Phenolic composition, antioxidant and antimicrobial activity of the extracts from *Prunus spinosa* L. fruit

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Abstract

Blackthorn (*Prunus spinosa* L.) is commonly used in food industry and phytotherapy. The contents of phenols, flavonoids, anthocyanins and antioxidative activity in extracts of blackthorn fruit were determined using spectrophotometric methods. The content of total phenol compounds varies from 15.33 to 20.94 mg GAE g^{-1} of fresh fruit. The content of total flavonoids is very low and ranges from 0.419 to 1.31 mg QE g^{-1} of fresh fruits. Anthocyanins content lies between 0.112 mg cyanidin 3-glucoside/g of fresh sample in ethanol extract and 0.265 mg of cyanidin 3-glucoside g^{-1} of fresh blackthorn fruit in methanol-water 50/50 (*V*/*V*) extract. The differences in total phenol compounds content depend on used extraction medium as a consequence of different polarity of used organic solvents and their mixtures, which selectively extract individual compounds. All explored extracts exhibited strong scavenging activity against DPPH radicals, which ranges from 32.05 to 89.10%. Phenolic acids (neochlorogenic and caffeic acids), flavonoids (quercetin and myricetin) and anthocyanins (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside and peonidin-3--*O*-glucoside) were identified in investigated ethanol extracts by HPLC analysis. Ethanol extract shows significant antimicrobial activity against S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017 and antifungal activity against *Candida albicans* ATCC 10231. Blackthorn fruit extract exhibits a high phenolic content and a high antioxidant activity, and can be used as an antioxidant in food and pharmaceutical industries.

Keywords: phenolic compounds content, antioxidants, antimicrobial activity.

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Blackthorn (*Prunus spinosa* L.) is a perennial plant growing as a shrub on slopes of wide uncultivated areas, making a thick thorny mass, but it can also be found beside roads, along the channels and in shelterbelts against the wind. It grows in moderate continental climate in northern chemisphere. Blackthorn (*Prunus spinosa* L.) is used in phytotherapy for the treatment of many diseases related to various forms of cough; it is mild laxative, diuretic, spasmolytic and anti-inflammatory agent. It has anti-septic (due to the presence of tannins) effect and shows activity against inflammation of the mucosal layer of the digestive system [1,2].

Medicinal characteristics of blackthorn were shown by fruit, flowers, bark and root of the plant. Apart from phytotherapy, blackthorn is also used in food industry for the production of jams and various beverages: liqueur, wine, juice, compote and tea.

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Antioxidative capacity and the content of biologically active compounds in wild fruit species have been previously examined. Due to the complexity of food composition, it is still unknown which dietary constituents are responsible for this behaviour, but it seems that antioxidants play a major role in the protection in plants. The main property of an antioxidant is its ability to trap free radicals which may oxidize nucleic acids, proteins, lipids [3,4]. Fresh fruit extracts are an excellent source of polyphenolic compounds, as free radicals scavengers, which can significantly alleviate the negative effect of free radicals in the organism. Therefore, they have an important role in the prevention of neurodegenerative diseases, cardiovascular diseases and cancer [5].

There is an increasing interest in the measurement and use of wild fruits as antioxidants for scientific research, as well as for industrial (dietary, pharmaceutics and cosmetics) purposes. Aqueous and aqueous-methanolic extracts obtained from six Bulgarian wild edible fruits have been studied for their antioxidant activity and polyphenol content [6]. *Sambucus ebulus* fruits exhibited the highest polyphenol content – 73.73±0.57 mg QE/g in aqueous extracts and 68.27±1.93 mg QE g^{-1} in aqueous-methanolic extracts, while *Prunus spinosa* showed the lowest polyphenol

content of 9.44 and 9.15 mg QE g^{-1}, respectively. In both types of extracts, the antioxidant activity decreased as follows: *Sambucus ebulus* > *Crataegus monogyna* > *Rosa canina* > *Berberis vulgaris* > *Vaccinium myrtillus* > *Prunus spinosa.* High positive correlation was found between the polyphenol content and the antioxidant activity of these extracts.

