Emirates Journal of Food and Agriculture. 2022. 34(2): 135-143
doi: 10.9755/ejfa.2022.v34.i2.2819
http://www.ejfa.me/

RESEARCH ARTICLE

Prunus padus L. as a source of functional compounds – antioxidant activity and antidiabetic effect

Aleksandra Telichowska[1]*, Joanna Kobus-Cisowska[2], Judyta Cielecka-Piontek[3], Szymon Sip[3], Kinga Stuper-Szablewska[4], Piotr Szulc[5]

[1] Foundation for the Education of Innovation and Implementation of Modern Technologies, 62-069 Dabrowka, Poland, [2] Department of Gastronomy Sciences and Functional Food, Poznan University of Life Sciences, 60-637 Poznan, Poland, [3] Department of Pharmacognosy, Poznan University of Medical Sciences, 61-701 Poznan, Poland, [4] Department of Chemistry, Poznan University of Life Sciences, 60-637 Poznan, Poland, [5] Department of Agronomy, Poznan University of Life Sciences, 60-637 Poznan, Poland

ABSTRACT

The search for natural raw materials and their beneficial properties has recently become very popular. Bird cherry (*Prunus padus* L.) is a plant that grows mainly in Europe. The properties of its individual anatomical parts stem from the content of numerous characteristic compounds. The aim of the paper was to assess the impact of the way of bird tree (*P.padus*) fruit and bark extraction on the antioxidant potential and the assessment of inhibitory activity against alpha-glucosidase, catalase and glutathione reductase and peroxidase. Bark extract had a greater antioxidant potential. The highest chelating activity was determined for acetone-water extract of black cherry bark (44.87%), and ethanolic bark extract showed the highest reducing power. These activities, however, were similar for all extracts tested. It was demonstrated that ferulic acid and gallic acid predominated among polyphenols in aqueous extracts, together with quercetin and catechin, whose presence and proportion of occurrence probably determined the inhibitory activity against alpha-glucosidase, whose activity for fruit extract was determined at the level of 27.11 IC_{50} mg/mL.

Keywords: Antidiabetic; Antioxidant; Alpha-glucosidase; Prunus padus; Catalase; Glutathione; Polyphenols

INTRODUCTION

Two fractions of bird tree, fruit and bark, are currently used in food technology and diet therapy. These raw materials have high antioxidant potential that stems from the high content of polyphenolic compounds. Although bird tree bark contains many bioactive chemical compounds, it is particularly rich in catechins (Telichowska et al., 2021). Catechin and its derivatives are believed to contribute to the beneficial effects of herbal and tea infusions and are effective scavengers of reactive oxygen species *in vitro*, and may indirectly act as antioxidants through their impact on transcription factors and enzyme activity (Higdon & Frei, 2003).

Type 2 diabetes mellitus is a chronic metabolic disorder that is characterized by high blood glucose levels (De Boer et al., 2017). Despite the availability of effective drug treatments of type 2 diabetes, drug resistance is increasingly often observed. In carbohydrate metabolism, it is important to delay glucose absorption by inhibiting enzymes such as alpha-glucosidase.

Alpha-glucosidase belongs to the class of hydrolases. It is produced by glands located in the puoches of the small intestine (Kim et al., 2008). The specificity of alpha-glucosidase is mainly directed towards hydrolysis of alpha-1,4-glycosidic bonds of oligosaccharides and polysaccharides, although in the case of the latter, the reactions occur more slowly or do not occur at all. The end product of the reaction is glucose, which is absorbed from the digestive system into the bloodstream. As a result of the activity of alpha-glucosidase inhibitors, breakdown of polysaccharides in the gastrointestinal tract is reduced, and, thus, so is the amount of glucose absorbed into the blood (Truscheit et al., 1981).

