

## Phenolic Composition, and Antioxidant and Antineurodegenerative Potential of Methanolic Extracts of Fruit Peel and Flesh of Pear Varieties from Serbia

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Pear (*Pyrus communis* L.) is consumed as fresh fruit, in numerous food products, and also used as a traditional remedy in various countries, including Serbia. In search of bioactive compounds, six traditional pear varieties (‘Vidovača’, ‘Lubeničarka’, ‘Karamanka’, ‘Jeribasma’, ‘Lončara’, ‘Takiša’) and wild pear from Serbia were investigated and compared with a commercial variety (‘Williams Bartlett’). The aim of this study was to determine the total phenolic and flavonoid contents, phenolic composition, antioxidant capacity, and antineurodegenerative activities of methanolic extracts of peel, flesh, and mixed peel and flesh of pear fruits. Phenolic composition of extracts was determined with HPLC-DAD, while the antioxidant activity of extracts was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), and ferric-reducing antioxidant power (FRAP) assays. Bearing in mind that oxidative stress is closely linked to neurodegeneration, the antineurodegenerative potential of the extracts was assessed by the inhibition of acetylcholinesterase (AChE) and tyrosinase (TYR) activities. The extracts of traditional varieties, particularly peel extracts, had a high content of phenolics, as well as significant antioxidant and moderate antineurodegenerative potential, compared to the commercial variety. The highest contents of total flavonoids and individual compounds, such as arbutin and chlorogenic acid, as well as the strongest antioxidant and TYR inhibitory activities were reported for the ‘Takiša’ peel extract. The performed analyses have revealed that fruits of traditional Serbian pear varieties are rich in bioactive components and could be used as functional food and for possible nutraceutical applications, to prevent diseases induced by oxidative stress.

### ABBREVIATIONS

TPC, total phenolic content; TFC, total flavonoid content; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2’-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP, ferric-reducing antioxidant power; BHA, 2(3)-tert-butyl-4-hydroxyanisole; BHT, 3,5-di-tert-butyl-4-hydroxytoluene; TPTZ, 2,4,6-tripyridyl-s-triazine; L-DOPA, 3,4-dihydroxy-L-phenylalanine; AAE, ascorbic acid equivalents; DTNB, 5,5’-dithiobis(2-nitrobenzoic acid); AChE, acetylcholinesterase; TYR, tyrosinase; HPLC-DAD, high-performance liquid chromatography with diode array detection.

### INTRODUCTION

Pear (*Pyrus communis* L.), belonging to the Rosaceae family, is one of the most widespread fruits in the world. It is the fifth most produced fruit worldwide, but, despite over 3000 pear cultivars, there are only a few dozen globally produced pear varieties [Brahem *et al.*, 2017]. Because of their desirable taste and good digestibility, pears are widely consumed as fresh or dry fruits, juices, and numerous products.

The pear has been used as a traditional remedy in China for more than 2000 years [Li *et al.*, 2012, 2014; Reiland & Slavin, 2015], and also in Ancient Greece [Parle & Arzoo, 2016]. Ethnobotanical surveys of the traditional use of *P. communis* L. fruit have shown similar usability of pear for consumption and medicinal purposes (against hypertension, diabetes, high cholesterol, constipation, as uroseptic,

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antirheumatic, for body mass reduction) also in the Balkan region [Dajić-Stevanović *et al.*, 2014; Savić *et al.*, 2019]. Several previous studies investigated fruits, their nutritional components, and bioactivities of flesh and peel of different pear varieties grown worldwide [Kolniak-Ostek, 2016; Kolniak-Ostek *et al.*, 2020; Li *et al.*, 2014, 2016b; Liaudanskas *et al.*, 2017; Öztürk *et al.*, 2015; Reiland & Slavin 2015; Salta *et al.*, 2010; Sharma *et al.*, 2015]. Additionally, several investigations proved that, besides fruit, each part of the pear tree (leaves, bark, root) possesses phenolics with notable antioxidant activities [Carbonaro *et al.*, 2002; Imeh & Khokhar, 2002], providing numerous health benefits [Kolniak-Ostek, 2016; Öztürk *et al.*, 2015; Parle & Arzoo 2016].

The health-promoting, nutritional, and sensorial quality of the pear fruit is influenced by the pear species and variety from which it originates. Chemical composition analyses of pear fruits have demonstrated that they represent essential sources of sugars, vitamins, amino acids, minerals, phenolics, organic acids, and other chemical constituents important for human nutrition and health boosting [Li *et al.*, 2012, 2014; Salta *et al.*, 2010]. Phenolics in the pear fruit contribute to its sensory quality and are strongly linked to many health benefits and antioxidant activity [Brahem *et al.*, 2017]. Phenolic compounds are more concentrated in the peel of the fruit, compared to flesh [Brahem *et al.*, 2017; Li *et al.*, 2014; Kolniak-Ostek, 2016].

The studies of chemical composition of pear fruit revealed the presence of phenolics, such as arbutin, (–)-epicatechin, quercitrin, isoquercitrin, kaempferol, astragalol, tannins, as well as triterpenoids including friedelin, squalene, sterols, and saponins [Kaur & Arya, 2012; Kolniak-Ostek, 2016; Liaudanskas *et al.*, 2017; Sharma *et al.*, 2015]. Phenolic acids (chlorogenic, syringic, ferulic, and coumaric acids) [Lin & Harnly, 2008; Salta *et al.*, 2010; Sharma *et al.*, 2015], organic acids (malic, citric, and shikimic acids), as well as vitamins C and E were also detected in pear fruits [Kolniak-Ostek, 2016; Öztürk *et al.*, 2015].

Methanol is commonly used to extract phenolics from fruit parts [Chel-Guerrero *et al.*, 2018]. The chemical analyses performed by Li *et al.* [2012] and Öztürk *et al.* [2015] revealed that methanolic extracts of pear fruit contained phenolic compounds, such as chlorogenic acid, arbutin, (+)-catechin, and (–)-epicatechin, followed by quercetin and rutin, predominantly in the peel. In addition to a high content of the phenolics, the peel is an excellent source of triterpenoids [Kolniak-Ostek, 2016; Li *et al.*, 2014].

The phenolic compounds are well known for their antioxidant activity. The antiviral activity of specific flavonoid groups, such as flavanones, flavonols, and catechins, has also been reported against various viral strains, as well as SARS-CoV and MERS-CoV, which suggests that functional food has a huge potential to strengthen the immune system and provide treatment for the ongoing epidemic of COVID-19 [Adem *et al.*, 2020]. Besides, phenolic compounds of various fruits can play a beneficial therapeutic role in the treatment of oxidative stress-induced neurodegenerative disorders, such as Alzheimer's disease and Parkinson's disease [Wilson *et al.*, 2017].

The aim of the present study was to evaluate and compare the total phenolic and flavonoid contents, the phenolic composition, as well as the antioxidant and antineurodegenerative

activities of methanolic extracts of peel, flesh, and mixed peel and flesh of six traditional and rare pear varieties from Serbia ('Vidovača', 'Lubeničarka', 'Karamanka', 'Jeribasma', 'Lončara', and 'Takiša'), and wild pear, and comparing them to one commercial variety ('Williams Bartlett'). Additionally, the correlations were established between the content of phenolics and flavonoids and bioactivities tested. The mentioned pear varieties have never been studied before for chemical composition and bioactivities, hence they were subjected to detailed analyses.

## MATERIALS AND METHODS

### Chemicals

Ascorbic acid, gallic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH<sup>•</sup>), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2(3)-tert-butyl-4-hydroxyanisole (BHA), 3,5-di-tert-butyl-4-hydroxytoluene (BHT), 2,4,6-tripyridyl-s-triazine (TPTZ), potassium acetate (C<sub>2</sub>H<sub>3</sub>KO<sub>2</sub>), potassium-persulfate (K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>), dimethyl sulfoxide, aluminum nitrate nonahydrate (Al(NO<sub>3</sub>)<sub>3</sub>×9H<sub>2</sub>O), Folin-Ciocalteu reagent, sodium acetate (CH<sub>3</sub>COONa), sodium carbonate anhydrous (Na<sub>2</sub>CO<sub>3</sub>), iron(III) chloride (FeCl<sub>3</sub>), iron(II) sulfate heptahydrate (FeSO<sub>4</sub>×7 H<sub>2</sub>O), sodium phosphate monobasic (NaH<sub>2</sub>PO<sub>4</sub>), sodium phosphate dibasic (Na<sub>2</sub>HPO<sub>4</sub>), 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholine iodide, acetylcholinesterase from *Electrophorus electricus* (AChE), galanthamine hydrobromide from *Lycoris* sp., tyrosinase from mushroom, kojic acid, 3,4-dihydroxy-L-phenylalanine (L-DOPA), rutin hydrate (94%, HPLC), chlorogenic acid (95%, titration), hyperoside (97%, HPLC), arbutin (96%, HPLC), isoquercitrin (90%, HPLC), procyanidin B<sub>1</sub> (≥90%, HPLC) and procyanidin B<sub>2</sub> (>90%, HPLC), formic acid (98–100%), and acetonitrile (99.8%) were purchased from Sigma Aldrich (St. Louis, MO, USA). Other used chemicals, such as ethanol, methanol, glacial acetic acid, and hydrochloric acid, were purchased from Zorka Pharma, Šabac (Serbia). Quercitrin (98.5%, HPLC) was purchased from Extrasynthese (Genay, France). Water was deionized using a MilliQ system (Millipore, Bedford, MA, USA).