In one of the previous studies, seven wild species from Poland were investigated: dog rose (*Rosa canina* L.), blackberry (*Rubus caesius* L.), elderberry (*Sambucus nigra* L.), blueberry (*Vaccinium myrtillus* L.), blackthorn (*Prunus spinosa* L.), rowan (*Sorbus aucuparia* L.) and wild strawberry (*Fragaria vesca* L.). The following order of polyphenol content was established: dog rose > elderberry > blueberry > blackthorn > blackberry > rowan > wild strawberry, while the order of antioxidant activity was: dog rose > blueberry > elderberry > blueberry > blackthorn > rowan > wild strawberry. Polyphenol content of fresh blackthorn fruit exhibited 402.67± ±12.44 mg GAE·100 g^{-1} f.w., and vitamin C content was 21.94±1.42 mg GAE·100 g^{-1} f.w. Fresh blackthorn fruit shows a significant antioxidant activity amounting to 14.17±3.06 mM Fe·100 g^{-1} f.w. measured by FRAP, and5.33±0.22 μM TE·g^{-1} f.w. by ABTS method. The achieved results indicated a high biological value of the analyzed wild fruit species. [7].

Fresh blackthorn flowers contain a cyanogenic glycoside, which makes investigated plants a mild laxative and diuretic agents. In addition, they contain flavonoids, rutin and hyperoside [3]. Among flavonoids, the following compounds have been isolated from the flowers of *Prunus spinosa* L.: kaempferol, quercetin, kaempferol 3-*O*-α-L-arabinofuranoside, quercetin 3-*O*-α-L-arabinofuranoside, quercetin3-*O*-α-D-xylopyranoside, kaempferol 3-*O*-α-L arabinofuranoside-7-*O*-α-L-ramnopyranoside [4,5], tannin, 2% of organic acids (malic, etc.), and water. Blackthorn stones are poisonous because they contain toxic glycoside amygdalin which produces hydrogen cyanide.

Quantitative studies revealed a high content of flavonoids in the flowers of the Polish population of blackthorn (about 2.7% as aglycones and 3.8% as glycosides) [8], while in the Romanian population of this plant it reached around 1.2% as aglycones [9]. Apart from flavonoids, the flowers of blackthorn contain A-type proanthocyanidins [10] and phenolic acids [11]. Studies conducted at the Department of Pharmacognosy, Medical University of Lodz, confirmed the presence of kaempferol, quercetin and their heterosides in the flowers and leaves of blackthorn. In flowers, flavonoids are present mostly in the form of monoglycosides, mainly kaempferol and quercetin 3-*O*-arabinosides. Leaves are abundant in diglycosides, mainly kaempferol 3,7-*O*-dirhamnoside [12]. The structure of these compounds was detected chemically and instrumentally (UV, IR, H-NMR, C-NMR and MS).

The content of flavonoids in the flowers of Romanian blackthorn population (1.16% of total flavone aglycones – quercetin) is the only well established value. It is known that flowers and leaves of blackthorn contain complex of flavonoids, derivatives of flavonol: kaempferol, quercetin and their glycosides with arabinose, rhamnose and xylose [9].

Fraternale *et al.* proved that the antioxidant activity of red berries has been correlated with their anthocyanin content. The results of this study indicate that the three most representative anthocyanins in *P. spinosa* fruit juice (cyanidin-3-rutinoside, peonidin-3-rutinoside and cyanidin-3-glucoside) are likely to play an important role in its antioxidant properties [14].

Despite the wide use of these wild fruits in Serbia, data regarding a complete phytochemical characterization are missing. Therefore, the aim of our work is further examination of phenolic composition (total phenols, flavonoids and anthocyanins), of fresh fruit extracts of *Prunus spinosa* L. from Southeast region of Serbia. The influence of solvent on the extraction of these compounds was investigated. Determination of compounds was performed using HPLC analysis. The antimicrobial activity of compounds from the ethanol extract was checked.