Natural resources represent a vast and highly diverse set of compounds in which potential therapeutic agents can be sought. Currently, scientists are looking for new,

***Corresponding author:**
Aleksandra Telichowska, Foundation for the Education of Innovation and Implementation of Modern Technologies, 62-069 Dabrowka, Poland, **E-mail:** aleksandra.telichowska@mail.up.poznan.pl

Received: 15 October 2021; **Accepted:** 14 January 2022

natural preparations that may have an impact on lowering the activity of alpha-glucosidase. Numerous medical and nutritional studies have shown that natural polyphenols play a key role in the prevention and control of various disease. Adequate content and proportion of phytochemicals in plant materials may have therapeutic significance and help the treatment of diabetes.

Insulin resistance is a key defect associated with obesity and type 2 diabetes. The exact factors that lead to insulin resistance have not been fully elucidated, but there is a strong association between insulin resistance and abnormal lipid accumulation in insulin target tissues. Chronic production of reactive oxygen species (ROS) by the mitochondria may also contribute to the development of insulin resistance. Oxidative imbalance in cells is a factor in increased susceptibility to the development of inflammation and disease. Therefore, protective and antioxidant enzymes such as glutathione peroxidase, glutathione oxidase, and catalase can be potential protection for cells. The enzyme catalase (CAT) is involved in the body's enzymatic defense against free radicals. Catalyzes the reduction reaction of hydrogen peroxide leading to the formation of molecular oxygen and water. Glutathione (GPx) in oxidized form (glutathione peroxidase) participates in the first and second lines of defense against free radicals. It protects cells against peroxides produced in the biochemical process. Glutathione peroxidase is a metalloenzyme and participates in the reduction of hydrogen peroxide with the simultaneous transformation of reduced glutathione into its oxidized form. The enzyme glutathione peroxidase (GPx) requires the presence of selenium and glutathione to function properly. Literature data show that the excess of free radicals decreased mitochondrial function associated with insulin resistance in model animal systems (Boudina et al., 2007; Harmon et al., 2009; Højlund et al., 2003) There are also studies in which the unexpected consequence of overexpression of GPx in mice is the development of insulin resistance, hyperinsulinemia, and obesity (McClung et al., 2004).

Extraction method - selection of process parameters as well as the extractant is an important step in the process of extracting bioactive components from the plant matrix. The extracted components vary in terms of their structure, and their chemical structure and interactions with other nutrients are not yet fully understood and are difficult to predict.

Polyphenols, which are the dominant group of bird tree phytochemicals, are also susceptible to oxidation (Telichowska, Kobus-Cisowska, Stuper-Szablewska, et al., 2020). High temperature and alkaline conditions cause their degradation. Studies on plants of the *Prunus* species conducted so far, have been focusing on phenolic acid content, flavonoids, antioxidant activity and many other issues. However, differences in antioxidant activity that depend on the extractants used have not yet been investigated for bird tree bark and fruit. In previous papers we demonstrated that due to the content of diverse groups of compounds, individual parts of the bird tree can exhibit many properties that are beneficial to human health (Telichowska, Kobus-Cisowska, Ligaj, et al., 2020; Telichowska, Kobus-Cisowska, Stuper-Szablewska, et al., 2020).

The aim of the paper was to assess the impact of the way of bird tree (*P.padus)* fruit and bark extraction on the antioxidant potential and the assessment of inhibitory activity against alpha-glucosidase, catalase and glutathione reductase and peroxidase.

Material

The study was conducted using bird cherry (*P.padus*) bark and fruits (Fig. 1) from an orchard in Ozierany Małe in the region of Podlasie, Poland (53º 13' 14.865" N 23º 51' 9.327" E). The mean rainfall during the growing season was 317 nm per square meter, with an average temperature of 14.4 °C, and the macronutrient content in the orchard soil was at a moderate level.

The bark and fruits was stored frozen (temperature = - 28 °C) until the freeze-drying and extract preparation process.

