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# Polyphenolic Profile, Antioxidant and Antidiabetic Potential of Medlar (*Mespilus germanica* L.), Blackthorn (*Prunus spinosa* L.) and Common Hawthorn (*Crataegus monogyna* Jacq.) Fruit Extracts from Serbia

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**Abstract:** Plant-based food represents an excellent source of different nutrients and bioactive compounds, such as phenolics, carotenoids, vitamins, etc., with proven health benefits for humans. The content of selected phytochemicals, polyphenolic profile, and biological activity (antioxidant potential and α-glucosidase inhibitory activity) of fruit extracts of medlar (*Mespilus germanica* L.), blackthorn (*Prunus spinosa* L.), and common hawthorn (*Crataegus monogyna* Jacq.), the neglected *Rosaceae* species originated from Serbia were studied. Targeted UHPLC/(−)HESI–MS/MS quantitative analysis of phenolic compounds revealed pinocembrin only in medlar fruit extract, and it is the first report of this flavanone in medlar fruits. Total phenolic content did not differ between extracts, whereas significant differences were observed for the contents of total flavonoids, total phenolic acids, and total gallotannins. Monomeric anthocyanins and total anthocyanins were significantly higher in blackthorn compared to medlar and hawthorn fruit extracts ($p < 0.05$). $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ scavenging activities for examined fruits were modest compared to other natural antioxidants and BHT. The most potent inhibitory activity toward α-glucosidase expressed medlar and blackthorn extracts with $IC_{50}$ values of 129.46 and 199.84 μg/mL, respectively, which was higher compared to the standard drug acarbose.

**Keywords:** Rosaceae; neglected fruits; polyphenols; flavonoids; pinocembrin; UHPLC-MS/MS; α-glucosidase



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## 1. Introduction

Plant-based food represents an excellent source of different nutrients (proteins, sugars, lipids) as well as phytochemicals with expressed biological activity including phenolic compounds, vitamins, and carotenoids. Currently, antioxidants are the most studied group of compounds with special emphasis on natural compounds which can replace artificial ones, like butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), etc., during food production [1]. Since phenolics and carotenoids are the most prominent antioxidants, plants could be considered the most important source of these compounds [2,3]. Apart from well-known, there is a significant number of rarely used wild fruits which are usually underestimated although they possess excellent adaptive properties to resident agroecological conditions and present a good source of nutritionally valuable and health beneficiary compounds. Several underutilized and/or scarcely studied plant species of the Rosaceae

family that can be used as good sources of different phytochemicals. These species, that are neglected nowadays, have been frequently disbursed previously, in particular during the years of famine. The Rosaceae family includes over 3000 species, several hundred of which belong to the genera Rosa and Rubus. Plants of this family are widespread, primarily in the extratropical regions of the northern hemisphere. Due to different environmental conditions, the Rosaceae family includes both woody (trees and shrubs) and herbaceous perennials and rarely annuals [4].

Medlar (*Mespilus germanica* L.) is a plant species belonging to the *Mespilus* genus and wide spread plant family- the Rosaceae, originates in the southeastern part of Europe, Turkey, Iran, and Iraq, and it is well known among folks in those parts of the world. Due to limited growing area, it is not recognized among the wide scientific community although it has been established that fruit is an excellent source of several phenolic acids (*p*-coumaric acid, protocatechuic acid, cholorgenic acid, gallic acid, caffeic acid), flavonoids (quercetin, rutin, vanillin), carbohydrates (sucrose, fructose, glucose) as well as vitamins, in particular ascorbic acid [5,6]. Besides, the study of these fruits from Serbia confirmed that the main volatile compounds in green and ripe fruits were hexanal and (*E*)-2-hexenal i.e., hexanol and (*Z*)-3-hexenol, respectively [7]. Among fatty acids, saturated ones, such as hexanoic and hexadecanoic acids, were predominant [7,8], as well as stearic, oleic, linoleic, and behenic acids [8]. The specificity of medlar fruit is that it can be eaten after some period of ripening when it becomes soft and dark. At the same time, this can be the reason why, for some consumers, it is unattractive. However, data about the chemical composition and biological properties of wild-growing and/or cultivated medlar fruits are very limited.

Blackthorn or sloe (*Prunus spinosa* L.) a tall bush or tiny tree that is part of *Prunus* genus, which is a part of the Rosaceae plant family, widespread across the world (Europe, parts of Australia, North America, Africa and New Zealand). The fruit of this plant has been recognized as good source of several phenolics (3-*O*-caffeoylquinic acid, quercetin-3-*O*-rutinoside known also as rutin, catechins), in particular procyanindins and anthocyanins which are responsible for its dark blue color [9–11]. Authors of a study from Russia have reported that epicatechin and chicoric acid (one of the hydroxycinnamic acids) were the most predominant phenolic compounds in fresh and dried fruit with no significant differences after the drying process [12]. Also, several types of the research reported blackthorn fruit as an outstanding source of vitamin C [10,13]. It was confirmed that the fruit of this plant possessed significant quantities of important biogenic macroelements, intensely potassium, calcium, and sulfur [14]. Through several studies, authors emphasized the antioxidant properties of blackthorn fruits [9,10,15] as well as possible anti-inflammatory and antimicrobial activity [15] against both Gram positive and Gram negative bacteria. It was reported that burgers enriched with *P. spinosa* extract expressed higher stability under lipid oxidation conditions [11].

Common hawthorn (*Crataegus monogyna* Jacq.), also known as one-seed hawthorn, may, maythorn, quickthorn, whitethorn, etc., is a shrub or small tree with intense crown and hermaphrodite flowers. The tree is innate to European continent and some parts of North America and Asia, with a tendency to spread around the world. However, it is still insufficiently investigated. Based on the available literature, the phenolic composition of the fruit of this tree is quite different compared to the previous ones. It is characterized by the predominance of the following compounds: kaempferol and quercitrin (flavonol), apigenin (flavone), and ursolic acid (phenolic acid) [16], flavan-3-ol monomers and dimmers [17]. Among fatty acids, the differences have been determined between unripe and ripened fruits where polyunsaturated fatty acids were predominant in unripe fruit while during ripening saturated fatty acids are formed and dominated. In addition, both unripe and ripened fruits contained significant quantities of tocopherols, β-carotene [18], as well as vitamin C [10,18]. Analysis of volatile compounds present in oil obtained from *C. monogyna* fruit performed via gas chromatography (with FID detector, GC-FID) confirmed occurrence of eighty one compounds with the predominance of benzaldehyde, butyraldehyde, and (*E*)-2-hexenal [19]. The most recent publication deals with the mineral composition of *C.*

*monogyna* fruit and presented data about the existence of potassium, phosphorus, calcium, magnesium, sodium, iron, and boron [20] as important biogenic elements. In addition to the chemical composition, all articles reported the antioxidant activity of hawthorn fruits [10,16,18].

Plants can be used to combat different diseases due to the presence of various bioactive compounds. Currently, diabetes mellitus (especially type 2, DMT2) is one of the most spread illnesses all around the world. However, apart from standard drugs, plants can be considered excellent sources of naturally derived compounds with significant antidiabetic activity [21,22].