### Plant material

Fruits of eight different pear varieties were analyzed in this study (Figure 1). Among them, six are traditional varieties from organic production: 'Vidovača', 'Lubeničarka', 'Karamanka', 'Jeribasma', 'Takiša', 'Lončara' (from the hoods in central Serbia – Šumadija region), wild pear was collected from southwest Serbia, while fruits of commercial variety 'Williams Bartlett' were purchased at a local market. Fruits were sampled in 2016, at their optimal ripening stage recommended for consumption (from July till October). The collected material was stored at -20°C until the extracts preparation.

The fruit sizes varied between varieties: from approximately 20–25 g (wild pear and 'Takiša'); 30–50 g ('Vidovača' and 'Lubeničarka'); 120–220 g ('Williams Bartlett', 'Karamanka', 'Jeribasma') to 220–280 g ('Lončara'). In the case of smaller fruits (wild pear, 'Takiša', 'Vidovača' and 'Lubeničarka'), 4–10 fruits were used for the extraction; and in the case of the larger ones ('Williams Bartlett', 'Karamanka', 'Jeribasma', 'Lončara'), 1–2 fruits were used.

### Preparation of extracts

Before extracts preparation, the frozen fruits were peeled (skin thickness approx. 0.5 mm) for the peel sample, and mashed in the jar. The mixed peel and flesh, and pure flesh samples were cut into cubes (1 cm<sup>3</sup>) and separately homogenized using a laboratory blender (No. 8010ES, Waring® Laboratory Science, Torrington, CT, USA). The pear extracts were prepared using methanol as followed: 10 g of peel were extracted with 10 mL of methanol; 30 g of flesh were extracted with 30 mL of methanol, and 30 g of peel and flesh were extracted with 30 mL of methanol. The extractions of the material were performed three times, every 24 h for 72 h at room temperature. The obtained liquid extracts were mixed and filtered (Whatman No.1) and subsequently concentrated under reduced pressure (rotavapor R-114, BÜCHI Labortechnik AG, Flawil, Switzerland). Yields of crude extracts were calculated and dry extracts were subsequently stored at +4°C. Stock solutions were prepared in methanol in a concentration of 1 mg/mL freshly before experiments.

### Determination of total phenolic content

The total phenolic content of pear extracts was measured spectrophotometrically [Singleton & Rossi, 1965] with slight modification. The reaction mixture containing 0.2 mL of the extract in concentration of 1 mg/mL, 1.0 mL of 10% Folin-Ciocalteu reagent, and 0.8 mL of 7.5% Na<sub>2</sub>CO<sub>3</sub> were incubated for 2 h, at room temperature in the dark. Distilled water was used as a blank, while control was prepared to contain the distilled water instead of the sample. Absorbances were recorded using a JENWAY 6305UV/Vis spectrophotometer (Jenway® Equipment for Analysis, Staffordshire, UK) at 740 nm. The phenolic content in extracts was determined using gallic acid as a standard and presented as gallic acid equivalents per gram of dry extract (mg GAE/g dry extract).

### Determination of total flavonoid content

Flavonoid contents of the samples were measured spectrophotometrically [Park *et al.*, 1997] with slight modification. The reaction mixture contained 1.0 mL of the pear extract (in the concentration of 1 mg/mL), 0.1 mL of 10% Al(NO<sub>3</sub>)<sub>3</sub>·9 H<sub>2</sub>O, 0.1 mL of 1 M CH<sub>3</sub>COOK, and 4.1 mL of 80% ethanol. Control was prepared using 96% ethanol instead of the extract, while 96% ethanol solution was used as blank. Absorbances were measured at 415 nm (JENWAY 6305UV/Vis spectrophotometer) after incubation for 40 min at room temperature. Sample flavonoid concentrations (mg/mL) were calculated using the standard curve equation, and expressed as quercetin equivalents per gram of dry extract (mg QE/g dry extract) from three measurements.

### HPLC analysis

Phenolic compounds in the extracts were determined by comparing the retention times and absorption spectra (200–400 nm) of unknown peaks with those of pure standards (arbutin, rutin, chlorogenic acid, isoquercitrin, quercitrin, hyperosid, procyanidin B<sub>1</sub>, procyanidin B<sub>2</sub>) injected under the same conditions. The HPLC-DAD analysis was performed on an Agilent 1200 Series HPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Lichrospher® 100 RP 18e column (5 μm, 250×4 mm, Agilent Technologies, Palo Alto, CA, USA). Mobile phase A was formic acid in water (1%, v/v) and mobile phase B was acetonitrile. The concentration of injected extracts varied from 1.5 to 55 mg/mL. The injection volume was 30 μL, and flow rate was 1 mL/min with the gradient program as follows: 5–15% B 0–5 min, 15–20% B 5–8 min, 20% B 8–12 min, 20–30% B 12–15 min, 30% B 15–17 min, 30–35% B 17–20 min, 35% B 20–22 min, 35–100% B 22–25 min. Stop time of the analysis was 25 min. The column temperature was kept at 25°C during

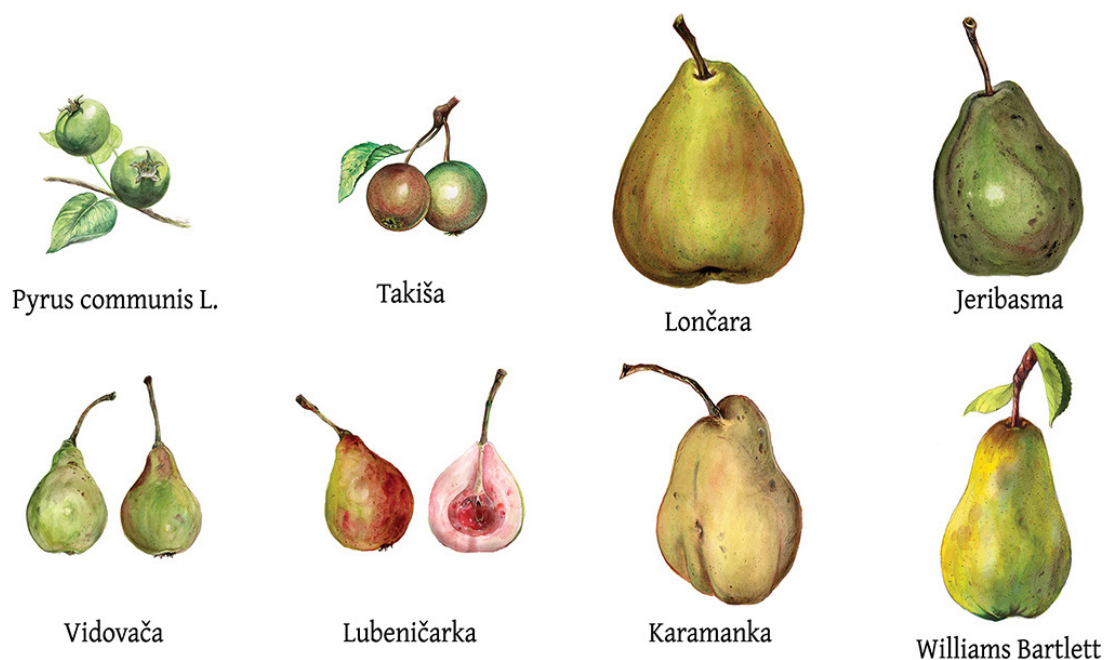


FIGURE 1. Traditional pear varieties ('Vidovača', 'Lubeničarka', 'Karamanka', 'Jeribasma', 'Takiša', 'Lončara'), commercial variety ('Williams Bartlett') and wild pear.

separation. The contents of individual phenolic compounds were expressed as  $\mu\text{g/g}$  of dry extract. The samples were analyzed in triplicate [Šavikin *et al.*, 2014].

