Phenolic composition, antioxidant activity, mineral content and antimicrobial activity of fresh fruit extracts of *Morus alba* L.

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Summary

The aim of this work was to evaluate the content of phenolic compounds (phenols, flavonoids and anthocyanins) of *Morus alba* L. fruit (white mulberry) as well as the antioxidant activities of its extract, heavy metals content and antimicrobial activity. The contents of phenols, flavonoids and antioxidant activity of extracts of white mulberry in the following solutes: water, ethanol, ethanol–water (1 : 1, v/v), methanol, methanol–water (1 : 1, v/v), acetone and acetone–water (1 : 1, v/v), were determined using spectrophotometric methods. Total phenol compounds in the extracts varied from 629.7 mg·kg^{-1} to 4 326.0 mg·kg^{-1} (expressed as gallic acid equivalents) of fresh mulberry fruit in acetone–water (1 : 1, v/v) and in ethanolic extract, respectively. The contents of flavonoids ranged from 290.0 mg·kg^{-1} to 1 378.6 mg·kg^{-1} of fresh fruit (expressed as catechin equivalent). The fruit extracts (1 ml) showed high antioxidant activity with 1,1-diphenyl-2-picrylhydrazyl radical transformation value of 87.2% in methanolic extract. Anthocyanins were not found. The fruit of white mulberry contained the highest amount of iron and the lowest amount of lead. Some of the tested extracts showed antimicrobial activity.

Keywords

Morus alba L.; phenolic compunds; antioxidants; antimicrobial activity; heavy metals

Phenol compounds are active as antioxidants in different ways, such as direct reaction with free radicals, scavenging of free radicals, increasing dismutation of free radicals to the compounds with much lower reactivity, chelation of pro-oxidant metals (mainly iron), delaying or strengthening activities of many enzymes. Fresh fruit extracts are an excellent source of polyphenolic compounds with antioxidant activity [1]. With the aim of finding new sources of natural antioxidants, plants, fruits, vegetables and other plant materials that are known to possess antioxidant activity, have been investigated [2–10].

The white mulberry, *Morus alba* L. (*Moraceae*), is native to eastern and central China. It became naturalized in Europe centuries ago. The tree was introduced to America for silkworm culture in early colonial times and naturalized and hybridized with the native red mulberry. The white mulberry is a deciduous tree, which can grow to a height of 24.38 m and is variable in form, including drooping and pyramidal shapes [11, 12]. Mulberry grows in the temperate and subtropical regions of the northern hemisphere and it can grow in a wide range of climatic, topographic and soil conditions. The white mulberry is so named for the colour of its buds, rather than for the colour of its fruit [11].

The total content of phenolic compounds depends on geographic location and soil on which the mulberry tree grows. Despite previous research on this plant, there is no information on phenolic content in fruit extracts of *Morus alba* L. grown in the southeast of Serbia. Additional aim of this investigation was to determine the contents of heavy metals in the fruit of white mulberry and its extracts, and to determine the coefficient of extraction of metals in different solvents and their mixtures.

Fruits and their extracts deserve special attention because of the important influence they have

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on human health. For the majority of the world population, fruits represent the primary source of health-promoting compounds. Although the effectiveness of fruits is mainly associated with their constituents such as essential oils, vitamins or glycosides, it was found that their prolonged intake can cause health problems due to the possible presence of heavy metals [13]. Heavy metals have a significant toxicity for human, animals, microorganisms and plants [14]. Thus, contamination of fruits with heavy metals seriously affects their quality and safety [15]. Lead and cadmium are very harmful to human body, in particular at high doses [16].

White mulberry is a wild fruit that has a great importance in nutrition and traditional medicine in Serbia, although losing its significance in recent years. Reviewing the literature we found no detailed examination of white mulberry and its extracts. The aim of this study was to examine the phenolic composition, antioxidant activity, mineral content and antimicrobial activity of fresh fruit extracts of *Morus alba* L. from southeastern Serbia.