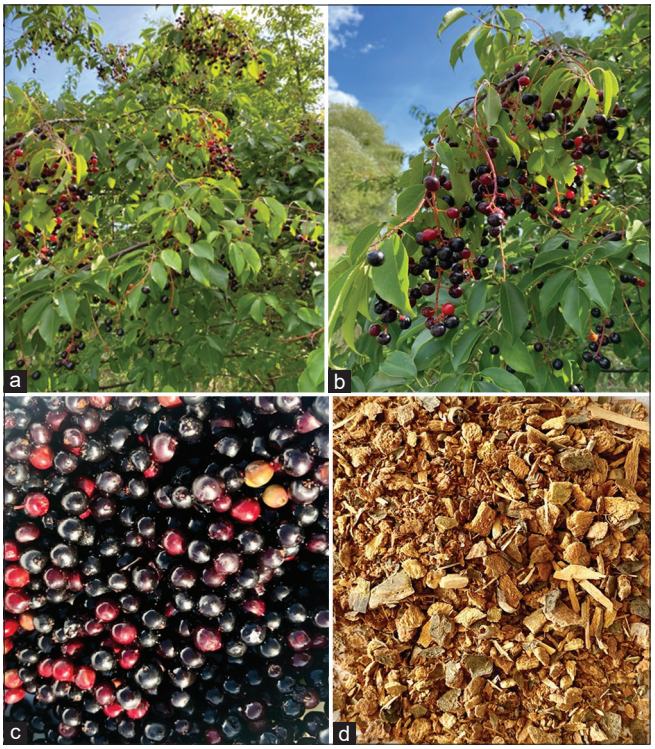

Fig 1. a) b) *Prunus padus* L. tree, c) *Prunus padus L.* fruit, d) *Prunus padus* L. bark.

Lyophilized bird cherry fruits were grated through a sieve with mesh diameter of 0.5 mm.

METHODS

Extraction

Dried and shredded fruit and bark were extracted using distilled water at 70 °C for 60 min, acetone-water solution (3:2 v/v) - at 40 °C for 60 min, and ethanol (96%) - at 40 °C for 60 min at a ratio of 2 g of shredded material per 100 ml of solvent. A single extraction was performed by shaking the whole at the specified temperature and over a specified period of time, followed by centrifugation for 5 min at 4500 rpm. In further stages of the study, we used a clear supernatant solution, which was evaporated and/or lyophilized, and stored under nitrogen in dark containers at a temperature of 4±1 °C until the time of conducting determination.

The tested extracts were designated as follows: KW - aqueous extract from bird cherry bark, KA - acetone-water extract from bird cherry bark, KE - ethanol extract from bird cherry bark, FW - aqueous extract from bird cherry fruit, FA - acetone-water extract from bird cherry fruit, and FE - ethanol extract from bird cherry fruit. Extraction parameters that included the time and temperature of the processes was were selected on the basis of literature data and preliminary results of the author's own analyses.

Antioxidant activity

Antioxidant activity with cation radical ABTS was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) assay, according to the methodology previously described by Kobus-Cisowska et al. (2020) (Kobus-Cisowska et al., 2020). The percent $ABTS^{\bullet+}$ sweep factor was calculated from the standard curve for y = 95.12x +31.54 (R2 =0.938) and expressed as mg Trolox Equivalent/g dry matter. extract.

The methodology with the DPPH radical involved a spectrophotometric measurement of the level of reduction of the absorbance of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution at 517 nm, in the presence of free radicals (Kobus-Cisowska et al., 2020). The percentage of DPPH radical scavenging was estimated on the basis of the standard curve for y = 185.21x + 19.33 (R2 = 0.941) and presented in mg Trolox Equivalent/1 g dry matter. extract.

Antioxidant properties of aqueous extracts were determined using an iron reduction assay (the FRAP method), in accordance the procedure described by O'Sullivan et al. (2013) (O'Sullivan et al., 2013). The method consisted in forming an antioxidant and iron complex ($Fe2^{+}$) by attaching a metal ion by an antioxidant molecule containing a free pair of electrons via a coordination bond (Tang et al., 2002), chelating activity was expressed in %.

The method consisted in forming an antioxidant and iron complex ($Fe2^{+}$) by attaching a metal ion by an antioxidant molecule containing a free pair of electrons via a coordination bond (Tang et al., 2002). Change in the colour of the reaction system was recorded spectrophotometrically at a wavelength of 562 nm, and chelating activity was expressed in %.