### Evaluation of antioxidant activity

#### DPPH assay

A slightly modified DPPH assay was employed [Blois, 1958] in order to evaluate free radical scavenging activity of the pear extracts. The reaction mixture (2000  $\mu\text{L}$ ) containing the pear extract and methanolic solution of DPPH $\cdot$  (40  $\mu\text{g/mL}$ ) was incubated in the dark at room temperature for 30 min. Ascorbic acid, BHA, and BHT were used as positive controls (tested at the concentrations from 1–20  $\mu\text{g/mL}$ ). The absorbances were measured using JENWAY 6305UV/Vis spectrophotometer at 517 nm. Methanol was used as a blank, while control was prepared to contain methanol instead of the pear extract/standard. DPPH $\cdot$  scavenging activity was determined using the following formula:

$$\text{DPPH}\cdot \text{ scavenging activity (\%)} = [(A_c - A_s)/A_c] \times 100\% \quad (1)$$

where:  $A_c$  is absorbance of control and  $A_s$  is absorbance of the reaction mixture. The results were presented as extract concentration providing 50% inhibition of DPPH $\cdot$  ( $\text{IC}_{50}$  value,  $\text{mg/mL}$ ).

#### ABTS assay

ABTS assay [Miller *et al.*, 1993] with slight modifications was also employed to evaluate radical scavenging activity of the pear extracts. ABTS $^{2+}$  stock solution (7 mM) in 2.46 mM potassium-persulfate was prepared 12 h before experiment and stored at room temperature in the dark, and then subsequently diluted by distilled water to obtain a working solution which absorbance at 734 nm was  $0.700 \pm 0.020$ . The reaction mixture consisted of 50  $\mu\text{L}$  of extract (1  $\text{mg/mL}$ ) or methanolic solutions of BHA and BHT standards (0.1  $\text{mg/mL}$ ) and 2000  $\mu\text{L}$  of a working ABTS $^{2+}$  solution. Distilled water was used as a blank, while control contained distilled water instead of the sample. After incubation (30 min at 30°C), absorbances were measured using the JENWAY 6305UV/Vis spectrophotometer at 734 nm. ABTS $^{2+}$  scavenging activity of the extracts was determined using ascorbic acid and presented as ascorbic acid equivalents (AAE) per gram of dry extract ( $\text{mg AAE/g dry extract}$ ).

#### Ferric-reducing antioxidant power (FRAP) assay

In order to prepare FRAP reagent, sodium acetate buffer (300 mM, pH 3.6), TPTZ (10 mM) in HCl (40 mM) and  $\text{FeCl}_3 \cdot 6 \text{H}_2\text{O}$  (20 mM) were mixed in the proportion of 10:1:1 (v/v/v) and subsequently heated to 37°C before usage as previously described [Benzie & Strain, 1996]. Pear extracts (100  $\mu\text{L}$  in concentration of 1  $\text{mg/mL}$ ) or positive controls (ascorbic acid, BHA, and BHT) in the concentration of 0.1  $\text{mg/mL}$ , were added to 3000  $\mu\text{L}$  of the FRAP reagent. After 4-min incubation at room temperature, absorbance of the reaction mixture was measured at 593 nm using the JENWAY 6305UV/Vis spectrophotometer. Distilled water was used as a blank, while control was prepared to contain

distilled water instead of the sample. FRAP values were calculated using  $\text{FeSO}_4 \cdot 7 \text{H}_2\text{O}$  standard curve and expressed as  $\mu\text{mol Fe (II)/g dry extract}$ .

### Antineurodegenerative activity

In the present study, AChE- and TYR-inhibitory effects of methanolic extracts of eight pear varieties were tested at concentrations of 25  $\mu\text{g/mL}$ , 50  $\mu\text{g/mL}$ , and 100  $\mu\text{g/mL}$ .

#### Inhibition of acetylcholinesterase activity

Acetylcholinesterase (AChE) inhibition by the tested pear extracts was determined spectrophotometrically using 96-well plates [Ellman *et al.*, 1961]. The reaction mixture (S) was prepared to contain 140  $\mu\text{L}$  of a sodium phosphate buffer (0.1 M, pH 7.0), 20  $\mu\text{L}$  of DTNB, 20  $\mu\text{L}$  of a sample-buffer solution, and 20  $\mu\text{L}$  of an AChE solution (5 units/mL). The commercial AChE inhibitor, galanthamine, was used as a positive control. Control (C) contained sodium buffer instead of the test sample, while blank (B) did not contain AChE. The acetylthiocholine iodide solution (10  $\mu\text{L}$ ) was added after incubation (25°C, 15 min) to initiate the reaction. Absorbances were measured at 412 nm using a Tecan Sunrise SN microplate reader equipped with XFluor4 software (Tecan Group Ltd., Männedorf, Switzerland). The recorded absorbances were used to calculate the percentage of inhibition of AChE according to the formula:

$$\text{AChE inhibition (\%)} = [C - (S - B)/C] \times 100 \quad (2)$$

#### Inhibition of tyrosinase activity

Inhibition of tyrosinase activity by the pear extracts was assessed spectrophotometrically using 96-well microtiter plates [Masuda *et al.*, 2005]. The wells were designed as: A (containing 120  $\mu\text{L}$  of 0.1 M sodium phosphate buffer, (pH 7) and 40  $\mu\text{L}$  of tyrosinase (46 U/L)), B (containing only phosphate buffer), C (containing 80  $\mu\text{L}$  of sodium phosphate buffer, 40  $\mu\text{L}$  of tyrosinase, and 40  $\mu\text{L}$  of the pear extract), and D (containing 120  $\mu\text{L}$  of phosphate buffer and 40  $\mu\text{L}$  of the pear extract). Subsequently, 40  $\mu\text{L}$  of L-DOPA was added and plates were incubated (30 min, 25°C). Absorbances were measured at 475 nm using the Tecan Sunrise SN microplate reader equipped with XFluor4 software. Kojic acid was used as a positive control. The recorded absorbances were used to calculate the percentage of inhibition of TYR according to the formula:

$$\text{TYR inhibition (\%)} = [(A - B) - (C - D)/(A - B)] \times 100\% \quad (3)$$

### Statistical analysis

All measurements were performed in triplicate and presented as the mean  $\pm$  standard deviation. Analysis of variance (one-way ANOVA) and Tukey's post-hoc test were carried out to test the significance of differences among mean values using PAST (PAleontological STatistics) 3.21. [Hammer *et al.*, 2001]. Pearson's correlation coefficients were calculated between the content of phenolic components and values obtained from different bioactivity assays [Taylor, 1990].

## RESULTS AND DISCUSSION

### Extraction yield, and total phenolic and flavonoid contents of the pear extracts

Extraction yield of mixed flesh and peel extracts varied from 7.63 to 13.5%, that of flesh extracts from 7.40 to 15.7%, while that of peel extracts between 8.60 and 13.0% (Table 1).

Total phenolic content (TPC) and total flavonoid content (TFC) of the methanolic extracts of peel, flesh, and mixed flesh and peel samples were evaluated applying frequently used colorimetric methods (Folin-Ciocalteu and  $AlCl_3$ , respectively), which are described in literature in detail. The HPLC-DAD technique was subsequently employed for more precise quantitative-qualitative analysis of phenolic compounds.

The TPC and TFC of fruit extracts of eight pear varieties are presented in Table 1. The methanolic extracts obtained from peel, flesh, and mixed flesh and peel showed significantly ( $p < 0.05$ ) different TPC and TFC for all tested pear varieties. Generally, the results showed that the traditional varieties 'Lončara' and 'Takiša' had the highest total phenolic contents, while 'Takiša' had the largest amount of total flavonoids.

TPC for the peel samples ranged from 10.7 to 272 mg GAE/g dry extract for 'Lubeničarka' and 'Lončara' varieties, respectively. The TPC of flesh varied from 3.10 mg GAE/g dry extract ('Lubeničarka') to 198 mg GAE/g dry extract ('Lončara'). TPC of mixed flesh and peel extracts varied from 6.21 to 250 mg GAE/g dry extract with extreme values for the same varieties as for both peel and flesh. The highest values of TPC in flesh and peel separately, and mixed flesh and peel were determined in the samples of traditional 'Lončara' and 'Takiša' varieties, while the lowest values – in 'Lubeničarka' and 'Vidovača'. Comparing to the traditional varieties, TPC of wild pear and 'Williams Bartlett' displayed the intermediate values. A wide range of variations of TPC values was observed for the varieties analyzed in this study, as it was reported for other pear varieties of different origin [Kolniak-Ostek, 2016; Li *et al.*, 2014].