Accordingly, the main goal of current research was to determine the content of selected phytochemicals (phenolic compounds) in fruits of *M. germanica*, *P. spinosa* and *C. monogyna* collected in Serbia, as well as UHPLC-MS analysis in order to establish the differences in polyphenolic profile between examined fruits. Furthermore, antioxidant properties through in vitro assays (phosphomolybdenum antioxidant assay, inhibition of DPPH radicals and ABTS radical-cations) as well as activity against $\alpha$-glucosidase enzyme were examined and discussed.

## 2. Materials and Methods

### *2.1. Chemicals and Instruments*

All chemicals for spectrophotometric methods were purchased from Sigma Aldrich (Deisenhofen, Germany) and Alfa Aesar (Karlsruhe, Germany). Acetonitrile and formic acid (MS grade) were obtained from Merck, Darmstadt, Germany. Phenolic standards (HPLC-grade $\geq$ 98%) of protocatechuic acid, syringic acid, chlorogenic acid, caffeic acid, aesculetin, rutin, *p*-coumaric acid, quercetin-3-*O*-glucoside, kaempferol-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin, and pinocembrin were purchased from Sigma-Aldrich (Steinheim, Germany). Ultrapure water (MicroPure water purification system, 0.055 µS/cm) was used to prepare standard solutions and dilutions. Syringe filters (25 mm, PTFE membrane 0.45 µm) were purchased from Supelco (Bellefonte, PA, USA). UV–Vis double beam spectrophotometer Halo DB-20S (Dynamica GmbH, Dietikon, Switzerland) with temperature control was used in all assays based on monitoring UV–Vis absorption spectra.

### *2.2. Plant Material*

In the autumn of 2020, samples of ripe medlar (*M. germanica*), blackthorn (*P. spinosa*), and hawthorn (*C. monogyna*) fruits (Figure 1) were collected from a rural area near the town of Pančevo (South Banat District, Autonomous Province of Vojvodina, Serbia). The fruits were collected in the period of full ripeness. Medlar fruits were collected at the end of October, whereas hawthorn and blackthorn were collected in the second half of November. Hawthorn fruits were harvested randomly from several trees, blackthorns fruits were also collected from several different bushes and medlar fruits were collected randomly from different trees from one plantation in the area. Samples of medlar, blackthorn, and hawthorn fruits weighed 250, 220, and 275 g, respectively. First, the fruits were washed and air-dried. After that, samples were packed in plastic boxes and stored in a freezer at a low temperature (−18 °C), until the moment of extraction.

Southern Banat is a part of the Pannonian Plain, with the relief units such as alluvial plains, loess terraces, loess plains, sandstones and rare mountains. The prevailing soil type in southern Banat is chernozem with increased content of $CaCO_3$. The rural area where the samples were collected is at the altitude 69–76 m above sea level (coordinates: latitude 44°45′ N, longitude 20°44′ E) (http://www.pancevo.rs/?wpfb_dl=882). Average temperatures in October and November 2020 were 13.4 and 6.8 °C, respectively (average annual temperature was 13.4 °C), whereas average precipitations in October and November 2020 were 81 and 10.8 mm, respectively, (average annual precipitation was 656.9 mm) (http://vreme.in.rs/pancevo-tesla/index.php; accessed on 27 October 2022).

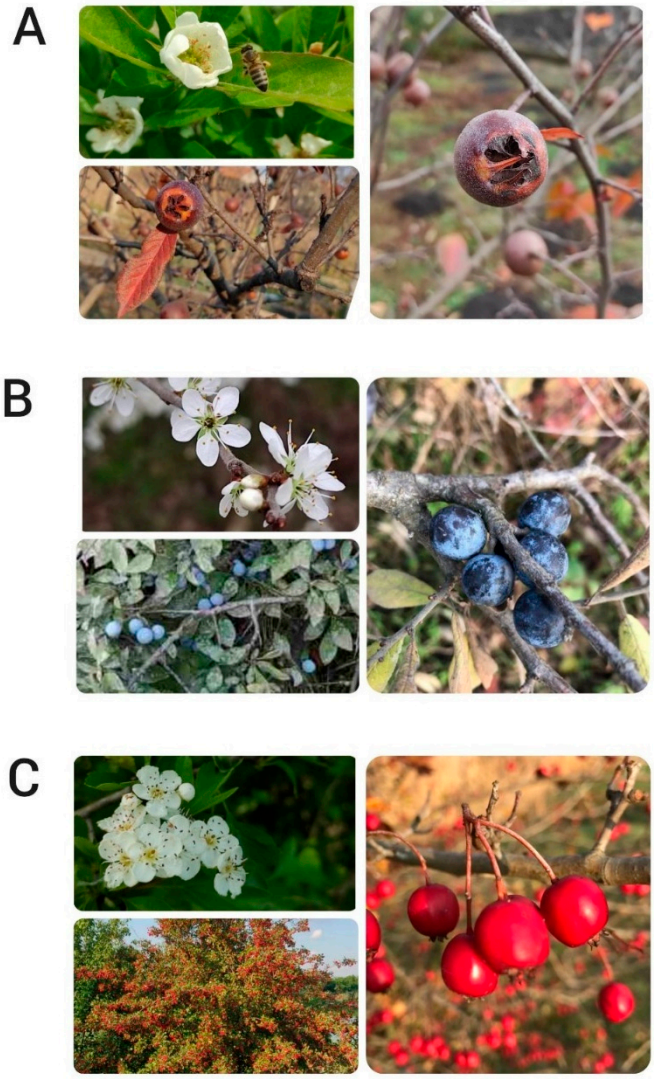


**Figure 1.** Developing stages and fruits of medlar (**A**), blackthorn (**B**) and common hawthorn (**C**) (photo by N. Mićanović).

### *2.3. Preparation of the Extracts*

The previously frozen fruits of *M. germanica*, *P. spinosa*, and *C. monogyna* were separated from seeds and finely ground in the blender. The samples were separately macerated with 75% ethanol (in a 1:5 ratio) in an ultrasound bath (240 W, 35 kHz) (Bandeln electronic, Berlin, Germany) with a temperature controller, at 25 °C for 30 min. Thereafter, the extracts were separated from plant material by vacuum filtration, and filtrates were concentrated employing a rotary evaporator (RV 10 basic, IKA, Staufen, Germany) to obtain dry extracts at maintaining temperature under 40 °C to protect phytochemical compounds from degradation and low pressure. The obtained dry extracts were kept in the refrigerator and used for further analyses.

### *2.4. Determination of Phenolic Compounds*

#### 2.4.1. Total Phenolic Content (TPC)

The method reported by Singleton et al. [23] was used to analyze total phenolic content in tested extracts. Ten-fold dilutedFolin-Ciocalteu reagent (2.5 mL) was added in 0.5 mL of tested extracts (2 mg/mL, in methanol) followed by 2 mL of $NaHCO_3$ (7.5%). The absorbance of a blue-green product was measured after 15 min of incubation at 765 nm.

Total phenolic content in samples was expressed using the constructed standard curve as equivalents of gallic acid (mg GAE/g dry extract).