MATERIALS AND METHODS

Preparation of the fresh fruit extracts

Plant materials were collected in the southeastern Serbia in early July 2011. Voucher specimens (*Morus alba* L. No 2-1753, Bela Palanka, UTM 34TDR2 01, determined by Mirjana Milenkovic, Faculty of Biology, University of Belgrade, Belgrade, Serbia) were deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Natural Sciences, University of Novi Sad, Novi Sad, Serbia. Samples were stored in plastic bags and kept frozen until extraction. The frozen fresh fruit material was homogenized using a blender. Fresh fruits (10 g) were extracted with water, acetone–water (1 : 1, v/v), acetone, methanol–water (1 : 1, v/v), methanol, ethanol–water (1 : 1, v/v) and ethanol. All solvents were acidified with 1 ml concentrated HCl. The extraction was performed with 100 ml of solvents in an ultrasonic bath during 30 min. The suspension was filtered through Whatman No. 1 filter paper (Sigma-Aldrich, St. Louis, Missouri, USA). Extracts were stored in a refrigerator until analysis.

For the metals, the working solutions were prepared immediately before the analysis from the basic solution with concentration 1000 $mg \cdot l^{-1}$ for all metals. For the preparation of standard solutions, Milli-Q water (Millipore, Schwalbach am Taunus, Germany) was used. The glassware and polyethylene containers used for analysis were washed with tap water, then soaked overnight in 6 $mol \cdot l^{-1}$ HNO_3 solution and rinsed several times with ultra pure water to eliminate absorbance due to detergent.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), catechin and $AlCl_3$ were purchased from Sigma-Aldrich. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Acros Organics (Morris Plains, New Jersey, USA). Folin-Ciocalteu's phenol reagent, sodium carbonate, sodium chlorate buffer (pH 1.0) and acetate buffer (pH 4.5) were purchased from Merck (Darmstadt, Germany). All other reagents were of analytical purity (Merck).

Aparatus

An Agilent 8453 UV/VIS spectrophotometer (Agilent Technologies, Santa Clara, Caligornia, USA) was used for absorbance measurements and spectra recording, using optical glass or quartz cuvettes of 1 cm optical path. The pH measurements were made with pH meter (Hanna Instruments, Woonsocket, Rhode Island, USA) equipped with a glass electrode. Atomic absorption measurements were made using a Varian SpectraAA 10 (Varian Medical Systems, Palo Alto, California, USA) with background correction and hollow cathode lamps. Air–acetylene flame was used for determination of all the elements.

Determination of total phenolics

Total phenol contents of the extracts were determined by a modified Folin-Ciocalteu method [2]. An aliquot of the extracts (1 ml) was mixed with 0.5 ml Folin-Ciocalteu reagent and 2 ml of sodium carbonate (20%). Absorbance was measured after 10 min incubation at room temperature at 760 nm. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per kilogram. The result of each assay was obtained from three replicate determinations.

Determination of the flavonoid content

Total flavonoid content was determined using a spectrophotmetric method based on formation of flavonoid complex with aluminum [10]. The liquid extract (1 ml) or standard solution of catechin (50–500 $mg \cdot l^{-1}$) was mixed with 3 ml deionized water and 0.3 ml $NaNO_2$. After standing at room temperature for 5 min, 3 ml of water solution of $AlCl_3$ (2%) was added to the solution, followed by 2 ml of 1 $mol \cdot l^{-1}$ NaOH after another 5 min. The solution was then made up to mark with deion-

ized water in a 10 ml flask. The absorbance of the solution was measured at 510 nm. Total flavonoid content was calculated as milligrams of catechin equivalent (CE) per kilogram of fresh fruit based on a calibration curve for catechin (R^2 = 0.999). All samples were analysed in triplicate.

Determination of monomeric anthocyanins

The total monomeric anthocyanin content in the fruit extracts was determined using the previously described pH-differential method [17]. Anthocyanins demonstrate maximum of absorbance at 520 nm at pH 1.0. The coloured oxonium form predominates at pH 1.0, and the colourless hemiketal form at pH 4.5. The pH-differential method is based on reaction producing oxonium forms and permits accurate and rapid measurement of the total monomeric anthocyanins. The result, considered as the monomeric anthocyanin pigment, was expressed as milligrams of cyanidin-3-O-glucoside. For this method, 1 ml of the extract was poured into two separate 10 ml volumetric flasks. Then, one was filled up to the line with a solution of KCl (pH 1.0), and the second with CH_3COONa (pH 4.5). The two diluted solutions were left to stand for 15 min at room temperature. Finally, the absorbance of both samples was measured at wavelengths of 520 nm and 700 nm. Absorbance (A) of the investigated extracts was calculated by Eq. 1.

$$A = (A_{520} - A_{700})_{\text{pH 1.0}} - (A_{520} - A_{700})_{\text{pH 4.5}} \quad (1)$$

