40 mmol/dm^3) and $FeCl_3$ x $6H_2O$ in ration 10:1:1. In 1.5 ml FRAP reagent was added 20 µl of extracts and the absorbance was read at 510 nm after 30 min.

The ABTS test was performed as described by Re et al. [28] with slight modifications. The ABTS reagent was prepared by mixing 7.4 mmol/dm^3 ABTS solution (2,2′-azinobis-(3-ethylbenzothiazoline-6 sulfonic acid) with 2.6 mmol/dm^3 potassium persulfate solution. The mixture was left in dark for 12 h. The stock solution was diluted until the absorbance of the working solution approached approximately 0.7. In 2 ml of regent was added 25 µl of extracts and the absorbance was read at 734 nm after 2 h.

The calibration curve was established using different concentrations of trolox and the results of DPPH, FRAP and ABTS tests were expressed as mg of trolox equivalents per gram of dry weight (mg TE/g DW).

TAA of cherry stalk extracts was assayed according to the phosphomolybdenum method described by Kalaskar and Surana [29] with minor modifications. To the mixture of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate (1 ml), 25 µl of extracts were added. After incubation in a boiling water bath at 95°C for 90 minutes, the absorbance was measured at 695 nm. Different concentrations of butylated hydroxytoluene were utilized to obtain the calibration curve, and results were expressed as mg of butylated hydroxytoluene equivalents per gram of dry weight (mg BHT/g DW).

The NBT assay was carried out according to the procedure described Mandal et al. [30] with slight modifications. The reaction mixture contained 50 mmol/dm^3 phosphate buffer (pH 7.8), 13 mmol/dm^3 L-methionine, 75 μmol/dm^3 NBT, 0.1 mmol/dm^3 EDTA, 2 μmol/dm^3 riboflavin and 20 μl of the extract. It was kept under a fluorescent lamp for 10 min, and then the absorbance was read at 560 nm. The results were expressed as percent of inhibition of superoxide anion generated (% inhibition).

Statistical analysis

All results were expressed as the mean of the values obtained for three replications ± standard deviation (SD). Statistical significance was tested using software STATISTICA ver. 13 (StatSoft, Inc., USA). The effect of genotype and extraction solvent on the tested biochemical parameters in cherry stalks extracts was evaluated by two-way analysis of variance (Factorial ANOVA), followed by comparison of means by Duncan's multiple range test (p<0.05). Correlation between the tested biochemical parameters was analyzed using the Pearson correlation coefficient.

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