#### 2.4.2. Total Flavonoid Content

For the determination of total flavonoid content in samples the aluminum trichloride method was employed [24]. In 1 mL of each sample (2 mg/mL, in methanol) the same volume of aluminum trichloride solution (2% $AlCl_3$) was added. The mixture was incubated for 1 h at room temperature. The absorbance of mixtures was measured at 415 nm and the results were shown in equivalents of rutin (mg RUE/g extract).

#### 2.4.3. Total Flavonol Content (TFlC)

The total content of flavonols in the tested extracts was determined using the method reported in the literature [25]. In 1 mL of tested extracts (2 mg/mL, in methanol) were added the same volume of 2% $AlCl_3$ and 3 mL of sodium acetate (50 mg/mL). After incubating the mixtures for 90 min, the absorbance of formed products was measured at 440 nm and the results were expressed in rutin equivalents (mg RUE/g extract).

#### 2.4.4. Total Phenolic Acid Content (TPAC)

The content of phenolic acids in three tested extracts was analyzed using the methodology from Polish Pharmacopoeia [26]. In 1 mL of samples (2 mg/mL, in methanol) were added 5 mL of distilled water and 1 mL of each HCl (0.1 M), Arnow reagent (10% $w/v$ of sodium molybdate and 10% $w/v$ sodium nitrite) and NaOH (1 M). The mixtures were filled up to 10 mL with distilled water and the absorbance was read at 490 nm, instantly. The content of phenolic acids was expressed in the equivalents of caffeic acid (mg CAE/g dry extract).

#### 2.4.5. Total Gallotannin Content (TGC)

The evaluation of gallotannin content was done according to the literature method [27]. In the reaction of potassium iodate ($KIO_3$) with galloyl esters, the red intermediate was formed leading to the final yellow compound. In 3.5 mL of samples (2 mg/mL, in methanol) was added 1.5 mL of saturated $KIO_3$, the mixture was maintained at >40 °C and the absorbance was measured at 550 nm until the maximum value was reached. Gallotannins content was expressed as gallic acid equivalents (mg GAE/g extract).

#### 2.4.6. Monomeric and Total Anthocyanin Contents (MAC and TAC)

The pH differential method was applied for the determination of monomeric anthocyanins as well as total anthocyanins (monomeric and polymerized) [28]. The samples were prepared by dissolving the extracts in water (10 mg/mL) and then in two dilutions were made with 0.025 M potassium chloride solution and with 0.4 M sodium acetate solution. The pH values of the solutions were adjusted respectively to pH 1.0 and 4.5 with HCl. The absorbance of all prepared samples was measured at two wavelengths, 520 and 700 nm. Monomeric and total anthocyanin contents were expressed as malvinidin-3-glucoside equivalents (mg Mv-3-glc/g dry extract). Absorbance (A) was calculated using the following equation:

$$A = (A_{\lambda max} - A_{700})_{pH=1.0} - (A_{\lambda max} - A_{700})_{pH=4.5}; \text{ where } \lambda_{max} = 520 \text{ nm}$$

The monomeric and total anthocyanin contents were calculated as follows:

$$\text{Monomeric anthocyanins (mg/L)} = (A \times MW \times DF \times 1000)/(\varepsilon \times L) \text{ and}$$
$$\text{Total anthocyanins (mg/L)} = (A' \times MW \times DF \times 1000)/(\varepsilon \times L);$$

where: $A' = (A_{\lambda max} - A_{700})_{pH=1.0}$; MW = molecular weight (493.43 g/mol malvinidin-3-glucoside)

DF = dilution factor; ε = molar extinction coefficient, L $mol^{-1}$ $cm^{-1}$ (28,000 L $mol^{-1}$ $cm^{-1}$ malvinidin-3-glucoside); L = path length (1 cm). All analyses of phenolic compounds content were done in triplicates.

### *2.5. Phytochemical Composition Analysis*

Chromatographic analysis was done by Dionex Ultimate 3000 UHPLC system connected to the triple-quadrupole mass spectrometer (TSQ Quantum Access Max, Thermo Fisher Scientific, Basel, Switzerland). Separation was done by Syncronis C18 column (100 × 2.1 mm, 1.7 µm particle size; Thermo Fisher Scientific, Waltham, MA, USA). All chromatographic and MS settings were the same as previously described in the literature [29]. Concentrations of target compounds in the samples were expressed as mg per kg of dry extract (dw).

### *2.6. Antioxidant Activity*

#### 2.6.1. Total Antioxidant Capacity (TAC)

The method of Prieto et al. was used for the evaluation of the total antioxidant capacity of tested fruit extracts [30]. In 0.3 mL of extract solution (2 mg/mL, in methanol) was added 3 mL of reagent solution (made of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was 90 min incubated at 95 °C and cooled to room temperature. The products' absorbance was read at 695 nm and TAC was expressed as ascorbic acid equivalents (mg AAE/g dry extract).

#### 2.6.2. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The potential scavenging activity of tested samples towards $DPPH^{\cdot}$ was monitored by the method of Kumarasamy et al. [31]. In 2 mL of eight double dilutions of extracts in methanol (from 2 mg/mL) was added 2 mL of DPPH solution (80 µg/mL). The incubation of mixtures was done at room temperature for 30 min, in the dark, and, thereafter, the absorbance was measured at 517 nm. Gallic acid, ellagic acid, caffeic acid, quercetin, rutin, ascorbic acid, and butylated hydroxytoluene (BHT) were used in this assay as the reference antioxidants (0.1 mg/mL).

The scavenging activity (%) was estimated by the following equation:

$$\text{Scavenging activity (\%)} = [(Ac - As)/Ac] \times 100;$$

where Ac is the absorbance of the $DPPH^{\cdot}$ in methanol and As is the absorbance of the samples. The $IC_{50}$ value, which represents the concentration of the samples that reduces 50% of the concentration of free-radicals, was calculated as µg/mL via a sigmoidal dose-response curve.

#### 2.6.3. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) Diammonium (ABTS) Radical-Cation Scavenging Activity

The potential scavenging activity of tested samples towards $ABTS^{\cdot+}$ was determined using previously generated radical cation using a 7 mM stock solution of ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) and 2.45 mM potassium persulfate. The mixture was left in the dark at room temperature so that the reaction could take a place, for 16 h before use [32]. The $ABTS^{\cdot+}$ solution should have absorbance at 734 nm of 0.70 ± 0.02 so it was properly diluted to get those values. The diluted $ABTS^{\cdot+}$ solution (900 µL) was added to sample solution (100 µL, eight double dilutions from 2 mg/mL, in methanol). A half hour of incubation at room temperature in a dark place followed and thereafter the absorbance was measured at 734 nm. The reference compounds were the same as previous. The same equation for the calculation of scavenging activity (%) was used and $IC_{50}$ values were expressed as previously, via a sigmoidal dose-response curve.