Lower TPC of European pear fruit extract of  $14.5 \mu\text{g}$  GAE/g was achieved in the study of Sharma *et al.* [2015]. Abaci *et al.* [2016] investigated fruit of ten Turkish pear varieties and concluded that peel (300.1 to 687.2 mg GAE/100 g) had 2–3 times higher content of phenolic compounds than flesh (112.6 to 230.5 mg GAE/100 g). Kolniak-Ostek [2016] found that European pear peel (917.6 mg GAE/100 g dry matter) had 4 times higher amount of phenolics than flesh (234.2 mg GAE/100 g dry matter). Li *et al.* [2014] presented results of TPC determination in ten Chinese pear varieties and showed it was higher in the peel (263.6 to 1121.5 mg GAE/100 g dry weight) than in the flesh. In our study, peel was 2–3 times, or even 5 times richer in TPC than flesh, ranging overall from 3.10 mg GAE/g dry extract (flesh of 'Lubeničarka') to 272 mg GAE/g dry extract (peel of 'Lončara'). Imeh & Khohar [2002] reported the results of TPC determination in fruits of four commercial pear varieties (1795 to 2566 mg GAE/100 g dry weight), which displayed similar amounts as apple fruit of commercial varieties.

The TFC of eight pear peel extracts ranged from 2.52 to 9.94 mg QE/g dry extract with the lowest and the highest values determined for 'Vidovača' and 'Takiša', respectively.

TABLE 1. Extraction yield, total phenolic content (TPC), and total flavonoid content (TFC) of methanolic extracts of pear varieties.

Variety	Fruit part	Yield (%)	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
Wild pear	flesh+peel	12.3	$16.4 \pm 0.5^{y,c}$	$2.54 \pm 0.10^{z,b}$
	flesh	12.0	$13.3 \pm 0.2^{z,c}$	$1.93 \pm 0.19^{z,b}$
	peel	9.00	$27.3 \pm 0.4^{x,c}$	$4.82 \pm 0.30^{x,b}$
Takiša	flesh+peel	7.63	$94.4 \pm 4.0^{y,b}$	$6.03 \pm 0.18^{z,a}$
	flesh	7.40	$79.5 \pm 2.1^{z,b}$	$3.21 \pm 0.20^{z,a}$
	peel	9.20	$242 \pm 6^{x,b}$	$9.94 \pm 0.19^{x,a}$
Lončara	flesh+peel	13.0	$250 \pm 7^{y,a}$	$1.83 \pm 0.11^{y,c}$
	flesh	8.40	$198 \pm 7^{z,a}$	$0.69 \pm 0.05^{z,d}$
	peel	10.9	$272 \pm 4^{x,a}$	$3.16 \pm 0.15^{x,d}$
Jeribasma	flesh+peel	9.10	$10.1 \pm 0.1^{y,d}$	$1.36 \pm 0.07^{y,d}$
	flesh	12.1	$3.78 \pm 0.05^{z,d}$	$1.22 \pm 0.09^{y,c}$
	peel	8.60	$23.2 \pm 0.7^{x,c}$	$2.73 \pm 0.13^{x,d,e}$
Vidovača	flesh+peel	11.1	$6.56 \pm 0.33^{y,d}$	$1.87 \pm 0.11^{y,c}$
	flesh	15.7	$5.39 \pm 0.06^{z,d}$	$1.32 \pm 0.08^{z,c}$
	peel	12.7	$14.4 \pm 0.1^{x,d}$	$2.52 \pm 0.07^{x,c}$
Lubeničarka	flesh+peel	13.5	$6.21 \pm 0.09^{y,d}$	$2.69 \pm 0.16^{y,b}$
	flesh	9.52	$3.10 \pm 0.02^{z,d}$	$1.72 \pm 0.01^{z,b}$
	peel	11.3	$10.7 \pm 0.1^{x,d}$	$4.30 \pm 0.09^{x,c}$
Karamanka	flesh+peel	11.4	$8.45 \pm 0.49^{y,c,d}$	$0.98 \pm 0.03^{y,d,e}$
	flesh	11.3	$4.51 \pm 0.24^{z,d}$	$0.46 \pm 0.06^{z,d}$
	peel	13.0	$21.1 \pm 0.9^{x,c,d}$	$2.98 \pm 0.19^{x,d,e}$
Williams Bartlett	flesh+peel	9.40	$10.1 \pm 0.1^{y,c,d}$	$1.07 \pm 0.83^{y,d}$
	flesh	10.7	$3.78 \pm 0.05^{z,d}$	$0.70 \pm 0.00^{z,d}$
	peel	10.9	$23.2 \pm 0.7^{x,c}$	$2.57 \pm 0.10^{x,c}$

Within each pear variety, mean values with different superscript letters (<sup>x-z</sup>) differ significantly; for each fruit part separately, mean values with different superscript letters (<sup>a-c</sup>) differ significantly between varieties (one-way ANOVA, Tukey's post hoc;  $p < 0.05$ ). The superscript letters are assigned to show values in the descending order, where (<sup>b</sup>) and (<sup>a</sup>) present the highest values. GAE – gallic acid equivalents; QE – quercetin equivalents.

TFC of the flesh extracts ranged from 0.46 to 3.21 mg QE/g dry extract for 'Karamanka' to 'Takiša', and that of the mixed flesh and peel extracts ranged from 0.98 to 6.03 mg QE/g dry extract with the extreme values noted for the same varieties. Li *et al.* [2014] analyzed TFC of ten pear fruit extracts of Chinese varieties and results showed that TFC was 6–20 times higher in the peel (281.2 to 1682.7 mg rutin/100 g) than in the flesh. In turn, Sharma *et al.* [2015] recorded  $10.30 \mu\text{g}$  catechin equivalents/mg in the sample of European pear fruit extract. As many researchers reported, pears contain high amounts of phenolics, including flavonoids, in the whole fruit, flesh, and especially in the peel. Brahem *et al.* [2017] presented the phenolic profile of peel and flesh crude extracts, and noted

TABLE 2. Content of individual phenolics in methanolic extracts of pear varieties ( $\mu\text{g/g}$  dry extract).