EXPERIMENTAL

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), quercetin and $AlCl_3$ were purchased from Sigma Chemical Co (St Louis, MO, USA). Folin–Ciocalteu's phenol reagent and sodium carbonate were purchased from Merck Chemical Suppliers (Darmstadt, Germany). Sodium chlorate buffer (pH 1.0) and acetate buffer (pH 4.5) were purchased from Merck Chemical Suppliers (Darmstadt, Germany).

All other chemicals used, including solvents, were of analytical grade. An Agilent 8453 UV/Vis spectrophotometer was used for absorbance measurements and spectra recording, using optical or quartz cuvettes with 1 cm of optical path. The pH measurements were made with Hanna Instruments pH-meter equipped with glass electrode.

Materials preparation

Blackthorn (*Prunus spinosa* L.) fruits (sloes) were collected in the phase of full ripeness in the region of Southeast Serbia, in October 2009. This region is classified as an ecological complex, with minor negative environmental influences.

Voucher specimens are deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevre-

movac", Faculty of Biology, University of Belgrade under the accession number: 16477, BEOU [15]. The plant species were identified by Mirjana Milenković, Faculty of Biology, University of Belgrade. Fresh blackthorn fruits were used for the extract preparation. The prepared extracts were kept in a refrigerator in dark bottles and were analyzed before use.

Preparation of herbal extracts

Fresh blackthorn fruits (*Prunus spinosa* L.) were ground up in a blender. Samples of 2 g of each fruit were weighed from the homogenized herb and extracted with solvents listed in Table 1.

The extraction was carried out in time intervals of 15 min three times with 30, 20 and 20 mL of solvent, respectively. The selected solvents were: water, ethanol (also ethanol–water: 50/50, *V*/*V*), and methanol (and methanol–water: 50/50, *V*/*V*). The extraction was performed in an ultrasonic bath. The extracts were filtered through a Buchner funnel and filter paper (blue collar), (CHMLAB, Spain), transferred into a standard 100 mL flask and the same solvent was added. The liquid extracts were stored in a refrigerator at 5 °C until analysis.

Determination of total phenolics

Total phenolic contents in the extracts were determined by the modified Folin–Ciocalteu method [16]. Then, the absorbencies were measured at 765 nm. Gallic acid was used as standard. The calibration line was linear in the range of 0 to 2 mg L^{-1}. The total phenolic content was expressed as mg gallic acid equivalent (GAE) on 1 g of fresh fruit (f.f.). The result of each assay was obtained from three parallel determinations.

Determination of total monomeric anthocyanins

Total monomeric anthocyanin content of the plant extracts was determined using pH-differential method [17]. The colored oxonium form of antocyanins predominates at pH 1.0, and the colorless hemiketal form at pH 4.5. Finally, the absorbance of each sample was measured at λ_{max} 520 and 700 nm, respectively. $A_{\lambda vis\text{-}max}$ and A_{700} were absorbances of each solution at above mentioned wavelengths.

The total absorbance of each solution (A) was calculated from Eq. (1):

$$A = (A_{\lambda vis\text{-}max} - A_{700})_{pH1.0} - (A_{\lambda vis\text{-}max} - A_{700})_{pH4.5} \quad (1)$$

The content of the monomeric anthocyanin pigment (MAP) was calculated from Eq. (2):

$$MAP \text{ (mg } L^{-1}) = (A \times MW \times DF \times 1000)/(\varepsilon l) \quad (2)$$

where ε is molar absorptivity (26.900), MW is the molecular weight of cyanidin-3-*O*-glucoside (449.2 g mol^{-1}), DF is the dilution factor for dilution of extracts by buffer, l is the length of cuvette. Monomeric anthocyanin pigment (MAP) was expressed as mg of cyanidin-3-*O*-glucoside L^{-1}.

Determination of total flavonoid content

The total flavonoid contents were determined using the spectrophotmetric method based on the formation of yellow flavonoid complex with aluminum [18]. The absorbance was measured at 420 nm. Quercetin was used as a standard. The calibration line was linear from 0 to 30 mg L^{-1}. The total flavonoid content was calculated using the equation based on the calibration curve and expressed as mg of quercetin equivalent (*QE*) per g of fresh fruits.