Content of the monomeric antocyanin pigment (*MAP*) was calculated by Eq 2.

$$MAP = \frac{A \times MW \times DF}{\varepsilon \times l} \times 1\,000 \quad (2)$$

where A is absorbance calculated by Eq. 1, ε is the molar absorptivity (26.900 $dm^3 \cdot mol^{-1} cm^{-1}$), *MW* is the molecular weight (449.2 $g \cdot mol^{-1}$), *DF* is the dilution factor, and l is the path length (1 cm). The result, taken as the monomeric anthocyanin pigment (*MAP*), was expressed as milligrams of cyanidin-3-O-glucoside per litre.

Free radical scavenging activity

The free radical scavenging activity (*RSC*) of the fruit extracts was determined by the DPPH assay [18–22]. This antioxidant assay is based on the measurement of the loss of colour of DPPH solution by a 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. Methanolic solution of Trolox (10–30 $mmol \cdot l^{-1}$) and 1.8 ml of freshly prepared DPPH in methanol (20 $mg \cdot l^{-1}$) were put into a cuvette at room temperature. After 30 min of incubation at room temperature, the absorbance was read against a blank at 515 nm. The measurements were performed in triplicate. The total antioxidant capacity was calculated as miiligrams of Trolox equivalent (TE) per kilogram of fresh fruit using the equation based on the calibration curve (R^2 = 0.996).

The plant extract (1 ml), 1.8 ml of freshly prepared DPPH in methanol and 1 ml methanol were placed in a cuvette at room temperature. After 30 min of reaction, the absorbance was read against a blank at 517 nm. All measurements were performed in triplicate. The ability of the extracts to inhibit DPPH, i.e. percentage of *RSC*, was calculated from the decrease in absorbance as in Eq. 3.

$$RSC = \left(1 - \frac{A_{sample}}{A_{blank}}\right) \times 10 \quad (3)$$

where A_{blank} is the absorbance of control (1×10^{-4} $mol \cdot dm^{-3}$ DPPH methanolic solution) and A_{sample} is the absorbance of the test sample.

Determination of mineral elements

The standard procedure described by Association of Official Analytical Chemists was followed for the preparation of the samples for the analysis of heavy metals [23]. Accurately weighed (2 g) sample was transferred into a silica crucible and kept in a muffle furnace for ashing at 450 °C for 3 h and then 5 ml of 6 $mol \cdot l^{-1}$ HCl was added to the crucible. Care was taken to ensure that all the ash came into contact with acid. Further, the crucible containing acid solution was kept on a hot plate and digested to obtain a clear solution. The final residue was dissolved in 0.1 $mol \cdot l^{-1}$ HNO_3 solution and made up to 50 ml. Working standard solutions were prepared by diluting the stock solution with 0.1 $mol \cdot l^{-1}$ nitric acid for checking the linearity.

Extraction coefficient (*EC*) is defined by Eq. 4:

$$EC = \frac{M_{extract}}{M_{fruit}} \times 100 \quad (4)$$

where $M_{extract}$ is content of the metal in the extract and M_{fruit} is content of the metal in the fruit.

Antimicrobial activity

The in vitro antimicrobial activity of water, methanol–water and methanolic extracts of *Morus alba* L. was tested against a panel of laboratory control strains from the American Type Culture Collection (ATCC; Gaithersburg, Maryland, USA) except one, belonging to National Collection of Type Cultures (NCTC, Public Health England, London, United Kingdom). Antibacterial ac-

tivity was evaluated against two Gram-positive and three Gram-negative bacteria. The Gram-positive bacteria used were *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 6538. The Gram-negative bacteria were *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027 and *Salmonella typhimurium* NCTC 6017. A disc-diffusion method was employed for the determination of the antimicrobial activity of the extracts, according to National Committee on Clinical Laboratory Standards (NCCLS) [24]. The inocula of the bacterial and fungal strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. A volume of 100 μl of the 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, Belgrade, Serbia) and Sabouraud dextrose agar (SDA, Torlak) respectively, in sterilized Petri dishes (90 mm in diameter). The discs (6 mm in diameter and 9 mm in diameter; Macherey-Nagel, Düren, Germany) were impregnated with 20 μl and 50 μl of the extracts (concentration 30 mg·ml^{-1}) and placed on the inoculated agar. Negative controls were prepared using the solvent (dimethyl sulfoxide). Tetracycline (30 μg, Torlak) was used as a positive reference standard 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. Antimicrobial activity was evaluated by measuring the zone of inhibition. Each assay was done in triplicate.