### *2.7. α-Glucosidase Inhibition*

Extracts were tested for α-glucosidase inhibitory activity using the method described by Matsui et al. [33] with slight modifications as reported in literature [34]. In brief, a solution of 400 mU/mL of α-glucosidase from *Saccharomyces cerevisiae* in 0.1 M phosphate buffer was prepared. Stock solutions of extracts in dimethylsulfoxide (DMSO, 50 mg/mL) were diluted in 0.1 M phosphate buffer. The final concentrations of the extracts were 10.42, 20.83, 41.67, 83.33, 166.67, and 333.33 μg/mL. The extract dilutions and control (10% DMSO) were incubated (prior to a treatment) with enzyme solution at 37 °C for 15 min. After the addition of *p*-nitrophenyl α-D-glucopyranoside (1.5 mg/mL in phosphate buffer) in each well, absorbance was measured at 405 nm. As a positive control antidiabetic drug acarbose was used. Experiments were done in duplicate. Concentration of extracts that produced 50% inhibition of α-glucosidase activity ($IC_{50}$ value) was defined using linear regression analysis.

### *2.8. MTT Assay*

Tested cell lines human cervical adenocarcinoma (HeLa), colorectal adenocarcinoma (LS-174T), and human melanoma cell line (FemX) were obtained from the ATCC (American Type Culture Collection, USA). For cells growth the RPMI-1640 medium was used. Stock solutions of extracts (50 mg/mL) were dissolved in DMSO. MTT assay was employed to determine the effects of the extracts on cell survival after 72 h of treatment [35]. Briefly, cells were seeded into 96-well microtiter plates as follows: HeLa 3000, FemX 2000, LS-174T 7000 cells per well. After overnight adherence cells were remediated with two-fold dilutions of extracts and fractions ranging from 1000 to 62.5 μg/mL. The final concentrations of DMSO were lower than 0.5% and non-toxic. Experiments were performed in triplicate and repeated three times. Concentrations of extracts that inhibited cell survival by 50% compared to control were defined as $IC_{50}$ values.

### *2.9. Statistical Analysis*

The $IC_{50}$ for the antioxidant activity was evaluated by nonlinear regression analysis from the sigmoidal dose-response inhibition curve using Origin 2019b Software (OriginLab, Northampton, MA, USA). The presented data are expressed as the mean ± standard deviation (SD) of three independent experiments. The one-way analysis of variance (ANOVA) was used for the statistical analyses accompanied by the least significant difference (LSD), Tukey, and Bonferroni tests. The results were considered statistically significant at $p < 0.05$. Pearson's correlation was performed using Excel 2010 package.

## 3. Results

### *3.1. Phytochemical Composition of Fruit Extracts*

The content of various groups of phenolic compounds of tested *M. germanica*, *P. spinosa,* and *C. monogyna* fruit extracts was measured using spectrophotometric methods and the obtained results are shown in Table 1. The results of total phenolic content (TPC), expressed in gallic acid equivalents, showed that blackthorn fruit extract was the richest in phenolic compounds (25.9 mg GAE/g) while medlar and hawthorn extracts had slightly lower TPC values (16.7 and 14.9 mg GAE/g, respectively). The blackthorn extract also had the highest content of flavonoids and flavonols (5.09 and 2.14 mg RUE/g, respectively), while medlar and hawthorn extracts had somewhat lower amounts of these compounds. Similarly, the results of total phenolic acid content showed that *P. spinosa* was the richest in phenolic acids (4.13 mg CAE/g), followed by *M. germanica* (3.20 mg CAE/g) and *C. monogyna* (2.05 mg CAE/g) extracts. The total content of gallotannins was the highest again in the *P. spinosa*, as expected, followed by *C. monogyna* and *M. germanica* extracts. Finally, *P. spinosa* ethanolic extract was the richest in the monomeric and total anthocyanins content.

**Table 1.** Phenolic compounds content in *M. germanica*, *P. spinosa* and *C. monogyna* fruit ethanolic extracts.

| Extract | Total Phenolic Content (mg GAE/g) | Total Flavonoid Content (mg RUE/g) | Total Flavonol Content (mg RUE/g) | Total Phenolic Acid Content (mg CAE/g) | Total Gallotannin Content (mg GAE/g) | Monomeric Anthocyanin Content (mg Mv-3-glc/g) | Total Anthocyanin Content (mg Mv-3-glc/g) |
|---|---|---|---|---|---|---|---|
| *M. germanica* | 16.7 ± 0.3 * [a] | 2.30 ± 0.07 [c] | 0.99 ± 0.13 [b] | 3.20 ± 0.11 [b] | 1.47 ± 0.07 [c] | 0.02 ± 0.001 [b] | 0.03 ± 0.004 [b] |
| *P. spinosa* | 25.9 ± 0.2 [a] | 5.09 ± 0.12 [a] | 2.14 ± 0.19 [a] | 4.13 ± 0.22 [a] | 2.54 ± 0.03 [a] | 0.13 ± 0.01 [a] | 0.16 ± 0.001 [a] |
| *C. monogyna* | 14.9 ± 0.7 [a] | 3.51 ± 0.05 [b] | 1.84 ± 0.23 [a] | 2.05 ± 0.07 [c] | 2.04 ± 0.13 [b] | 0.02 ± 0.001 [b] | 0.03 ± 0.001 [b] |

* Data represented as means ± SD ($n$ = 3); Means in the same column with different letters in superscript are significantly different at $p < 0.05$. GAE—gallic acid equivalents; RUE—rutin equivalents; CAE—caffeic acid equivalents; Mv-3-glc—malvinidin-3-glucoside.

The qualitative composition of phenolic compounds in ethanol extracts of *M. germanica*, *P. spinosa,* and *C. monogyna* fruits was evaluated by UHPLC-MS$^2$. The results showed the presence of eleven phenolic compounds in all three extracts and pinocembrin only in *M. germanica* extract (Table 2). Five phenolic acids were shown to be present in all tested extracts, namely protocatechuic, syringic, chlorogenic, caffeic, and *p*-coumaric acids. The protocatechuic and syringic acids were the most present in hawthorn (*C. monogyna*) extract with values of 50.75 and 40.29 mg/kg, respectively. Chlorogenic acid was the most dominant in *M. germanica* extract (78.81 mg/kg) while in other extracts it was present in a much lower amount. Caffeic and *p*-coumaric acids were represented by a smaller amount compared to other phenolic acids; again more in medlar extract than the other two (8.55 and 3.53 mg/kg, respectively). A derivative of coumarin, esculetin, 6,7-dihydroxycoumarin, or simply aesculetin, was detected in all three extracts in smaller amounts, with the highest concentration in medlar fruits (4.65 mg/kg). Another group of phenolic compounds detected in tested extracts using spectrophotometric methods were flavonoids. Using UHPLC-MS$^2$ analysis was confirmed that there were quercetin and its three derivatives as well as kaempferol glucoside. Generally, quercetin was present in a lower amount compared to its derivatives, with the highest concentration in blackthorn extract (13.71 mg/kg). The same extract had the highest concentration of rutin (quercetin-3-*O*-rutinoside) and quercetin-3-*O*-rhamnoside (104.12 and 79.66 mg/kg, respectively) compared with a much lower quantity of these compounds in medlar and hawthorn extracts. Quercetin-3-*O*-glucoside was the dominant compound in hawthorn extract with 148.86 mg/kg. Moreover, the same extracts had the highest amount of kaempferol-3-*O*-glucoside (42.58 mg/kg) compared to medlar and hawthorn. Flavanone pinocembrin was detected only in medlar fruit extract.