In the last two methods, the samples were prepared and tested at five different concentrations: 200 ppm, 300 ppm, 400 ppm, 600 ppm and 800 ppm for each raw material (ppm = part per million).

HPLC determination of phenolic acids and flavonols

Phenolic compounds in water samples only were analyzed after alkaline and acidic hydrolysis. The procedure was based on the method published by Telichowska et al. (2021) (Telichowska et al., 2021). The analysis was performed using an Aquity H class UPLC system equipped with Waters Acquity PDA detector (Waters, USA). Chromatographic separation was performed using Acquity UPLC® BEH C18 column (100mm×2.1mm, particle size 1.7μm) (Waters, Ireland). The results were expressed as percentage share of the conent of each component.

Inhibition of alpha-glucosidase

The inhibition of alpha-glucosidase by *P.padus* bark and fruit aqueous extracts was determined using the spectrophotometric method described by Telagari et al. (2015) (Telagari & Hullatti, 2015) with minor modifications. Briefly, 50 μl of sample solution or acarbose (positive control) with various concentrations, 50 μl of 0.1 M phosphate buffer (pH 6.8) and 30 μL alfa-glucosidase solution (1.0 U/ml) were pre-incubated in 96 well plates at 37 °C for 15 min. Then, 20 μl of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added and incubated at 37 °C for 20 min. The reaction was terminated by adding 100 μL of sodium carbonate Na_2CO_3 (0.2 M) into the mixture. The absorbance of the liberated *p*-nitrophenol was measured at 405 nm (Multiskan GO 1510, Thermo Fisher Scientific, Vantaa, Finland). The absorbance of enzyme solution but without plant extracts/acarbose served as the control with total enzyme activity. The absorbance in the absence of the enzyme was used as the blind control. The enzyme inhibition rate expressed as a percentage of inhibition was calculated using the following formula: % inhibition activity = $((A_C - A_S)/A_C)*100$, where A_C is the absorbance of the control (100 % enzyme activity) and A_S is the absorbance of the analysed sample. Two independent

experiments were carried out in triplicate for the analysed extracts. Results were expressed as means ± SD. The IC_{50} values were calculated using OriginPro 9 software with nonlinear regression. IC values$_{50}$ (the amount of lyophilizate/reference substance needed to inhibit 50% of alpha-glucosidase activity) were calculated using the linear interpolation method between values above and below 50% of activity.

Inhibition of glutathione reductase, inhibition of glutathione peroxidase and catalase

The ability to inhibit glutathione reductase was performed according to the method of Moreira et al. (2014) (Moreira et al., 2014). One unit of enzyme activity was defined as nmol NADPH consumed/min • mL of sample, compared to nmol NADPH consumed/min in the blank.

Inhibition of glutathione peroxidase was performed according to the method described by Singh et al. (2000) (Singh et al., 2000) with its own modifications previously described by Telichowska et al. 2021 (Telichowska et al., 2021). One unit of enzyme activity was defined as nmol of NADPH consumed/min • mL of sample, compared to nmol of NADPH consumed/min in the blank (reagent) (Singh, Padmavathi & Rao, 2000). Samples were analyzed in at least four replicates.

Catalase activity inhibition by a modified method according to Watanabe et al. (2007) (Watanabe et al., 2007), modifications are described in Telichowska et al. 2021 (Telichowska et al., 2021). The inhibitory activity was calculated from the formula: Inhibition [%] = 100 - 100 × (Sample Abs. 0 min - Sample Abs. 2 min)/(Control Abs. 0 min - Control Abs. 2 min.).

Statistical analysis

The routine statistical tests (average values and standard deviation) were tested. Statistical diffrences were calculated using Turkey's HSD test with significant diffrences identified at $p<0.05$ (Statistica Software ver.13.1 StatSoft, Cracow, Polnad).