Statistical analysis

The experimental results were expressed as mean value ± standard error of mean value of three replicates. In order to estimate statistically any significant differences among mean values, where 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 (SAS 9) software (SAS Institute, Cary, North Carolina, USA).

RESULTS AND DISCUSSION

Total phenols, flavonoids and monomeric anthocyanins contents, as well as antioxidant activity of *Morus alba* L. fruit extracts are given in Tab. 1. The results show that the content of total phenols in the investigated extracts of fresh fruit of *Morus alba* L. ranged from 629.7 mg·kg^{-1} to 4325.0 mg·kg^{-1} (expressed as GAE equivalent). Ethanolic extract displayed the highest content of phenol compounds. Total flavonoids (expressed as CE equivalent) were in the range from 290.0 mg·kg^{-1} (methanol–water extract) to 1378.6 mg·kg^{-1} (methanolic extract), while no anthocyanin was not found to be contained in these extracts. All the extracts exhibited a good scavenging activity against DPPH radicals (44.5% to 87.2%). Graphically, total phenols, flavonoids and monomeric anthocyanins contents, and antioxidant activity are given in Fig. 1.

The contents of heavy elements iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), nickel (Ni), lead (Pb) and cadmium (Cd) in white mulberry fruit is given in Tab. 2. Presence of all metals was confirmed except for cadmium (Fig. 2). The highest content of metal in the mulberry fruit and extracts was that of iron. It ranged from 0.231 g·kg^{-1} in the methanolic extract of white mul-

Tab. 1. Total phenols, flavonoids and monomeric anthocyanins contents, and antioxidant activity of white mulberry extracts.

Solvent	Total phenols [mg·kg^{-1}]	Flavonoids [mg·kg^{-1}]	Monomeric anthocyanins	Antioxidant activity [mg·kg^{-1}]	*RSC* [%]
Water	1461.1 ± 0.21	527.3 ± 2.45	not found	1045.0 ± 0.33	44.5 ± 4.2
Ethanol–water	1175.3 ± 2.17	599.7 ± 0.01	not found	1492.1 ± 7.10	65.3 ± 3.0
Ethanol	4325.0 ± 9.12	1035.0 ± 0.15	not found	1671.0 ± 3.33	72.9 ± 7.1
Methanol–water	708.7 ± 4.56	290.0 ± 0.02	not found	339.3 ± 1.40	76.7 ± 0.4
Methanol	1091.0 ± 3.30	1378.6 ± 6.15	not found	541.1 ± 2.03	87.2 ± 6.3
Acetone–water	629.7 ± 1.00	875.2 ± 2.20	not found	596.0 ± 1.14	88.1 ± 4.2
Acetone	1189.0 ± 1.12	1008.0 ± 1.14	not found	655.0 ± 9.12	83.0 ± 0.0

Total phenols are expressed as milligrams of gallic acid equivalents per kilogram of fresh fruit. Flavonoids are expressed as milligrams of catechin equivalents per kilogram of fresh fruit. Antioxidant activity is expressed as milligrams of Trolox equivalents per kilogram of fresh fruit.
RSC – radical scavenging activity.

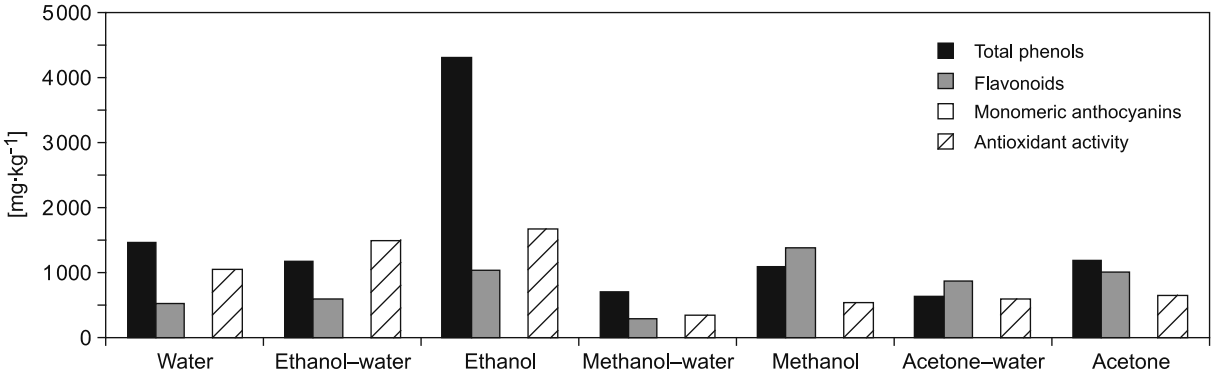

Fig. 1. Total phenols, flavonoids, monomeric anthocyanins contents, and antioxidant activity of white mulberry extracts.