**Table 2.** UHPLC/(−)HESI–MS/MS quantitative analysis of phenolic compounds in *M. germanica*, *P. spinosa* and *C. monogyna* fruit ethanolic extracts.

| mg/kg dw | *M. germanica* | *P. spinosa* | *C. monogyna* |
|---|---|---|---|
| Protocatechuic acid | 12.91 ± 0.39 * | 14.38 ± 0.29 | 50.75 ± 0.93 |
| Syringic acid | 10.24 ± 0.33 | 11.38 ± 1.86 | 40.29 ± 4.84 |
| Chlorogenic acid | 78.81 ± 1.45 | 22.32 ± 0.47 | 12.19 ± 0.19 |
| Caffeic acid | 8.55 ± 0.40 | 1.82 ± 0.01 | 0.48 ± 0.10 |
| Aesculetin | 4.65 ± 0.34 | 0.86 ± 0.10 | 0.11 ± 0.03 |
| Rutin | 2.92 ± 0.25 | 104.12 ± 13.11 | 3.54 ± 0.24 |
| *p*-Coumaric acid | 3.53 ± 0.18 | 1.53 ± 0.42 | 1.41 ± 0.17 |
| Quercetin-3-*O*-glucoside | 11.71 ± 0.41 | 22.86 ± 0.46 | 148.86 ± 0.61 |
| Kaempferol-3-*O*-glucoside | 3.12 ± 0.06 | 5.94 ± 0.14 | 42.58 ± 0.68 |
| Quercetin-3-*O*-rhamnoside | 14.04 ± 1.22 | 79.66 ± 4.33 | 1.23 ± 0.09 |
| Quercetin | 2.00 ± 0.11 | 13.71 ± 0.91 | 3.13 ± 0.47 |
| Pinocembrin | 0.44 ± 0.03 | ND ** | ND |

* Concentration is presented as mg per kg of dry weight (mg/kg dw); Values are means of three replicates ± SD; ** ND—not detected.

### 3.2. Antioxidant Activity

The antioxidant activity of *M. germanica*, *P. spinosa*, and *C. monogyna* fruit ethanolic extracts was evaluated using three in vitro spectrophotometric assays to demonstrate the total antioxidant capacity and scavenging potential of extracts towards DPPH free radicals and ABTS free radical cations. The results are presented in Table 3 and compared to the activities of phenolic compounds gallic, ellagic, and caffeic acids, quercetin, and rutin as well as standard antioxidants vitamin C (ascorbic acid) and synthetic compound butyl hydroxytoluene (BHT).

**Table 3.** Antioxidant activity of *M. germanica*, *P. spinosa* and *C. monogyna* fruit ethanolic extracts and standard antioxidants.

| Fruit Extracts | Total Antioxidant Capacity (mg AAE/g) | $IC_{50}$ (μg/mL) | |
|---|---|---|---|
| | | $DPPH^{\cdot}$ Scavenging Activity | $ABTS^{\cdot+}$ Scavenging Activity |
| *M. germanica* | 238.2 ± 12.3 * [a] | 884 ± 12 [d] | 2048 ± 144 [c] |
| *P. spinosa* | 159.0 ± 23.1 [b] | 610 ± 21 [b] | 1153 ± 23 [b] |
| *C. monogyna* | 100.4 ± 6.7 [c] | 718 ± 31 [c] | 2964 ± 375 [d] |
| **Standards** | | | |
| Gallic acid | - | 0.91 ± 0.10 [a] | 4.42 ± 0.09 [a] |
| Ellagic acid | - | 0.46 ± 0.05 [a] | 5.74 ± 0.28 [a] |
| Caffeic acid | - | 2.84 ± 0.19 [a] | 10.9 ± 1.7 [a] |
| Quercetin | - | 1.51 ± 0.14 [a] | 8.15 ± 0.31 [a] |
| Rutin | - | 4.95 ± 0.26 [a] | 56.6 ± 1.5 [a] |
| Ascorbic acid | - | 3.16 ± 0.09 [a] | 16.7 ± 1.9 [a] |
| BHT | - | 12.9 ± 0.9 [a] | 24.0 ± 2.9 [a] |

* $IC_{50}$ values were determined by nonlinear regression analysis. AAE—ascorbic acid equivalents; The results are mean values ± SD from three independent experiments; - not tested; Means in the same column with different letters in superscript are significantly different at $p < 0.05$.

The total antioxidant activity of tested extracts showed that *M. germanica* fruits had the highest capacity to reduce molybdenum, so 1 g of this extract had the same activity as 238.2 mg of ascorbic acid activity. This result was followed by *P. spinosa* extracts with 159.0 mg AAE/g and *C. monogyna* with 100.4 mg AAE/g of activity. Among all tested extracts the lowest $IC_{50}$ value for $DPPH^{\cdot}$ scavenging activity exerted *P. spinosa* extract, followed by hawthorn and medlar extracts ($IC_{50}$ values 610, 718, and 884 μg/mL, respectively). Nevertheless, all pure reference compounds showed much higher and statistically significant ($p < 0.05$) scavenging effects, with $IC_{50}$ values in a range from 0.46 μg/mL for ellagic acid to 12.9 μg/mL for BHT. In the case of ABTS radical-cation, a similar trend was followed as *P. spinosa* extract again had the highest scavenging potential ($IC_{50}$ value 1153 μg/mL). The activities of medlar and hawthorn were two or almost three times lower than blackthorn extract. Further, the scavenging capacity results of reference compounds against $ABTS^{\cdot+}$ were significantly higher than those from the tested extracts. The $IC_{50}$ was in range from 4.42 μg/mL for gallic acid to 56.6 μg/mL for rutin.

### 3.3. α-Glucosidase Inhibition and In Vitro Cytotoxicity

Potential antidiabetic activities of the ethanol extracts of *M. germanica*, *P. spinosa* and *C. monogyna* fruits were measured through their ability to inhibit carbohydrate hydrolyzing enzyme α-glucosidase (α-Glc). The $IC_{50}$ values for the α-Glc inhibitory activity of the extracts significantly differed ($p < 0.05$), as shown in Table 4. Extract of *P. spinosa* exhibited the most potent inhibitory activity with an $IC_{50}$ value of 129.46 μg/mL, followed by *M. germanica* extract with an $IC_{50}$ value of 199.84 μg/mL. Both extracts showed better α-glucosidase inhibitory activity when compared with the activity of the standard drug acarbose ($IC_{50}$ 201.38 μg/mL), although the difference was significant only for *P. spinosa*.

The extract of *C. monogyna* also exhibited α-glucosidase inhibitory activity, but significantly lower compared to other two investigated extracts as well as standard ($p < 0.05$).