RESULTS

Antioxidant activity of the tested extracts from the bark and fruit of *P.padus*

The prepared extracts were screened for antiradical activity using spectrophotometric methods. Water, acetone/water mixture and ethanol were used as extractants. All tested extracts showed antioxidant activity that depended on the type of the raw material and extractant. The highest antioxidant activity measured using the ABTS test was observed in the case of ethanolic and acetone-water extract from bird cherry fruits (FE - 1.23^{c}±0.01mM Trolox/g d.m. and FA - 1.22^{c}±0.02 mM Trolox/g d.m.), there were also two other extracts that showed a high value, i.e. KA extract - acetone-water extract from bird cherry bark (1.22^{c}±0.04 mM Trolox/g d.m.) and water extract from bark - KW 0.94^{b}±0.02mM Trolox/g d.m.

In addition, the study included an evaluation of the activity concerning the scavenging of DPPH radicals. The study showed that the ethanolic extract from fruits was most active against DPPH radicals (FE 2.43^{d}±0.13 mM Trolox/g d.m.), two other extracts also showed high capacity in the test (FA - 2.32^{c}±0.04 mM Trolox/g d.m. and KW - 2.01^{c}±0.01 mM Trolox/g d.m.). On the other hand, the aqueous extract of bird cherry fruit (FW) had the lowest antiradical activity, both in the ABTS and DPPH test, and showed values of, respectively, 0.89^{a}±0.02 mM Trolox/g d.m. and 1.76^{b}±0.09 mM Trolox/g d.m. (table 1).

Extracts of bird cherry fruit and bark were characterized with regard to their ability to reduce Fe(III) ions to Fe(II). Results of analyses were expressed by absorbance at λ = 765 nm, and are shown in Fig. 2. Concentrations of the extracts were determined experimentally in such a way that the absorbance of the samples be in the range of 0.1-0.9.

All analysed extracts showed reducing power dependent on an extract concentration. The bark ethanolic extract (KE) at a concentration of 800 ppm had the highest reducing power, while the aqueous extract of bird cherry fruit (FW) showed the lowest activity. However, the highest absorbance value, indicating a high capacity to reduce iron ions, was shown for the ethanol extract of bark (KE) at all analysed concentrations. It was found that the activity of the extract of bark (KE) hiked at the third analysed concentration and further remained stable. Perhaps the reason is that compounds act up to a certain point as antioxidants, however, plant phenols (like vitamin C) sometimes stimulate processes of an oxidative nature by reducing transition metals.

Fig. 3 shows the results of evaluating the chelating activity of extracts of bird cherry bark and fruit. It was found that the analysed extracts showed activity to chelate iron ions, which depended on both the solvent used for extraction and the type of material from which the extract was obtained. The highest chelating activity was found for acetone-water extract of bird cherry bark (KA) – 44.87 % at 800 ppm concentration. When it comes to the analysed fruit extracts, the aqueous extract of bird cherry fruit and the ethanol extract chelated the least iron ions, i.e. from 12.65 % (FW) at the lowest concentration (200 ppm) to 38.87 % (FE) at the highest concentration (800 ppm). Out of all the analysed bird cherry extracts, the lowest metal chelating capacity was shown for the acetone-water extract

Table 1: Ability to scavenge ABTS radicals and DPPH radicals by extracts from bark and fruit of bird cherry

Sample	KW	KA	KE	FW	FA	FE
ABTS (mM Trolox/ g d.m.)	$0.94^{b}\pm0.02$	$1.22^{c}\pm0.04$	$0.76^{a}\pm0.11$	$0.89^{a}\pm0.02$	$1.22^{c}\pm0.02$	$1.23^{c}\pm0.01$
DPPH (mM Trolox/ g d.m.)	$2.01^{c}\pm0.01$	$1.23^{a}\pm0.02$	$1.32^{a}\pm0.02$	$1.76^{b}\pm0.09$	$2.32^{c}\pm0.04$	$2.43^{d}\pm0.13$

Data represent the mean values from three repetitions of two series and standard deviation. Mean values marked with different capital letters in the same row indicate significance of differences ($p \leq 0.05$)