Free radical scavenging activity

The free radical scavenging activity of the fruit extracts was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay [19,20]. The antioxidant assay is based on the measurement of the loss of colour of DPPH solution by change of absorbance at 517 nm caused by the reaction of DPPH with the test sample. The reaction was monitored with a UV–Vis spectrophotometer.

The ability of extracts to inhibit DPPH (*RSC*, %) was calculated from the decrease of absorbance:

$$RSC\ (\%) = (1 - A_{sample}/A_{blank}) \times 100 \quad (3)$$

where A_{blank} is the absorbance of control (1×10^{-4} mol L^{-1} DPPH methanol solution) and A_{sample} is the absorbance of the test sample. The data were expressed as milligrams of quercetin equivalent (*QE*) per 100 g of fresh fruit (mg *QE* 100 g^{-1} f.f.).

Antimicrobial activity

The *in vitro* antimicrobial activity of ethanol extract of *Prunus spinosa* L. was tested against a range of laboratory control strains belonging to the American Type Culture Collection, Maryland, USA (except one, belonging to National Collection of Type Cultures, see below). The antibacterial activity was evaluated against two Gram-positive and three Gram-negative bacteria. Used Gram-positive bacteria were: *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The Gram-negative bacteria in the assay were: *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017. The antifungal activity was tested against two organisms *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231.

A disc-diffusion method was used for the determination of the antimicrobial activity of the extracts, according to NCCLS [20]. The inocula of the bacterial and fungal strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. 100 µL of suspension containing 1.0×10^8 CFU mL^{-1} of bacteria and 1.0×10^4 CFU mL^{-1} of fungal spores spread on Mueller-Hinton agar (MHA,

Torlak) and sabouraud dextrose agar (SDA, Torlak) respectively, in sterilized Petri dishes (90 mm in diameter). The discs (9 mm in diameter, Macherey–Nagel, Düren, Germany) were impregnated with 20 and 50 μL of extracts (conc. 30 mg mL^{-1}) and placed on the inoculated agar. Negative controls were prepared using the same solvent (ethanol). Tetracycline (30 μg, Torlak) and Nystatin (30 μg, Torlak) were used as positive reference standards to determine the sensitivity of a strain of each tested microbial species. The inoculated plates were kept at 4 °C for 2 h and incubated at 37 °C (24 h) for bacterial strains and at 28 °C (48 h) for fungal strains. The antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Each assay in this experiment was repeated in triplicates.

High-performance liquid chromatography (HPLC) analysis

The fruit extracts were analyzed by the direct injection of the extracts, previously filtered through a 0.45 μm pore size membrane filter, into the Agilent Technologies 1200 chromatographic system equipped with the Agilent photodiode array detector (DAD) 1200 with RFID tracking technology for flow cells and a UV lamp, an automatic injector, and a Chemstation software. The column was termostated at 30 °C. After injecting 5 μL of the sample extract, the separation was performed in an Agilent-Eclipse XDB C-18 4.6 mm×150 mm column. Two solvents were used for the gradient elution: A – (H_2O + 5% HCOOH) and B – (80% ACN + 5% HCOOH + H_2O). The used elution programme was as follows: from 0 to 28 min, 0.0% B, from 28 to 35 min, 25% B, from 35 to 40 min, 50% B, from 40 to 45 min, 80% B, and finally for the last 10 min again 0% B. The detection wavelengths were 320 and 520 nm. The identification and quantification of phenolic compounds were performed using calibration curves obtained with standard solutions. The results are expressed as mg L^{-1} of fruit extracts.

Statistical analysis

The experimental results were expressed as mean value ± standard error of mean value of three replicates. In order to statistically estimate any significant differences among mean values wherever it was applicable, the data were subjected to a one-way analysis of variance (ANOVA test), and differences among samples were determined by Duncan's Multiple Range test using the Statistical Analysis System software (SAS) [22].

The data were reported as mean ± standard deviation (*SD*) for triplicates. The significance of inter-group differences was determined by the analysis of variance (ANOVA). The p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The total phenol content in the investigated samples of blackthorn (*Prunus spinosa* L.) fruits extracts was determined by Folin–Ciocalteu method and shown in Table 1.