Variety	Fruit part	Arbutin	Chlorogenic acid	Rutin	Hyperoside	Isoquercitrin	Quercitrin	Procyanidin B <sub>1</sub>	Procyanidin B <sub>2</sub>
Wild pear	flesh+peel	485±20 <sup>z,b</sup>	221±10 <sup>x,d</sup>	tr	tr	20.7±5.05 <sup>y,c</sup>	100±6 <sup>d</sup>	950±40 <sup>x,a</sup>	213±10 <sup>z,b</sup>
	flesh	695±31 <sup>x,b</sup>	61.2±4.8 <sup>y,c</sup>	tr	tr	8.01±1.03 <sup>z,b</sup>	92.8±4.6 <sup>y,c</sup>	972±58 <sup>x,a</sup>	275±14 <sup>z,b</sup>
	peel	555±29 <sup>y,c</sup>	25.8±4.0 <sup>z,c</sup>	tr	38.4±2.1 <sup>c</sup>	125±7 <sup>x,d</sup>	264±13 <sup>x,c</sup>	258±10 <sup>y,a</sup>	245±11 <sup>y,b</sup>
Takiša	flesh+peel	1708±62 <sup>y,a</sup>	11763±63 <sup>y,a</sup>	tr	5820±76	12216±46 <sup>x,a</sup>	tr	671±30 <sup>z,b</sup>	2065±62 <sup>x,a</sup>
	flesh	1157±44 <sup>z,a</sup>	10795±49 <sup>z,a</sup>	tr	tr	72.8±4.4 <sup>z,a</sup>	nd	476±29 <sup>y,b</sup>	393±17 <sup>y,a</sup>
	peel	3749±72 <sup>x,a</sup>	16147±60 <sup>x,a</sup>	104±6 <sup>c</sup>	215±10 <sup>a</sup>	657±21 <sup>y,a</sup>	tr	161±4 <sup>z,b</sup>	276±10 <sup>z,b</sup>
Lončara	flesh+peel	114±5 <sup>y,d</sup>	957±39 <sup>y,b</sup>	tr	tr	10.1±1.0 <sup>y,c</sup>	77.6±3.0 <sup>c</sup>	63.3±2.4 <sup>z,c,d</sup>	85.4±4.5 <sup>z,c</sup>
	flesh	100±5 <sup>y,c,d,e</sup>	890±40 <sup>y,b</sup>	tr	tr	1.70±0.30 <sup>y,d</sup>	tr	50.1±2.1 <sup>y,c</sup>	134±6 <sup>y,c</sup>
	peel	767±21 <sup>x,b</sup>	1108±44 <sup>x,b</sup>	84.9±4.0 <sup>c,d</sup>	118±6 <sup>b</sup>	216±9 <sup>x,c</sup>	275±11 <sup>c</sup>	296±13 <sup>x,a</sup>	576±25 <sup>x,a</sup>
Jeribasma	flesh+peel	17.6±1 <sup>z,e</sup>	25.3±2.1 <sup>z,f</sup>	24.2±1.1	tr	3.55±0.21 <sup>c</sup>	264±12 <sup>y,a</sup>	40.9±3.0 <sup>z,d</sup>	86.1±3.5 <sup>y,c</sup>
	flesh	122±7 <sup>x,c,d</sup>	36.3±2.9 <sup>y,c</sup>	tr	tr	tr	70.7±3.7 <sup>z,d</sup>	55.5±2.4 <sup>y,c</sup>	93.3±4.0 <sup>y,d</sup>
	peel	60.9±2.9 <sup>y,f</sup>	80.1±3.7 <sup>x,c</sup>	571±25 <sup>a</sup>	tr	93.8±3.4 <sup>f</sup>	1778±48 <sup>x,a</sup>	76.1±3.4 <sup>x,c</sup>	175±6 <sup>x,c</sup>
Vidovača	flesh+peel	175±6 <sup>y,c,d</sup>	27.1±1.7 <sup>y,f</sup>	tr	tr	36.3±1.0 <sup>y,c</sup>	145±3 <sup>c,c</sup>	37.0±15.2 <sup>d</sup>	25.1±1.1 <sup>z,c</sup>
	flesh	104±5 <sup>z,c,d,e</sup>	37.5±2.0 <sup>x,c</sup>	tr	tr	2.15±0.21 <sup>z,d</sup>	192±7 <sup>y,a</sup>	nd	110±5 <sup>y,c,d</sup>
	peel	374±14 <sup>x,d</sup>	30.2±1.1 <sup>y,c</sup>	43.6±1.6 <sup>c</sup>	tr	134±4 <sup>x,d</sup>	376±12 <sup>x,d</sup>	40.7±2.2 <sup>d</sup>	169±5 <sup>x,c</sup>
Lubeničarka	flesh+peel	86.9±3.8 <sup>z,d,e</sup>	62.6±3.1 <sup>y</sup>	tr	0.10±0.03	191±8 <sup>x,c</sup>	108±4 <sup>y,d</sup>	101±3 <sup>y,c</sup>	52.0±1.3 <sup>z,c</sup>
	flesh	141±5 <sup>y,c</sup>	64.4±2.9 <sup>y,c</sup>	tr	tr	14.4±0.7 <sup>z,c</sup>	95.1±3.0 <sup>z,c</sup>	105±4 <sup>y,c</sup>	76.0±2.8 <sup>y,d</sup>
	peel	308±13 <sup>x,d,e</sup>	88.4±4.0 <sup>x,c</sup>	tr	38.3±1.9 <sup>c</sup>	80.7±3.2 <sup>y,f</sup>	131±5 <sup>x,f</sup>	150±6 <sup>x,b</sup>	99.2±3.3 <sup>x,c</sup>
Karamanka	flesh+peel	84.3±3.3 <sup>y,d,e</sup>	116±6 <sup>y,c</sup>	tr	tr	20.0±1.1 <sup>c</sup>	210±8 <sup>y,b</sup>	tr	tr
	flesh	71.9±3.0 <sup>y,d,e</sup>	92.9±3.7 <sup>z,c</sup>	tr	nd	tr	134±6 <sup>z,b</sup>	tr	tr
	peel	247±8 <sup>x,c</sup>	395±15 <sup>x,d</sup>	367±13 <sup>b</sup>	tr	276±10 <sup>b</sup>	829±36 <sup>x,b</sup>	tr	tr
Williams Bartlett	flesh+peel	226±10 <sup>y,c</sup>	356±12 <sup>y,c</sup>	tr	tr	36.9±2.7 <sup>c</sup>	266±10 <sup>a</sup>	tr	tr
	flesh	64.4±2.5 <sup>z,e</sup>	38.6±2.8 <sup>z,c</sup>	tr	tr	tr	tr	tr	tr
	peel	710±21 <sup>x,b</sup>	883±22 <sup>x,c</sup>	49.4±2.7 <sup>c</sup>	tr	81.0±3.2 <sup>f</sup>	463±20 <sup>c</sup>	tr	tr

Within each pear variety, mean values with different superscript letters (<sup>x-z</sup>) differ significantly; for each fruit part separately, mean values with different superscript letters (<sup>a-f</sup>) differ significantly between varieties (one-way ANOVA, Tukey's post hoc;  $p < 0.05$ ). The superscript letters are assigned to show values in the descending order, where (<sup>s</sup>) and (<sup>a</sup>) present the highest values. tr – traces, nd – not detected.

a higher phenolic content in the peel than in the flesh of pear fruit. In the mentioned studies, the TPC and TFC were measured predominantly in methanolic extracts, and varied depending on species or variety and employed methods of extraction.

The TPC and TFC values determined in the peel of all pear varieties were higher than those found in the flesh and mixed peel and flesh, which could be important data for the consumption of the fruits, because the peel is often discarded, resulting in the loss of valuable compounds [Abaci *et al.*, 2016; Kolniak-Ostek, 2016; Lin & Harnly, 2008; Öztürk *et al.*, 2015].

### Phenolic composition of pear extracts

Determination of the qualitative and quantitative composition of phenolics of the extracts of eight pear varieties was performed using the HPLC technique and the results are presented in Table 2. Among the eight pear varieties, chlorogenic

acid in the peel (25.8 to 16147  $\mu\text{g/g}$  dry extract) and arbutin in the peel (122 to 3748  $\mu\text{g/g}$  dry extract) were the major compounds, followed by quercitrin in the peel (131 to 1778  $\mu\text{g/g}$  dry extract), and isoquercitrin (3.55 to 12216  $\mu\text{g/g}$  dry extract) in the mixed flesh and peel extracts.

The peel of the most pear varieties had the higher content of chlorogenic acid compared to flesh, such as those obtained from 'Takiša', 'Lončara' and 'Williams Bartlett'. In the previous study of eight Chinese pear varieties, chlorogenic acid content in the whole fruits varied between 10.3 and 263.8  $\mu\text{g/g}$  [Li *et al.*, 2012], while in our study the range was notably wider. Other varieties showed a high chlorogenic acid content in the peel; however, with the content differing among the analyzed varieties [Li *et al.*, 2014; Salta *et al.*, 2010]. As a potential chemoprotective agent, chlorogenic acid has been reported to elicit antioxidant, antitumor, and immune system-enhancing effects [Li *et al.*, 2014].

The content of arbutin in the pear methanol extracts varied, being predominantly higher in the peel compared to the flesh (except for wild pear), ranging from 60.9 to 3749  $\mu\text{g/g}$  dry extract of peel; from 64.4 to 1157  $\mu\text{g/g}$  dry extract of flesh; and from 17.6 to 1708  $\mu\text{g/g}$  dry extract of the mixed flesh and peel. The highest content of arbutin was detected in 'Takiša' variety, while the lowest one in 'Jeribasma'. In the previous studies, some pear varieties showed arbutin contents 2 to 3 times higher in the peel than in the flesh [Kolniak-Ostek, 2016; Li *et al.*, 2014; Salta *et al.*, 2010]. As the major phenolic compound found in the different parts of plant and fruit, arbutin exhibits strong free radical scavenging properties. Additionally, it has been proved to elicit antibacterial, anti-inflammatory, antitussive, and skin-whitening effects and to be effective against urinary infections [Li *et al.*, 2014]. Dadgar *et al.* [2018] also demonstrated its ability to reduce oxidative stress in Parkinson's disease animal model.

In the present study, the contents of isoquercitrin, quercitrin, procyanidin B<sub>1</sub>, and procyanidin B<sub>2</sub> differed depending on the variety and a fruit part used, with higher values determined in the peel. Quercitrin and isoquercitrin have been also identified in pears in some previous studies, which showed that their content varied greatly among cultivars and within different tissues [Liaudanskas *et al.*, 2017]. Kaur & Arya [2012] identified quercitrin and isoquercitrin in *Pyrus communis*, while in this study, quercitrin was found in the highest amount in 'Jeribasma' peel extract and isoquercitrin in mixed flesh and peel extract of 'Takiša'. Li *et al.* [2016a] compared the antioxidant effects of quercitrin and isoquercitrin and concluded that isoquercitrin exhibited higher reactive oxygen species (ROS) scavenging activity than quercitrin, protecting mesenchymal stem cells from ROS-induced oxidative damage. Jeong *et al.* [2017] isolated five proanthocyanidins from *Pyrus pyrifolia* peel, while in the current study procyanidin B<sub>2</sub> was detected in the noticeably high amount in mixed flesh and peel extract of 'Takiša'. Fruits of wild pear were the richest in procyanidin B<sub>1</sub>.

Rutin and hyperoside were detected in traces in all pear varieties. Some natural polyphenolics, such as rutin, have been shown as potent inhibitors of COVID-19 main protease (Mpro), which is considered a potential therapeutic drug target [Adem *et al.*, 2020].