**Table 4.** The $IC_{50}$ values of the α-glucosidase inhibitory activity and cytotoxic activity of the fruit extracts.

| Fruit Extract | α-Glucosidase Inhibition | HeLa * | FemX | LS174T |
|---|---|---|---|---|
| *M. germanica* | 199.84 ± 0.18 $^{b,**}$ | 624.83 ± 4.96 | 854.98 ± 9.97 $^{b}$ | >1000 |
| *P. spinosa* | 129.46 ± 0.73 $^{a}$ | >1000 | 868.25 ± 8.45 $^{b}$ | >1000 |
| *C. monogyna* | 335.71 ± 6.68 $^{c}$ | 651.80 ± 6.80 | 724.30 ± 9.42 $^{a}$ | >1000 |
| Acarbose *** | 201.38 ± 0.50 $^{b}$ | | | |

* Human tumour cell lines: cervical adenocarcinoma (HeLa), colorectal adenocarcinoma (LS-174T) and melanoma cell line (FemX); ** $IC_{50}$ (μg/mL)—Concentrations of examined extracts inducing a 50% decrease in cells survival rate; Results are presented as the mean value ± SD of three independent experiments; Means in the same column with different letters in superscript are significantly different at $p < 0.05$. *** Acarbose—antidiabetic drug used as standard.

Human cervical adenocarcinoma cells (HeLa) were the most susceptible to the extracts' cytotoxic effects (Table 4). Medlar fruits extract showed the strongest cytotoxic activity on HeLa cells with an $IC_{50}$ value of 624.83 μg/mL, followed by the extract of hawthorn fruits with an $IC_{50}$ value of 651.80 μg/mL. All three of the tested extracts exerted weak cytotoxic activity against human melanoma cells (FemX), in the following order of potency: medlar > blackthorn > hawthorn. Cytotoxic activity of medlar extract toward FemX was significantly higher compared to other two extracts ($p < 0.05$, Table 4). None of the tested extracts showed any cytotoxic activity against human colon carcinoma cells (LS-174-T) at the highest tested concentrations of 1000 μg/mL. The rate of HeLa and FemX cells survival after 72 h of cell growth in the presence of different concentrations of studied fruit extracts was decreasing with the increasing concentrations. The lowest rates were observed for medlar (HeLa cells) and common hawthorn extracts (FemX cells) at the concentration of 1000 mg/mL (Figure 2).

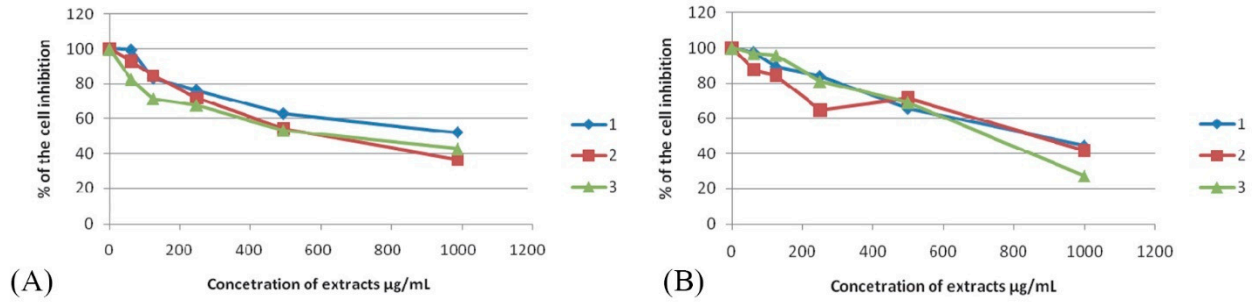


**Figure 2.** Representative graph of HeLa (**A**) and FemX (**B**) cells survival after 72 h cell growth in the presence of increasing concentrations of studied extracts *P. spinosa* (1), *M. germanica* (2), and *C. monogyna* (3).

### 3.4. Pearson's Correlation

Correlation between studied species (*M. germanica*, *P. spinosa* and *C. monogyna*) performed by using chemical parameters, polyphenolic compounds, and antioxidant assays, indicated a high degree of correlation between species (Table 5). Hence, based on the obtained results, Pearson's correlation was done to gain an insight into contribution of total phenolic content (TPC), total flavonoid content (TFC), total phenolic acid content (TPAC), total anthocyanin content (TAC), $DPPH^{\cdot}$ scavenging activity, $ABTS^{\cdot+}$ scavenging activity and total antioxidant capacity (TAntioxC) in plant material to α-Glc inhibition (α-GlcI).

**Table 5.** Correlation coefficients obtained for relationships between phenolic compounds content and antioxidant activity in *M. germanica*, *P. spinosa* and *C. monogyna* fruit ethanolic extracts.

| | **TPC *** | **TFC** | **TPAC** | **TAC** | **DPPH·** | **ABTS·+** | **TAntioxC** |
|---|---|---|---|---|---|---|---|
| TPC | 1.000 | | | | | | |
| TFC | 0.825 ** | 1.000 | | | | | |
| TPAC | 0.908 | 0.513 | 1.000 | | | | |
| TAC | 0.988 | 0.902 | 0.834 | 1.000 | | | |
| DPPH· | −0.698 | −0.980 | −0.334 | −0.799 | 1.000 | | |
| ABTS·+ | −0.930 | −0.559 | −0.999 | −0.863 | 0.385 | 1.000 | |
| TAntioxC | 0.067 | −0.508 | 0.478 | −0.086 | 0.668 | −0.430 | 1.000 |

* Parameters: TPC-Total phenolic content; TFC—Total flavonoid content; TPAC—Total phenolic acid content; TAC—Total anthocyanin content; DPPH·—2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity; ABTS+—2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium radical-cation scavenging activity; TAntioxC—Total antioxidant capacity; ** Values higher than 0.800 were considered as high positive correlations.

Based on the results presented in Table 6, high positive correlations were observed for the following parameters: TFC: TPC (0.825), TPAC: TPC (0.908), TAC: TFC, TAC:TFC and TAC:TPAC (0.988, 0.902 and 0.834, respectively). Chlorogenic acid, caffeic acid and aesculetin were positively correlated to DPPH· scavenging activity and total antioxidant capacity, whereas protocatechuic acid and syringic acid were positively correlated with ABTS·+ scavenging activity. High positive correlations were found for: rutin: TPC (0.987), rutin:TFC (0.904), rutin: TPAC (0.831) and rutin: TAC (1.00). A negative correlation between rutin and DPPH· and ABTS·+ scavenging activities (−0.80 and −0.86, respectively) proves a positive linkage between this bioactive compound and fruit extracts since in these assays antioxidative activity is expressed as $IC_{50}$ values. *p*-Coumaric acid was correlated positively to DPPH and total antioxidant capacity, as well as to chlorogenic acid, caffeic acid, and aesculetin. acid. Quercetin and its derivative quercetin-3-*O*-rhamnoside were highly correlated to TPC, TFC, TPAC and TAC. Moreover, quercetin was in high correlation with quercetin-3-*O*-rhamnoside (0.97). Glucosides quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside were correlated to DPPH· and ABTS·+ scavenging activities and two phenolic acids, protocatechuic and syringic. Kaempferol-3-*O*-glucoside showed high positive correlation with quercetin-3-*O*-glucoside. Inhibitory activity toward α-Glc (α-GlcI) was in positive correlations with some phenolic acids (protocatechuic, syringic, and *p*-coumaric), ABTS·+ scavenging activity, quercetin-3-*O*-glucoside, and kaempferol-3-*O*-glucoside. Negative correlations were found between α-GlcI and TPC, TPAC, rutin, quercetin, and quercetin-3-*O*-rhamnoside.