Total phenols are expressed as milligrams of GAE per kilogram of fresh fruit. Flavonoids are expressed as milligrams of CE per kilogram of fresh fruit. Antioxidant activity is expressed as milligrams of TE per kilogram of fresh fruit.

berry to 23.06 g·kg^{-1} in the fruit of white mulberry. Copper content ranged from 0.038 g·kg^{-1} in methanol–water and ethanol–water to 0.86 g·kg^{-1} in the fruit. The content of manganese was the highest in acetone extract (0.596 g·kg^{-1}) and the lowest was in methanol–water extract (0.151 g·kg^{-1}). Nickel content ranged from 0.035 g·kg^{-1} in acetone extract to 0.36 g·kg^{-1} in the fruit. The content of zinc was the highest in the fruit (2.234 g·kg^{-1}) and the lowest in ethanol–water extract (0.59 g·kg^{-1}). TRICHOPOULOS [25] reported that Pb has a toxic effect for human metabolism even in low amounts and may have carcinogenic effects. Lead was found only in the fruit in a content of 0.09 g·kg^{-1}. The extracts of

Tab. 2. Content of heavy metals in white mulberry and different extracts.

Solvent	Fe [g·kg^{-1}]	Cu [g·kg^{-1}]	Mn [g·kg^{-1}]	Cd [g·kg^{-1}]	Ni [g·kg^{-1}]	Zn [g·kg^{-1}]	Pb [g·kg^{-1}]
Fruit	2.31 ± 1.02	0.86 ± 3.11	2.33 ± 0.18	nd	0.36 ± 0.01	2.23 ± 0.17	0.09 ± 0.01
Water	0.32 ± 0.01	0.08 ± 0.02	0.28 ± 0.02	nd	0.10 ± 0.01	0.73 ± 0.05	nd
Ethanol–water	0.15 ± 0.16	0.04 ± 0.02	0.27 ± 0.01	nd	0.05 ± 0.01	0.59 ± 0.04	nd
Ethanol	0.71 ± 0.14	0.08 ± 1.36	0.44 ± 0.03	nd	0.04 ± 0.01	0.86 ± 0.07	nd
Methanol–water	1.44 ± 0.04	0.08 ± 0.01	0.42 ± 0.04	nd	0.05 ± 0.01	1.05 ± 0.08	nd
Methanol	5.38 ± 4.11	0.26 ± 0.19	0.59 ± 0.04	nd	0.03 ± 0.01	1.63 ± 0.23	nd
Acetone–water	0.35 ± 0.30	0.04 ± 0.54	0.15 ± 0.01	nd	0.14 ± 0.02	0.91 ± 0.07	nd
Acetone	0.23 ± 0.18	0.11 ± 0.03	0.46 ± 0.01	nd	0.10 ± 0.01	1.12 ± 0.08	nd

nd – not detected

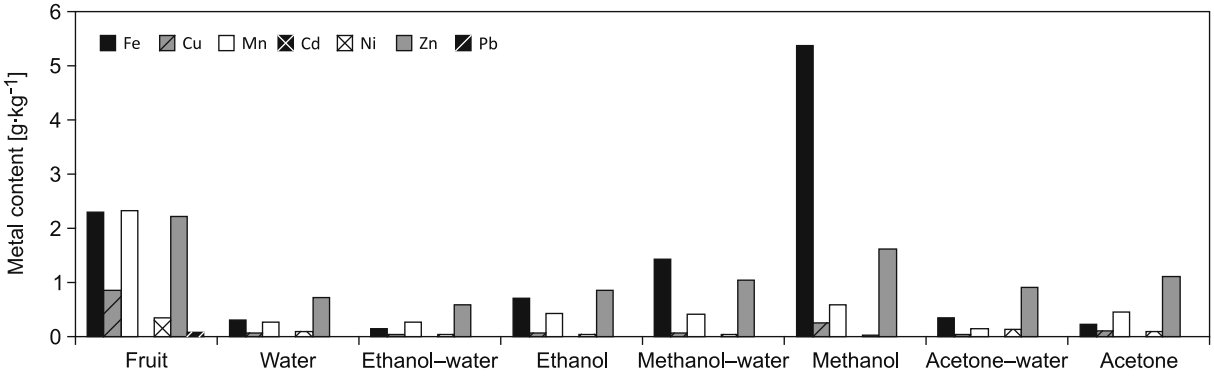

Fig. 2. Content of heavy metals in white mulberry and different extracts.