Variations in the content of phenolics among different pear varieties were reported in the previous studies [Kolniak-Ostek *et al.*, 2020; Li *et al.*, 2014; Öztürk *et al.*, 2015]. The content of individual phenolic compounds in the extracts varied widely among the tested varieties, which could be attributed to the sampling locality, different pre- or post-harvest conditions, or, as it was demonstrated for apple cultivars, genetics can be supposed to play a major role causing very high phenolic content variability [McClure *et al.*, 2019]. Additionally, the phenolics are not equally distributed in the fruit [Li *et al.*, 2014].

Recent research indicates that long-term polyphenol consumption may play a vital role in promoting health through the regulation of metabolism and protection against various types of cancer, cardiovascular diseases, type 2 diabetes, gastrointestinal disorders, lung impairment, neurodegenerative diseases, *etc.* These benefits could be explained by the "biochemical scavenger theory", which suggests that phenolic

compounds inactivate free radicals by forming stabilized chemical complexes, thus preventing further adverse reactions in the body [Cory *et al.*, 2018].

The next step of the study was to investigate the antioxidant activity of the extracts and their potential to inhibit the enzymes involved in oxidative stress-induced neurodegenerative disorders.

### Antioxidant activity of pear extracts

Three different assays: DPPH, ABTS and FRAP, were used to achieve more complete evaluation of the antioxidant activity of peel, flesh, and mixed peel and flesh fruit extracts. The results are presented in Table 3. In terms of the scavenging activity against DPPH<sup>•</sup>, all pear fruit extracts showed strong to low activity, depending on the variety. The DPPH<sup>•</sup> scavenging activity was as follows: 'Takiša' > wild pear > 'Lončara' > 'Vidovača' > 'Jeribasma' > 'Lubeničarka' > 'Karamanka' > 'Williams Bartlett'. The peel showed the highest DPPH<sup>•</sup> scavenging activity (IC<sub>50</sub> values ranging from 0.37 to 5.50 mg/mL). With some exceptions, methanolic extracts obtained from peel, flesh, and mixed flesh and peel showed significantly different IC<sub>50</sub> values for all tested pear varieties (Table 3). The highest antioxidant potential was determined in the peel of 'Takiša' (IC<sub>50</sub>=0.37 mg/mL), followed by wild pear peel (IC<sub>50</sub> = 0.80 mg/mL), and 'Lončara' peel (IC<sub>50</sub>=1.56 mg/mL). In all samples, the mixture of flesh and peel showed intermediate activity (0.79 to 6.91 mg/mL), while flesh extracts showed the lowest activity (3.71 to 22.39 mg/mL). Compared to BHA and BHT standards and particularly ascorbic acid, the tested pear extracts showed notably lower DPPH<sup>•</sup> scavenging activity.

Similar DPPH<sup>•</sup> scavenging activity to certain varieties in this study, was reported for the pear fruit extracts of some Chinese varieties [Li *et al.*, 2012], *i.e.* Xuehua pear fruit (IC<sub>50</sub>=0.59 mg/mL) and Nanguo pear fruit (IC<sub>50</sub>=0.70 mg/mL). The extract of apple shaped pear (*P. pyrifolia* var. *pinggouli*) showed DPPH<sup>•</sup> scavenging activity expressed as IC<sub>50</sub>=38.30 mg/mL [Ma *et al.*, 2012], which is lower than the activity presented in this paper. Salta *et al.* [2010] investigated the scavenging activity of 'Rocha' pear fruit extract (IC<sub>50</sub>=0.11 mg/mL) and also of a few commercial pear fruits, and obtained significantly higher antiradical activity against DPPH<sup>•</sup> compared to results found in this research. Results of previous studies have indicated that pear fruit possesses a strong DPPH<sup>•</sup> antiradical potential, and that peel has a stronger antioxidant activity than flesh [Kolniak-Ostek, 2016; Li *et al.*, 2012, 2014; Salta *et al.*, 2010].

In the ABTS assay, the highest values were achieved for the peel extracts (1.17 to 2.91 mg AAE/g dry extract); followed by mixed flesh and peel extracts (0.44 to 2.09 mg AAE/g dry extract) and flesh (0.26 to 1.18 mg AAE/g dry extract). For most of the tested pear varieties (Table 3), peel, flesh, and mixed flesh and peel methanolic extracts showed significantly ( $p < 0.05$ ) different antioxidant activities in the ABTS assay. The results obtained using this assay showed that 'Takiša' and 'Lončara' extracts exhibited the highest antioxidant activity. Compared to BHA and BHT standards, the tested extracts showed lower activity (Table 3). High ABTS<sup>•+</sup> scavenging activity of the European pear fruit methanolic extract (IC<sub>50</sub>=15.90 mg) was presented by Sharma *et al.* [2015].

TABLE 3. Antioxidant activity of methanolic extracts of pear varieties.

Variety/ Standard	Fruit part	DPPH <sup>•</sup> scavenging activity (IC <sub>50</sub> , mg/mL)	ABTS <sup>•+</sup> scavenging activity (mg AAE/g)	Ferric-reducing antioxidant power (μmol Fe(II)/g)
Wild pear	flesh+peel	2.85±0.19 <sup>y,d</sup>	0.77±0.05 <sup>y,e</sup>	743±19 <sup>y,a</sup>
	flesh	4.29±0.33 <sup>x,d</sup>	0.76±0.07 <sup>y,c</sup>	618±19 <sup>z,a</sup>
	peel	0.80±0.05 <sup>z,d</sup>	1.10±0.03 <sup>x,d</sup>	880±21 <sup>x,b</sup>
Takiša	flesh+peel	0.79±0.05 <sup>y,e</sup>	1.54±0.09 <sup>y,b</sup>	711±12 <sup>z,a</sup>
	flesh	3.85±0.23 <sup>x,c</sup>	1.18±0.01 <sup>z,b</sup>	606±8 <sup>z,a</sup>
	peel	0.37±0.03 <sup>z,d</sup>	2.91±0.10 <sup>x,a</sup>	933±22 <sup>x,a</sup>
Lončara	flesh+peel	2.90±0.16 <sup>y,d</sup>	2.09±0.10 <sup>x,a</sup>	739±16 <sup>y,a</sup>
	flesh	3.71±0.14 <sup>x,e</sup>	1.65±0.32 <sup>xy,a</sup>	631±7 <sup>z,a</sup>
	peel	1.56±0.12 <sup>z,c,d</sup>	1.22±0.06 <sup>y,d</sup>	882±23 <sup>x,b</sup>
Jeribasma	flesh+peel	3.28±0.03 <sup>x,d</sup>	0.44±0.03 <sup>y,f</sup>	206±10 <sup>y,d</sup>
	flesh	3.71±0.07 <sup>x,c</sup>	0.41±0.03 <sup>y,d</sup>	190±9 <sup>y,d</sup>
	peel	2.72±0.09 <sup>y,c</sup>	2.67±0.04 <sup>x,b</sup>	737±14 <sup>x,c</sup>
Vidovača	flesh+peel	6.91±0.25 <sup>x,a</sup>	0.96±0.02 <sup>y,d</sup>	409±13 <sup>y,c</sup>
	flesh	5.97±0.52 <sup>x,c</sup>	0.59±0.03 <sup>z,c,d</sup>	330±8 <sup>z,b</sup>
	peel	2.28±0.68 <sup>y,c</sup>	1.69±0.06 <sup>x,c</sup>	647±2 <sup>x,d</sup>
Lubeničarka	flesh+peel	4.99±0.07 <sup>x,c</sup>	0.95±0.04 <sup>y,d</sup>	393±18 <sup>y,c</sup>
	flesh	5.00±0.02 <sup>x,c,d</sup>	1.00±0.06 <sup>y,b,c</sup>	248±10 <sup>z,c</sup>
	peel	3.55±0.07 <sup>y,b</sup>	1.17±0.03 <sup>x,d</sup>	486±9 <sup>x,e</sup>
Karamanka	flesh+peel	5.06±0.26 <sup>y,c</sup>	1.18±0.06 <sup>y,c</sup>	391±8 <sup>y,c</sup>
	flesh	11.4±0.7 <sup>x,b</sup>	0.54±0.04 <sup>z,c,d</sup>	178±2 <sup>z,d</sup>
	peel	5.50±0.38 <sup>y,a</sup>	2.52±0.03 <sup>x,ab</sup>	638±10 <sup>x,d</sup>
Williams Bartlett	flesh+peel	5.96±0.40 <sup>y,b</sup>	1.21±0.03 <sup>y,c</sup>	456±4 <sup>y,b</sup>
	flesh	22.4±1.1 <sup>x,a</sup>	0.26±0.04 <sup>z,d</sup>	221±14 <sup>z,c</sup>
	peel	4.57±1.09 <sup>y,ab</sup>	2.78±0.04 <sup>x,bc</sup>	645±10 <sup>x,d</sup>
		(IC <sub>50</sub> , μg/mL)		
BHT	–	17.9±0.2	27.5±0.2	4450±78
BHA	–	13.8±0.4	28.2±0.1	5840±53
Ascorbic acid	–	5.1±0.1	nt	1810±86

Within each pear variety, mean values with different superscript letters (<sup>x-z</sup>) differ significantly; for each fruit part separately, mean values with different superscript letters (<sup>a-e</sup>) differ significantly between varieties (one-way ANOVA, Tukey's post hoc;  $p < 0.05$ ). The superscript letters are assigned to show values in the descending order, where (<sup>b</sup>) and (<sup>a</sup>) present the highest values. AAE – ascorbic acid equivalents, BHT – 3,5-di-tert-butyl-4-hydroxytoluene; BHA – 2(3)-tert-butyl-4-hydroxyanisole. nt – not tested.