**Table 6.** Pearson's correlation between studied traits in *M. germanica*, *P. spinosa* and *C. monogyna* fruit ethanolic extracts.

| | TPC * | TFC | TPAC | TAC | DPPH· | ABTS·+ | TAntioxC | Protocatechuic Acid | Syringic Acid | Chlorogenic Acid | Caffeic Acid | Aesculetin | Rutin | *p*-Coumaric Acid | Quercetin-3-*O*-glucoside | Kaempferol-3-*O*-glucoside | Quercetin-3-*O*-rhamnoside | Quercetin | α-GlcI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| TPC | 1.000 | | | | | | | | | | | | | | | | | | |
| TFC | 0.825 ** | 1.000 | | | | | | | | | | | | | | | | | |
| TPAC | 0.908 | 0.513 | 1.000 | | | | | | | | | | | | | | | | |
| TAC | 0.988 | 0.902 | 0.834 | 1.000 | | | | | | | | | | | | | | | |
| DPPH· | −0.698 | −0.980 | −0.334 | −0.799 | 1.000 | | | | | | | | | | | | | | |
| ABTS·+ | −0.930 | −0.559 | −0.999 | −0.863 | 0.385 | 1.000 | | | | | | | | | | | | | |
| TAntioxC | 0.067 | −0.508 | 0.478 | −0.086 | 0.668 | −0.430 | 1.000 | | | | | | | | | | | | |
| Protocatechuic acid | −0.599 | −0.042 | −0.879 | −0.470 | −0.155 | 0.852 | −0.839 | 1.000 | | | | | | | | | | | |
| Syringic acid | −0.600 | −0.043 | −0.879 | −0.471 | −0.154 | 0.852 | −0.839 | 1.000 | 1.000 | | | | | | | | | | |
| Chlorogenic acid | −0.227 | −0.737 | 0.201 | −0.373 | 0.856 | −0.148 | 0.957 | −0.644 | −0.643 | 1.000 | | | | | | | | | |
| Caffeic acid | −0.213 | −0.728 | 0.215 | −0.360 | 0.849 | −0.162 | 0.961 | −0.654 | −0.654 | 1.000 | 1.000 | | | | | | | | |
| Aesculetin | −0.214 | −0.728 | 0.214 | −0.361 | 0.849 | −0.161 | 0.960 | −0.654 | −0.653 | 1.000 | 1.000 | 1.000 | | | | | | | |
| Rutin | 0.987 | 0.904 | 0.831 | 1.000 | −0.802 | −0.860 | −0.091 | −0.465 | −0.466 | −0.378 | −0.365 | −0.366 | 1.000 | | | | | | |
| *p*-Coumaric acid | −0.315 | −0.796 | 0.111 | −0.456 | 0.899 | −0.057 | 0.926 | −0.571 | −0.571 | 0.996 | 0.994 | 0.995 | −0.460 | 1.000 | | | | | |
| Quercetin-3-*O*-glucoside | −0.568 | −0.003 | −0.860 | −0.435 | −0.194 | 0.831 | −0.860 | 0.999 | 0.999 | −0.673 | −0.683 | −0.683 | −0.430 | −0.603 | 1.000 | | | | |
| Kaempferol-3-*O*-glucoside | −0.575 | −0.012 | −0.864 | −0.444 | −0.185 | 0.836 | −0.855 | 1.000 | 1.000 | −0.666 | −0.677 | −0.676 | −0.439 | −0.596 | 1.000 | 1.000 | | | |
| Quercetin-3-*O*-rhamnoside | 1.000 | 0.825 | 0.908 | 0.988 | −0.698 | −0.930 | 0.067 | −0.599 | −0.600 | −0.227 | −0.214 | −0.214 | 0.988 | −0.315 | −0.567 | −0.575 | 1.000 | | |
| Quercetin | 0.971 | 0.936 | 0.782 | 0.996 | −0.849 | −0.815 | −0.173 | −0.391 | −0.392 | −0.453 | −0.440 | −0.441 | 0.997 | −0.532 | −0.355 | −0.363 | 0.971 | 1.000 | |
| α-GlcI | −0.852 | −0.407 | −0.993 | −0.762 | 0.219 | 0.985 | −0.580 | 0.930 | 0.930 | −0.317 | −0.331 | −0.330 | −0.758 | −0.230 | 0.915 | 0.919 | −0.851 | −0.702 | 1.000 |

* Parameters: TPC—Total phenolic content; TFC—Total flavonoid content; TPAC—Total phenolic acid content; TAC—Total anthocyanin content; DPPH· scavenging activity; ABTS·+ radical-cation scavenging activity; TAntioxC—Total antioxidant capacity; α-GlcI—α-glucosidase inhibition; ** Values higher than 0.800 were considered as high positive correlations.

## 4. Discussion

The presented comparative study of phytochemical composition and biological activities of selected wild fruit extracts showed significant differences in polyphenolic content as well as the various scale of bioactivities. Blackthorn (*P. spinosa*) possessed the highest content of TPC and individual groups of phenolic compounds, but also exerted the highest free radical scavenging activity. In the territory of Europe and the Near East, the blackthorn fruits are in long-term use in the treatment of cardiovascular diseases, like myocarditis and atherosclerosis, which are not diminished by taking into account their pungent taste [15,36]. In the Balkans, blackthorn fruits are used in various forms, prepared as juice, wine, jams, or tea [37]. Principally, the phytochemicals that are the most responsible for the pharmacological activity of blackthorn are various classes of phenolic compounds, such as flavonoids, phenolic acids, anthocyanins, proanthocyanidins, and tannins [38].