fruits did not contain lead. Cadmium was found neither in the fruit nor in the extracts.

The extraction coefficients *EC* obtained in this study varied, from 0% to 25.6%, for all test solutions except for Zn. Zinc had much higher coefficients of extraction. The acetone and acetone–water extracts had the highest coefficients of extraction. Due to the very low coefficients of extraction and no toxicity of ethanolic and ethanol–water extracts, these are the most suitable for use in human nutrition and medicinal treatment (Tab. 3).

Tab. 4 presents data on antimicrobial activity of the extracts of white mulberry fresh fruit. The water extract was active against the bacteria *Salmonella typhimurium* and *Staphylococcus aureus.* The methanol–water extract was active only against the bacteria *S. typhimurium.* The methanolic extract showed the highest antioxidant activity and was active against the bacteria *S. typhimurium*, *Staph. aureus*, *Bacillus subtilis* and *Escherichia coli.*

Tab. 5 presents data on phenolic compound in fruits from different countries. Extracts of fresh white mulberry fruit from southeastern Serbia contained high levels of total phenols and flavonoids, more than 1000 mg·kg^{-1} fresh fruit, while the extracts did not contain anthocyanins. The highest content of phenols was in the ethanolic extract. The highest content of flavonoids was in the methanolic extract. All investigated extracts showed high antioxidant activity. Highest activity against DPPH showed the methanolic extract. Considering that all extracts had high contents of phenolic compounds and high antioxidant activities, they can be used as potential antioxidant agents. Many studies showed that the physiological functions of natural ingredients can be attributed to the antioxidant activity of phenolic compounds [25, 31–33].

Ercisli et al. found a similar content of total phenols (expressed as GAE equivalent) in the methanolic extract of *Morus alba* L. (1810 mg·kg^{-1} fresh fruit) [26]. Ethanolic extract of *Morus alba* L. from Korea contained 2570 mg·kg^{-1} fresh fruit of total phenols [27]. *Morus alba* L. from Taiwan had a higher content of total phenols, 15150 mg·kg^{-1} fresh fruit in water extract [30]. In previous studies [27], content of flavonoids in ethanolic extract (expressed as CE equivalent) ranged from 5.6 mg·kg^{-1} to 65.4 mg·kg^{-1} fresh fruit, which was quite different from our findings. Differences in the contents of total phenolic compounds in fresh fruit extracts of white mulberry from southeastern Serbia and from other countries may be a consequence of different growing conditions, the climate in the first place, and then depend on the extraction solvent, extraction methods and extraction time.

Our results regarding the heavy metals content in the mulberries from the region of Southeast Serbia could be compared with the results of other authors in Tab. 6.

Based on the results, the analysed elements can be classified into three groups: elements with a low extraction coefficient (lower than 10%); elements with a medium extraction coefficient

Tab. 3. Extraction coefficients of heavy metals from white mulberry by different solvents.

Solvent	*EC* [%]				
	Fe	Cu	Mn	Ni	Zn
Water	1.4	1.0	12.2	–	32.7
Ethanol–water	–	0.5	11.6	–	26.8
Ethanol	3.1	1.0	18.9	12.1	38.6
Methanol–water	6.2	1.0	18.3	15.6	47.0
Methanol	23.3	3.0	25.6	9.7	73.3
Acetone–water	–	0.4	6.5	–	41.1
Acetone	1.0	1.3	20.0	3.8	50.3

Tab. 4. Diameters of growth inhibition zones caused by the action of different crude extracts.