The experimental results show that the content of total phenols in the investigated extracts was significant, ranging from 15.33 mg GAE g^{-1} f.f. (water) to 20.94 mg GAE g^{-1} f.f. (ethanol–water 50/50, *V*/*V*) extract.

Earlier, the total phenolic content in plum fruits which was reported ranged from 0.42–4.13 mg GAE g^{-1} f.f. [23]. Uzelac reported data regarding the phenol content in blackthorn (*Prunus spinosa* L.) fruits. It is considerably lower than our values and amounts from 0.546 to 0.86 mg GAE g^{-1} f.f. (obtained by extraction with 80% ethanol solution) [13]. The results of this study support the results of our work regarding the values of total phenols. Fraternale et al. [14] proved in their study that the polyphenolic content in *Prunus spinosa* L. was 83.5±2.5 mg/g DW, that is significantly higher than our values, which may be due to differences in the expression of results sincethey are expressed in relation to dry substance, and the results of the present study are expressed in the relation to the fresh fruit.

Egea *et al*. investigated the antioxidant activity and the phenolic composition of several wild fruits including *P. spinosa*. Based on the fact that the plants were collected from different areas of the Mediterranean region, the results obtained in our study showed differences in total phenolics content per gram of fresh fruit [24].

Barros *et al.* investigated the dried fruits of strawberry-tree, blackthorn and dog rose and found that the

Table 1. The total phenolic, anthocyanin and flavonoid content and antioxidant activity of fruit extracts (Prunus spinosa *L.)*

Sample	Total phenols content[a]	Anthocyanin content[b]	Flavonoid content[c]	*RSC* / %	RSC[d]
Ethanol extract	15.33±0.19	0.11±0.008	0.700±0.10	47.38±0.02	2.76±0.36
Ethanol–water extract (1:1)	20.94±0.74	0.238±0.03	1.242±0.09	72.12±0.05	4.25±0.06
Methanol extract	15.33±0.98	0.17±0.01	1.31±0.17	89.10±0,02	5.24±0.83
Methanol–water extract (1:1)	17.69±0.41	0.265±0.01	1.18±0.18	75.69±0.22	4.45±0.45
Water extract	12.17±0.19	0.12±0.005	0.42±0.013	32.05±0,85	0.86±0.08

[a]Expressed as mg GAE g^{-1} f.f.; [b]expressed as mg of cyanidin-3-*O*-glucoside g^{-1} f.f.; [c,d]expressed as mg QE g^{-1} f.f.

ethanolic extracts of the fruits contain considerable amounts of phenolic compounds, affecting the antioxidant capacity of the fruits. Due to the fact that in the Southeast region of Serbia, the local people consume the investigated fruits in their fresh form, we examined the free radical scavenging capacity, as well as the bioactive compounds content of the fresh fruits in our study [25].

The antioxidant activity of the plant extracts was determined by DPPH method. All tested extracts exhibited strong scavenging activity against DPPH radicals, which ranged from 32.05 to 89.10%. Correlation coefficient between the total phenols and antioxidant activity was very low (R^2= 0.325).

Nevertheless, there are reports with low correlation between total phenolic contents and radical scavenging capacity. The high free radical scavenging capacity of the wild plants might be attributed not only to phenolic composition, but also to the presence of other bioactive compounds, such as vitamins (ascorbic acid, tocopherols) and pigments (anthocyanins), as well as the structural interaction among these compounds [26].

The colour of blackthorn (*Prunus spinosa* L.) fruits extracts originates from the anthocyan content. Anthocyan content lies between 0.112 mg cyanidin 3-glucoside g^{-1} of fresh sample in ethanol extract, to 0.265 mg of cyanidin 3-glucoside g^{-1} of fresh blackthorn fruit sample in methanol-water 50/50, *V*/*V* extract (Table 1). The content of monomer anthocyans in all extracts is very similar. The correlation coefficient for monomer anthocyans and antioxidant activity is R^2 = 0.5072. This value is lower than the value reported by Uzelac (0.305 compared to 0.497 mg cyanidin-3-*O*-glucoside g^{-1} f.f., 80% ethanol solution). The highest amount of total anthocyanins was observed in dark purple fruits: 0.413 mg cyanidin-3-*O*-glucoside g^{-1} f.f. [13].