Considering the FRAP assay results, the peel extracts showed significantly higher values than flesh, and flesh and peel ones. The FRAP values of the tested extracts were lower than those of the tested standards (Table 3). The highest

FRAP value was determined for the 'Takiša' peel extract (933 μmol Fe(II)/g dry extract) followed by 'Lončara' peel extract (882 μmol Fe(II)/g dry extract), and wild pear peel extract (880 μmol Fe(II)/g dry extract). With exception of 'Jeribasma' extracts, all methanolic extracts obtained from peel, flesh, and mixed flesh and peel of all other varieties tested showed significantly different FRAP values (Table 3). The FRAP of four commercial pears showed similar values to commercial apple fruit varieties, and slightly higher ones compared to those reported for peach and kiwi fruits [Imeh & Khokhar, 2012]. Kolniak-Ostek [2016] compared leaves, seeds, peel, and pulp of the 'Radana' pear and obtained the highest FRAP for leaves, probably because of the highest content of phenolics in this plant part.

To the best of our knowledge, there are a few studies on the ABTS and FRAP assays used to determine the antioxidant activity of pear fruits. However, due to the differences in the applied methods and presentation of results, it is difficult to directly compare our results with those obtained in other studies [Kolniak-Ostek, 2016; Kolniak-Ostek *et al.*, 2020; Liaudanskas *et al.*, 2017; Sharma *et al.*, 2015].

The highest antioxidant activity of 'Takiša', especially of the outer part of the fruit, could be attributed to the highest content of detected phenolics, particularly chlorogenic acid which is known for its antioxidant potential [Li *et al.*, 2014].

#### Antineurodegenerative activity of pear extracts

The results of the evaluation of the antineurodegenerative activities of pear extracts are presented in Table 4. The extracts did not show enzyme inhibitory activity in the concentration-dependent manner. Methanolic extracts obtained from peel, flesh, and mixed flesh and peel of the pear varieties tested, showed significantly different AChE- and TYR-inhibiting activities (Table 4). No correlation was established between inhibiting effects against both enzymes and fruit parts of all varieties.

The AChE inhibition by the tested extracts, compared to galanthamine (42.38 to 57.11%), was low to moderate. The highest AChE inhibitory activity displayed mixed flesh and peel extracts of 'Lončara' (40.4%) at 50 μg/mL and 'Takiša' (39.5%) at 100 μg/mL. In some cases, the mixed flesh and peel extracts ('Takiša', 'Lončara', 'Vidovača') showed higher AChE inhibitory activity than flesh or peel, but in some cases the peel extract showed the highest activity (wild pear).

The TYR inhibition by the studied extracts was lower than the inhibition displayed by kojic acid (33.93 to 51.81%). The highest inhibition of TYR activity exhibited 'Takiša' peel extract (32.8%) at the concentration of 50 μg/mL, which is close to kojic acid at the same concentration.

Previous studies have shown the significant inhibition of AChE and TYR by various fruit extracts [Szwajgier & Borowiec, 2012; Šavikin *et al.*, 2018]. Antineurodegenerative activity of 70% ethanolic extract of peel of *Punica granatum* L. was studied by Šavikin *et al.* [2018], who obtained the highest AChE inhibition of 33.46% at extract concentration of 100 μg/mL, which is moderate compared to galanthamine (57.11%), while TYR inhibitory activity of 78.46% was higher than the inhibition ensured by kojic acid (51.81%).



TABLE 4. Antineurodegenerative activities of methanolic extracts with different concentrations obtained from pear varieties.

Variety/ Standard	Fruit part	AChE inhibition (%)			TYR inhibition (%)		
		25 µg/mL	50 µg/mL	100 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Wild pear	flesh + peel	21.8±0.7 <sup>ya</sup>	34.5±3.0 <sup>xb</sup>	31.6±3.1 <sup>xb</sup>	17.7±1.4 <sup>yc</sup>	21.2±3.2 <sup>yb</sup>	13.4±0.8 <sup>zc</sup>
	flesh	9.53±3.37 <sup>zd</sup>	36.9±0.4 <sup>xa</sup>	12.5±0.8 <sup>yc</sup>	17.8±2.1 <sup>yb</sup>	20.7±1.6 <sup>yb</sup>	23.7±2.2 <sup>xa</sup>
	peel	34.7±2.43 <sup>xa</sup>	38.9±1.4 <sup>xa</sup>	32.4±2.6 <sup>xab</sup>	24.6±1.8 <sup>xa</sup>	27.4±2.1 <sup>xb</sup>	19.4±1.8 <sup>yab</sup>
Takiša	flesh + peel	30.1±2.7 <sup>xa</sup>	33.5±3.2 <sup>xb</sup>	39.5±2.4 <sup>xa</sup>	27.8±1.0 <sup>ya</sup>	28.2±2.5 <sup>ya</sup>	22.9±1.8 <sup>xab</sup>
	flesh	26.3±2.7 <sup>xc</sup>	34.4±1.5 <sup>xa</sup>	28.4±1.5 <sup>yb</sup>	29.2±2.7 <sup>xa</sup>	23.7±2.3 <sup>yab</sup>	21.1±2.1 <sup>xab</sup>
	peel	7.10±0.91 <sup>yc</sup>	32.4±3.4 <sup>xb</sup>	9.19±0.94 <sup>zd</sup>	22.3±2.9 <sup>yab</sup>	32.8±0.7 <sup>xa</sup>	24.6±2.8 <sup>xa</sup>
Lončara	flesh + peel	31.5±3.1 <sup>xa</sup>	40.4±0.9 <sup>xa</sup>	30.9±2.2 <sup>xb</sup>	24.5±2.1 <sup>xab</sup>	21.1±1.8 <sup>yb</sup>	26.5±1.0 <sup>xa</sup>
	flesh	31.3±1.0 <sup>xb</sup>	36.1±4.6 <sup>xa</sup>	35.7±4.5 <sup>xa</sup>	26.6±1.7 <sup>xa</sup>	27.0±1.9 <sup>xa</sup>	17.8±1.7 <sup>bc</sup>
	peel	29.4±0.4 <sup>xb</sup>	36.4±3.2 <sup>xab</sup>	30.2±3.3 <sup>xab</sup>	17.4±1.5 <sup>yb</sup>	21.8±0.8 <sup>yc</sup>	20.1±2.1 <sup>yab</sup>
Jeribasma	flesh + peel	7.66 ±0.95 <sup>yb</sup>	36.3±0.6 <sup>xa</sup>	10.3±1.7 <sup>zd</sup>	22.8±1.6 <sup>xb</sup>	25.0±2.1 <sup>xab</sup>	17.4±2.7 <sup>bc</sup>
	flesh	7.34±0.69 <sup>yd</sup>	7.92±1.0 <sup>xb</sup>	29.9±3.6 <sup>xab</sup>	21.1±1.8 <sup>xb</sup>	11.8±0.5 <sup>zd</sup>	16.8±1.1 <sup>ybc</sup>
	peel	37.8±1.0 <sup>xa</sup>	35.6±2.6 <sup>xab</sup>	24.0±0.1 <sup>ybc</sup>	18.5±2.2 <sup>xb</sup>	16.6±2.1 <sup>yde</sup>	12.7±1.1 <sup>yc</sup>
Vidovača	flesh + peel	27.7±1.8 <sup>xa</sup>	36.6±1.0 <sup>xa</sup>	24.9±0.3 <sup>xc</sup>	14.9±1.9 <sup>cd</sup>	20.7±3.0 <sup>yyb</sup>	20.2±0.6 <sup>b</sup>
	flesh	8.47±0.44 <sup>zd</sup>	10.3±2.0 <sup>xb</sup>	27.6±1.7 <sup>xb</sup>	27.4±2.1 <sup>xa</sup>	27.1±2.4 <sup>xa</sup>	21.7±2.2 <sup>xab</sup>
	peel	12.6±0.3 <sup>yd</sup>	7.99±0.84 <sup>yd</sup>	20.5±0.8 <sup>yc</sup>	11.9±1.7 <sup>yc</sup>	15.0±2.3 <sup>yde</sup>	23.9±2.0 <sup>xa</sup>
Lubeničarka	flesh + peel	35.0±0.8 <sup>xa</sup>	37.5±1.4 <sup>xa</sup>	24.2±0.8 <sup>yc</sup>	25.3±0.9 <sup>xab</sup>	24.4±0.7 <sup>xab</sup>	25.0±2.7 <sup>xa</sup>
	flesh	37.4±1.8 <sup>xa</sup>	36.6±2.0 <sup>xa</sup>	35.2±3.3 <sup>xa</sup>	10.4±0.6 <sup>zc</sup>	13.2±0.6 <sup>yed</sup>	16.5±0.7 <sup>ybc</sup>
	peel	34.0±2.4 <sup>xab</sup>	28.8±2.5 <sup>yb</sup>	28.7±1.8 <sup>ybc</sup>	20.4±2.1 <sup>yab</sup>	13.7±1.0 <sup>yc</sup>	8.56±1.00 <sup>zd</sup>
Karamanka	flesh + peel	8.99±0.85 <sup>yb</sup>	9.83±0.67 <sup>yc</sup>	12.3±1.5 <sup>yd</sup>	17.4±2.1 <sup>xc</sup>	13.5±0.6 <sup>zc</sup>	9.80±1.12 <sup>yd</sup>
	flesh	9.57±0.31 <sup>yd</sup>	10.7±1.1 <sup>yb</sup>	9.60±0.12 <sup>yc</sup>	18.7±1.9 <sup>xb</sup>	17.2±1.2 <sup>yc</sup>	16.5±1.3 <sup>bc</sup>
	peel	30.6±0.6 <sup>xb</sup>	34.5±1.2 <sup>xab</sup>	36.7±2.1 <sup>xa</sup>	19.4±0.7 <sup>xab</sup>	25.6±1.0 <sup>xbc</sup>	14.8±0.8 <sup>bc</sup>
Williams Bartlett	flesh + peel	9.56±0.82 <sup>yb</sup>	8.86±0.82 <sup>yc</sup>	20.1±2.5 <sup>zc</sup>	10.6±0.96 <sup>yd</sup>	13.0±1.9 <sup>yc</sup>	9.81±1.00 <sup>yd</sup>
	flesh	23.8±2.9 <sup>xc</sup>	34.3±1.1 <sup>xa</sup>	39.4±1.1 <sup>xa</sup>	15.1±1.66 <sup>xbc</sup>	14.5±1.2 <sup>xyed</sup>	14.8±1.9 <sup>xc</sup>
	peel	23.4±0.9 <sup>xc</sup>	34.5±1.6 <sup>xab</sup>	28.4±1.7 <sup>ybc</sup>	8.83±1.29 <sup>yc</sup>	18.5±1.9 <sup>xd</sup>	16.1±1.6 <sup>xb</sup>
Galanthamine	–	42.4±0.7	50.6±0.5	57.1±1.7	–	–	–
Kojic acid	–	–	–	–	35.7±5.5	33.9±3.8	51.8±2.6