Microorganism	*Morus alba* L. extract						Dimethyl sulfoxide		Tetracycline
	Water		Methanol–water		Methanol				
	20 µl	50 µl	20 µl	50 µl	20 µl	50 µl	20 µl	50 µl	30 µg
Bacillus subtilis	na	na	na	na	11 mm	16 mm	na	na	36 mm
Staphylococcus aureus	na	16 mm	na	na	12 mm	17 mm	na	na	34 mm
Escherichia coli	na	na	na	na	na	14 mm	na	na	30 mm
Pseudomonas aeruginosa	na	na	na	na	na	na	na	na	19 mm
Salmonella typhimurium	na	15 mm	na	16 mm	12 mm	17 mm	na	na	28 mm

Diameter of growth inhibition zone is expressed including the diameter of the disc.
na – not active.

Tab. 5. Total phenols, flavonoids and anthocyanins contents, and antioxidant activity of extracts of white mulberry fruit from different countries.

Country	Part of plant	Solvent	Total phenols [mg·kg^{-1}]	Total flavonoids [mg·kg^{-1}]	Monomeric anthocyanins [mg·kg^{-1}]	Antioxidant activity	Reference
Turkey	Fruit	Methanol	1810[a]	290[e]	nd	nd	[26]
Korea	Fruit	Ethanol	960–2570[a]	5.6–65.4[d]	137–2057	90%[g]	[27]
Pakistan	Fruit	Methanol	16500[b]	nd	nd	95%[g]	[28]
Pakistan	Fruit	Methanol	7750[a]	nd	nd	228.5 μmol·kg^{-1}[h]	[28]
Serbia	Fruit	Ethanol–water	4130[c]	890[f]	nd	nd	[29]
Taiwan	Fruit	Water	15150[a]	2501[e]	nd	nd	[30]

Expression of total phenols: a – as gallic acid equivalents per kilogram of fresh fruit, b – as tannin acid equivalents, c – as chlorogenic acid equivalents per kilogram of dry extract.
Expression of total flavonoids: d – as catechin equivalents, e – as quercetin equivalents, f – as rutin equivalents per kilogram of dry extract.
Total anthocyanins are expressed as malvidin-3-glucoside equivalents.
Expression of antioxidant activity: g – as percentage of DPPH inhibition, h – as micromoles of quercetin equivalent.
nd – not determined.

(10–30%), and elements with a high extraction coefficient (higher than 30%). The extraction coefficient depends mostly on the extraction medium. The lowest transfer of heavy metals was in the methanol–water and ethanol–water extracts for Cu (0.5%), and the highest in the acetone extract for Zn (50.3%). The extraction coefficient also depends on the solvent and the properties of the individual metal.

Tab. 6. Contents of macro- and micro-elements in white mulberry fruit from different countries.

Element [g·kg^{-1}]	Turkey	Pakistan	Turkey
N	0.75	nd	nd
P	24.7	nd	nd
K	166.8	173.1	nd
Ca	15.2	57.4	nd
Mg	10.6	2.4	nd
Na	6.0	28.0	nd
Fe	0.42	7.3	nd
Cu	0.05	nd	0.03
Mn	0.38	nd	1.07
Ni	nd	0.22	0.01
Zn	0.28	5.02	0.08
Co	nd	nd	nf
Cr	nd	nd	nf
Cd	nd	nd	nf
Pb	nd	nd	nf
References	[26]	[34]	[35]

nd – not determined, nf – not found.

The methanol extract showed the highest antimicrobial activity. Results of Omidiran et al. showed that the ethanolic extract had antimicrobial activity against a wider range of microorganisms when compared to the standard antibiotics [36].

This study supports the folklore use of plants (herbal extracts) in traditional medicines to cure various diseases caused by microorganisms, like diarrhea and other intestinal tract diseases, throat and ear infections, fever and skin diseases. The study provides data supporting the potential use of white mulberry extracts as food supplements.

CONCLUSION

The high phenolic content and high antioxidant activity of white mulberry from southeastern Serbia underline the nutritive and phytomedicinal potentials of the fruit. From among heavy metals (iron, copper, zinc, manganese, nickel and lead), white mulberry fruit contained the highest amount of iron and the lowest amount of lead. Cadmium was not found. Analysis of mulberries extracts showed a significant transfer of heavy metals during the extraction procedure. It was established that such coefficients mostly depend on the solvent nature and the type of metal. Metal content in the white mulberry and tested extracts was in acceptable limits. Some of tested extracts showed antimicrobial activity, especially the methanolic extract. This study suggested the use of mulberry as a potential healthy food, or an important antioxidant carrier for application in food and pharmaceutical industries.

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