A significant concentration of anthocyanins in ethyl acetate extract of blackthorn fruits from Serbia (461.27 mg cyanidin-3-glucoside eq/kg fresh fruit), comparable to Aronia fruits were reported in the literature [39]. In the same study, the authors showed a high level of antioxidant activity of blackthorn fruits in the terms of neutralizing free radicals (DPPH radicals and ABTS radical cations) as well as significant reducing power, comparable to those of raspberry and Aronia. It was demonstrated that the biological activity of blackthorn fruits is well correlated with the content of anthocyanins. Another study with blackthorn fruits from Serbia [40] reported quite high total phenolic, total tartaric esters, and flavonols contents in blackthorn extract, with particularly abundant phenolic acids, gallic and syringic acids (150.21 and 48.14 mg/kg fresh fruit, respectively), along with quercetin-3-glycoside, rutinoside, and procyanidin B2. The analysis of different genotypes of blackthorn fruits from North Serbia showed a great amount of hydroxycinnamic acids, particularly neochlorogenic acid (cca. 300–500 mg/100 g dw), flavonoids like quercetin and its derivatives, as well as cyanidin derivatives at high concentrations (cyaniding-3-glucoside and cyaniding-3-rutinoside with over 100 mg/100 g dw in cases of some genotypes) [41]. The tested genotypes exerted antioxidant properties similar to those presented here, the inhibitory activity of α-amylase and α-glucosidase, as well as anti-proliferative potential on HT29 colorectal cancer cell line. Similar chemical composition was reported in the literature [42] with a high concentration of neochlorogenic and caffeic acids and cyanidin-3-*O*-derivatives. Ethanol-water extract of blackthorn fruits from Southeast Serbia had the highest content of TPC (20.94 mg GAE/g), total flavonoids (1.24 mg QUE/g), and anthocyanins (0.24 mg cyanidin-3-*O*-glucoside/g) [42], similar to this study, compared with ethanol, methanol, methanol-water, and water extracts, while the highest antioxidant capacity was demonstrated by methanol extracts. Besides phenolic compounds, the high content of tocopherols and vitamin C contributes to blackthorns' high antioxidant activity [43]. The comparative study of blackthorn and hawthorn fruit extracts from Serbia showed consistency with the results of the present study in terms of total phenolic content and antioxidant effects. In the aforementioned study phytochemical analysis of *P. spinosa* and *C. monogyna* fruit extracts from Central Serbia revealed the presence of the following compounds in both species: protocatechuic acid, aesculin, chlorogenic (5-*O*-caffeoylquinic) acid, vanillic acid, ellagic acid, ferulic acid, rutin, quercetin 3-*O*-galactoside, naringin, naringenin, and apigenin [44]. Hawthorn fruits had a much higher content of kaempferol and its derivatives compared with blackthorn extract, as reported in this study as well. It was demonstrated that blackthorn extract was dominant in terms of the highest content of phenolic compounds, compared to extracts of hawthorn and medlar. Regarding the total content of phenolics, it was reported that blackthorn, in comparison to hawthorn, had much higher total phenolic compounds content, including phenolic acids and anthocyanins, while hawthorn had a substantially higher content of vitamin C, which was certainly reflected in the strong correlation of their antioxidant performance in ABTS, DPPH, and FRAP assays with the content of polyphenolics [10]. Another study also confirmed almost two-fold higher levels of vitamin C in hawthorn fruits, compared to blackthorn (15.19 and 7.73 mg of total vitamin C per 100 g fresh weight, respectively) [45]. The biological

effects also depend on the state of fruit ripeness in the case of hawthorn fruits [18]. They came to the interesting conclusion that unripe hawthorn fruits had the most prominent antioxidant properties in comparison to ripe and overripe fruits. The medlar fruit extract possessed the highest total antioxidant capacity but lower free radical scavenging ability compared with the other two extracts. It possessed somewhat higher TPC compared with hawthorn, but lower than blackthorn, and the lowest levels of all other groups of phenolics except total phenolic acids. Medlar extracts also had the highest contents of chlorogenic, *p*-coumaric, and caffeic acids among all tested samples. The high content of minerals Ca, K, P, Mg, and Na in ripened medlar fruits was reported in the literature [46]. The medlar fruit is particularly important as a rich source of K and Ca [47]. The most abundant phenolic compound in medlar was chlorogenic acid (78.81 mg/kg d.e.), which was confirmed by multiple research groups [47–49]. Out of three tested fruits in the present study, medlar extracts had the highest content of chlorogenic acid although it showed lower TPC and total phenolic acid content compared to blackthorn extract. The tests on medlar leaf, bud, and fruit extracts showed that fruit extract had the lowest content of phenolic compounds and consequently the lowest antiradical potential [50]. The content of phenolic compounds may vary between genotypes [51]. Tessa et al. [49] reported that medlar fruits contained a low amount of polyphenolics compared to a significantly high content of organic acids and monoterpenes. These compounds may also be responsible for the biological activity of medlar fruits.

α-Glucosidase (α-Glc) is an enzyme that hydrolyzes starch and disaccharides to a single D-glucose. Inhibitors of α-glucosidase constitute an important class of antidiabetic drugs used in the treatment of DMT2 and obesity, as they reduce postprandial hyperglycemia by suppressing the absorption of glucose [52]. An array of chemical compounds recognized from numerous plants exhibit inhibitory activities against the α-Glc enzyme. Alkaloids, phenolic acids, flavonoids, terpenoids, anthocyanins, and their glycosides are secondary metabolites are that are considered natural α-Glc inhibitors [21,53]. It is reported in the literature that some flavonoids (quercetin, rutin, luteolin, quercetin-3-*O*-α-l-rhamnopyranoside, epicatechin gallate, etc), anthocyanins (cyanidin, delphinidin, and pelargonidin) [54], as well as phenolic acids (*p*-hydroxycinnamic acid, protocatechuic acid, caffeic acid, syringic acid, ferulic acid, and ellagic acid), inhibit α-Glc [21,50]. Papers reporting the antidiabetic potential of medlar fruit are scarce. Antidiabetic activity of medlar fruit extract expressed as percent of inhibition of α-amylase and α-glucosidase was reported in the work of Isbilir et al. [50], whereas Żołnierczyk et al. [47] reported data on the inhibition of α-amylase with medlar fruit extracts. The ethanolic extract of *P. spinosa* fruit showed the highest anti-α-Glc activity among the studied fruit extracts. Furthermore, it had a higher $IC_{50}$ value compared to the standard antidiabetic drug acarbose. The obtained results were in line with the literature, which showed higher α-Glc inhibitory activity of hawthorn fruit extracts than acarbose and a positive correlation with the total content of phenolics and individual polyphenols [4,55]. In vitro and in vivo experiments proved the antidiabetic activity of hawthorn fruit extracts [4]. Antidiabetic activity of the medlar and hawthorn fruit extracts obtained in this work could be attributed to the content of total phenolic compounds, total phenolic acids, rutin, quercetin, and quercetin-3-*O*-rhamnoside, as a positive linkage between the antidiabetic activity of fruit extracts and these compounds was observed.

Cytotoxicity of studied fruit extracts toward three human tumor cell lines (HeLa, FemX, and LS174T) was rather low. The results obtained for the antitumor activity of blackthorn extract toward HeLa were in line with the literature [41]. The authors reported that cytotoxicity potential varies between the *P. spinosa* genotypes. Literature data also showed that blackthorn fruit extract exerts non to significant cytotoxicity depending on the human tumor cell line [41,56–58]. The extract of hawthorn fruits also expressed antitumor activity toward several cell lines [4].

## 5. Conclusions

The examination of extracts obtained from the fruits of medlar (*M. germanica*), blackthorn (*P. spinosa*), and hawthorn (*C. monogyna*) showed that each fruit extract was distinguished by a specific composition of polyphenolic compounds. Pinocembrin in medlar fruit is the first time reported in this paper, along with data about the polyphenolic composition and antioxidant activity of medlar fruit from Serbia. The blackthorn extract stood out for the pronounced amount of rutin and quercetin-3-*O*-rhamnoside. The results of antioxidant activity assays showed that medlar fruit had the highest total antioxidant capacity whereas blackthorn extract had the highest scavenging potential ($DPPH^{\cdot}$ and $ABTS^{\cdot+}$). The antidiabetic potential of medlar and blackthorn was notable, and the extract of these fruits showed the most potent inhibitory activity with $IC_{50}$ values comparable to the standard antidiabetic drug acarbose. Studied medlar, blackthorn, and hawthorn fruits are good natural sources of bioactive compounds which provide numerous opportunities for their application in the food industry for the development of new value-added foods or the improvement of existing food products.

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