Within each pear variety, mean values with different superscript letters (<sup>x-z</sup>) differ significantly; for each fruit part separately and each tested concentration, mean values with different superscript letters (<sup>a-e</sup>) differ significantly between varieties (one-way ANOVA, Tukey's post hoc;  $p < 0.05$ ). The superscript letters are assigned to show values in the descending order, where (<sup>x</sup>) and (<sup>a</sup>) present the highest values. GAE – gallic acid equivalents; AChE – acetylcholinesterase; TYR – tyrosinase.

Szwajgier & Borowiec [2012] investigated anti-AChE activities of numerous fruits, and found significant activity of extracts of whole fruits, or juices. Peach juice (*Prunus persica* L.) and wild strawberry fruit extract (*Fragaria vesca* L.) showed the highest AChE inhibitory activity (6.10 eserine µM), followed by apple juice varieties (*Mallus domestica* Borkh.) (2.91 to 6.10 eserine µM), and plum extract (*Prunus domestica* L.) (3.09 eserine µM). Their results proved that different fruits can be promising sources of inhibitors of enzymes implicated in neurodegeneration, potentially preventing Alzheimer's and Parkinson's diseases [Szwajgier & Borowiec, 2012; Šavikin et al., 2018].

### Correlation among TPC, TFC, and individual phenolic compounds and bioactivity assays

As presented in Table 5, results of ABTS and FRAP assays were moderately correlated with TPC and TFC. Results of those assays showed weak correlation to arbutin and chlorogenic acid, while rutin and quercitrin and ABTS<sup>•+</sup> scavenging activity were moderately correlated. A negative correlation was determined between DPPH<sup>•</sup> scavenging activity and TPC, TFC and phenolic constituents because of presenting results of DPPH assay using IC<sub>50</sub> values. The results obtained by DPPH assay were moderately correlated to TPC, TFC, and arbutin content. AChE inhibitory activity showed

TABLE 5. Correlation coefficients ( $r$ ) among total phenolic content (TPC), total flavonoid content (TFC), and individual phenolic compound contents and results of bioactivity assays.

	DPPH· scavenging activity	ABTS· <sup>+</sup> scavenging activity	Ferric-reducing antioxidant power	AChE inhibition	TYR inhibition
TPC	-0.36	0.39	0.52	0.34	0.46
TFC	-0.50	0.44	0.43	0.30	0.54
Arbutin	-0.36	0.36	0.29	0.17	0.53
Chlorogenic acid	-0.30	0.30	0.06	0.16	0.53
Rutin	-0.13	0.61	0.35	0.18	0.04
Hyperoside	-0.20	0.08	-0.07	0.09	0.28
Isoquercitrin	-0.21	0.10	-0.06	0.09	0.30
Quercitrin	-0.13	0.55	0.33	0.06	-0.13
Procyanidin B <sub>1</sub>	-0.29	-0.16	0.26	0.33	0.23
Procyanidin B <sub>2</sub>	-0.34	0.08	0.08	0.18	0.36

According to Taylor (1990):  $r \leq 0.35$  weak correlation;  $0.36 < r < 0.67$  moderate correlation;  $0.68 < r < 1$  strong correlation; AChE – acetylcholinesterase; TYR – tyrosinase.

weak correlation to TPC, TFC, and individual components. TYR inhibitory activity displayed moderate correlation to TPC, TFC, arbutin and chlorogenic acid contents. In some other studies of pear fruit extracts, peel-flesh phenolic content, peel phenolic content, and flesh phenolic content were significantly correlated [Abaci *et al.*, 2016]. Many researchers have found that antioxidant capacity of pear fruit appears to be strongly influenced by TPC and TFC, and highly significant linear correlations were shown between phenolic contents and antioxidant capacity [Abaci *et al.*, 2016; Imeh & Khohar, 2002; Kolniak-Ostek, 2016; Kolniak-Ostek *et al.*, 2020; Li *et al.*, 2012, 2014; Öztürk *et al.*, 2015; Salta *et al.*, 2010; Sharma *et al.*, 2016].

## CONCLUSION

This study evaluated phenolic composition, total phenolic and flavonoid contents, and biological activities (antioxidant and antineurodegenerative) of peel, flesh, and mixed flesh and peel methanolic extracts of six traditional pear varieties ('Vidovača', 'Lubeničarka', 'Karamanka', 'Jeribasma', 'Takiša', 'Lončara'), one commercial variety ('Williams Bartlett'), and wild pear from Serbia. All varieties, as well as wild pear, were analyzed for the first time from this perspective. Methanolic extracts of traditional pear fruit varieties showed a high content of total phenolics and flavonoids, significant antioxidant potential, and moderate antineurodegenerative activity. The significant level of phenolics and flavonoids, and the highest antioxidant activity were shown by 'Takiša', 'Lončara', and 'Jeribasma'. Wild pear had average or high antioxidant activity, while commercial 'Williams

Bartlett' displayed average or lower activities compared to traditional pear varieties from Serbia.

This study revealed that the fruits of the investigated traditional pears are rich in bioactive components and can be used in nutrition, for possible medical applications, and to prevent diseases induced by oxidative stress. The results are important to enhance their production as a significant source of functional food and potential medicinal remedy. Because of vulnerability of the gene pool, it is necessary to preserve the traditional pear varieties, as a favorable way to increase health benefits and agriculture sustainability. In the food processing industry, it is essential to develop technologies that will enable to process food, in order to keep high nutritional quality, but, also to retain its functional properties and biological activity. The COVID-19 pandemic affects the whole food sector, including food safety, bioactive food ingredients, food security, and sustainability. One of the promising alternatives in post-pandemic world includes the development of nutritional and immune-boosting plant-based products which could support the human health [Galanakis *et al.*, 2021].

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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