The total level of anthocyanins in *Prunus spinosa* L. juice was 55.1±5.6 mg g^{-1} DW, where the determined values were considerably higher than ours. In accordance with reported data for different fruits and vegetables, the total polyphenol and anthocyanin content appeared to contribute significantly to the antioxidant activity of sloe fruits (cyanidin-3-rutinoside, peonidin-3--rutinoside and cyanidin-3-glucoside) [14].

The content of total flavonoids in the investigated extracts of the plant fruit is low. The flavonid content ranges from 0.4 (water) to 1.3 mg (methanol) QE g^{-1} f.f. The correlation coefficient between the total flavonoids content and antioxidant activity is very high (R^2 = = 0.9618). On the basis of the obtained results we can conclude that the flavonoid compounds have significant contribution as antioxidants in the investigated plant fruits.

The data reported by Uzelac are lower than our values and amounts vary from 0.437 to 0.656 mg QE g^{-1} f.f. obtained by the extraction with 80% ethanolic solution [13].

The differences in phenolic compounds content depend on the used extraction medium as a consequence of different polarity of used organic solvents and their mixtures.

Using HPLC-DAD techniques, a comparative analysis of the quantities of phenolic compounds in investigated extracts was performed. The peaks were identified comparing their HPLC retention times and UV-Vis absorption spectra. Individual compounds and their content in tested extracts are shown in Table 2.

Phenolic acids (neochlorogenic and caffeic acids), flavonoids (quercetin and myricetin) and anthocyans (cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside, peonidin-3-*O*-glucoside) were identified in investigated extracts by HPLC analysis. Only anthocyanins were found in water extract, while in ethanol and ethanol-water extract, phenolic acids and flavonoids were determined.

Fraternale *et al.* [14] identified the following anthocyanins using HPLC analysis: cyanidin-3-*O*-glucoside, cyanidin-3-*O*-rutinoside chloride and peonidin-3-*O*-rutinoside chloride, which is in agreement with the results obtained in this study.

We chose the ethanol extract, which approximately has the same content of active substances as methanol extract, but it is not toxic, for the antimicrobial analysis.

The antimicrobial activity was analyzed by the measurement of the inhibition zone. Antimicrobial activity of the prepared ethanol fruit extracts (*Prunis spinosa* L.) is given in Table 3.

Table 2. HPLC Analysis of the fruit extracts (Prunus spinosa L.)

Time, min	Compound	Ethanol extract contents mg L^{-1}	Ethanol–water extract content mg L^{-1}	Water extract content mg L^{-1}
15.6	Neochlorogenic acids	12.26±0.2	16.95±0.3	–
19.8	Caffeic acids	2.12±0.1	9.73±0.2	–
20.8	Myricetin	–	8.86±0.2	–
23	Cyanidin-3-*O*-glucoside	1.1±0.1	0.9±0.1	1.1±0.1
24	Cyanidin-3-*O*-rutinoside	1.1±0.1	3.1±0.2	1.5±0.1
25	Peonidin-3-*O*-glucoside	–	1.2±0.1	2.2±0.1
26.5	Quercetin	4.02±0.2	3.83±0.2	–

Table 3. Antimicrobial activity of ethanol fruit extracts (Prunus spinosa L.); Zone of inhibition (mm); Data are expressed as the mean of three replicates ± standard deviation

Test microorganism	Extract (10 mg mL^{-1})		Reference antibiotic	Reference antimicotic	Negative control
	20 µL	50 µL	Tetracycline (30 µg)	Nistatin (30 µg)	Ethanol, 96%
Salmonella abony NCTC 6017	–	19.0±0.2	28.0±0.3	–	–
Escherichia coli ATCC 25922	11.0±0.1	24.0±0.3	30.0±0.3	–	–
Pseudomonas aeruginosa ATCC 9027	13.0±0.15	23.0±0.3	19.0±0.2	–	–
Bacillus subtilis ATCC 6633	–	–	36.0±0.4	–	–
Staphylococcus aureus ATCC 6538	13.0±0.2	18.0±0.2	34.0±0.3	–	–
Candida albicans ATCC 10231	14.0±0.2	23.0±0.3	–	20.0±0.3	–
Aspergillus niger ATCC 16404	–	–	–	19.0±0.3	–

Ethanol fruit extract (*Prunus spinosa* L.) showed antimicrobial activity against all tested microorganisms (*Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella abony* NCTC 6017) except *Bacillus subtilis* ATCC 6633. The antifungal activity was tested against two organisms *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231. The investigated extract exhibits antifungal activity against *Candida albicans* ATCC 10231.

The antimicrobial activity of tested extracts was directly correlated to the quantity and structure of extracted compounds. Significant antimicrobial activity against S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella abony* NCTC 6017 was detected. The ethanol extracts exhibited antifungal activity against *Candida albicans* ATCC 10231.

CONCLUSION

From the medical point of view, the investigated extracts contain a high percentage of polyphenolic compounds and show a significant antioxidant and antimicrobial activity. Alcohol solutions have a higher content of phenols than water extracts. All extracts contain significant amount of anthocyanins. The content of flavonoids in alcohol solutions is uniformed and it is higher than the content of flavonoids in water extract. Therefore, alcohol solutions are more suitable for extraction processes. Ethanol extracts are more suitable than methanolic because they are not toxic. All tested extracts exhibited strong scavenging activity against DPPH radicals. Ethanol solution of the investigated plant showed antimicrobial activity against all tested microorganisms except *Bacillus subtilis*, and a species of mold, *Aspergillus niger*. On the basis of obtained results, we concluded that the investigated blackthorn (*Prunis spinosa* L.) fruit extracts have significant biological importance as antioxidant and microbiological agent.

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IZVOD

SADRŽAJ FENOLA, ANTIOKSIDATIVNA I ANTIMIKROBNA AKTIVNOST EKSTRAKATA PLODA TRNJINE (*Prunus spinosa* L.)

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Trnjina (*Prunus spinosa L*.) se najčešće koristi u prehrambenoj industriji i fitoterapiji. Sadržaj fenola, flavonoida, antocijana i antioksidativna aktivnost određeni su primenom spektrofotometrijske metode. Sadržaj ukupnih fenolnih jedinjenja varira od 15,33 do 20,94 mg GAE g^{-1} svežeg voća. Sadržaj ukupnih flavonoida je veoma nizak, i kreće se od 0,419 do 1,31 mg QE g^{-1} svežeg voća. Sadržaj antocijana je od 0,112 mg cijanidin 3-glikozida g^{-1} svežeg uzorka u etanolnom ekstraktu do 0,265 mg cijanidin 3-glikozida g^{-1} svežeg voća u methanol–voda (50/50, *V*/*V*) ekstraktu. Svi testirani ekstrakti pokazuju visoku antioksidativnu aktivnost u odnosu na DPPH radikale, koja se kreće od 32,05 do 89,10%. Fenolne kiseline (neohlorogenska i kafeinske kiseline), flavonoidi (kvercetin i miricetin) i antocijani (cijanidin-3-*O*-glikozid, cijanidin-3-*O*-rutinozid i peonidin-3-*O*-glikozid) su identifikovani u ispitivanim ekstraktima HPLC analizom. Etanolni ekstrakti pokazuju značajnu antimikrobnu aktivnost prema bakterijama: S*taphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 i *Salmonella abony* NCTC 6017 i antifungicidnu aktivnost prema *Candida albicans* ATCC 10231. Ekstrakti svežeg ploda trnjine pokazuju visok sadržaj fenolnih jedinjenja i visoku antioksidativnu aktivnost, pa se mogu koristiti kao antioksidansi u prehrambenoj i farmaceutskoj industriji.

Ključne reči: Sadržaj fenola • Antioksidanti • Antimikrobna aktivnost