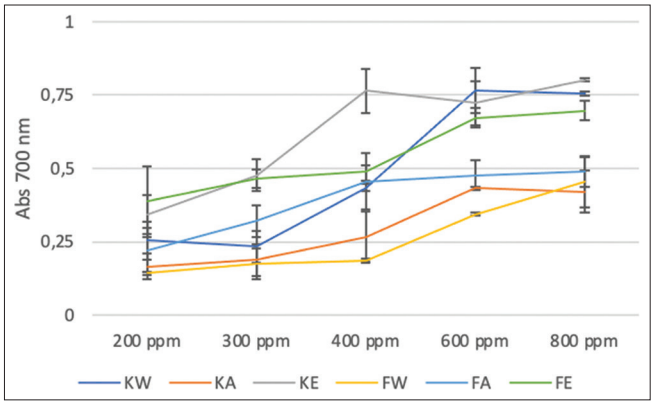

Fig 2. The reducing activity of iron ions of bird cherry bark and fruit extracts.

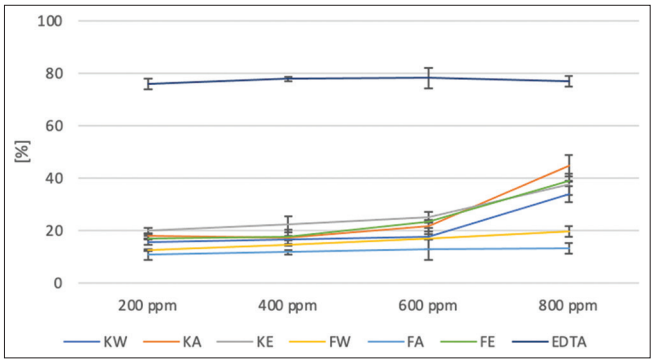

Fig 3. Chelating activity of extracts of bird cherry bark and fruit.

of bird cherry fruit (FA) – 10.87%. It was also found that an increase in the concentration of extracts from 200 ppm to 800 ppm had the greatest effect on the acetone-water extract of bird cherry bark (KA), and the chelating activity of this extract increased 2.5-fold.

The polyphenolic content in aqueous extracts of *P.padus* bark and fruit

Aqueous extracts of bird cherry bark and fruit were investigated for polyphenolic content. When it comes to flavonols, the extract of bird cherry fruit contained the most compounds such as catechin (over 75%) and quercetin (over 18%) (Fig. 4). Naringenin and kaempferol were smaller contributors. Chlorogenic acid (more than 25%), p-coumaric acid (more than 25%), and ferulic acid (more than 20%) were found to be dominant phenolic acids, while syringic acid was found to be the least dominant phenolic acid.

In the extract of bird cherry bark, however, the dominant polyphenols included catechin (over 65%), quercetin (over 40%), ferulic acid (over 45%), hydroxybenzoic acid (over 25%), and p-coumaric acid (over 20%). In contrast, the bark had low levels of compounds such as caffeic acid, rutin, kaempferol and apigenin in its composition.

Inhibition of alpha-glucosidase activity

Alpha-glucosidase is an enzyme involved in break-down of complex carbohydrates (di-, oligo- and polysaccharides) into simple sugars (e.g. into glucose). On the other hand, alpha-glucosidase inhibitors inhibit the breakdown of the alpha bonds of carbohydrates, reducing the absorption of glucose from the gastrointestinal tract into the blood, which ultimately contributes to a reduction in postprandial glycaemia. Three inhibitory drug compounds are currently used in the treatment of diabetes: acarbose, voglibose and miglitol. Acarbose acts primarily in the intestines and is virtually not absorbed from the gastrointestinal tract into the blood. Importantly, due to its mechanism of action, acarbose is only effective when consumed with food that includes products composed of polysaccharides. The extracts of analysed raw materials were tested at concentrations of 165-300 μg/mL. Moreover, IC_{50} was measured for these extracts and acarbose.

The analysis of the obtained results showed that the analysed bark extracts had a higher alpha-glucosidase inhibition capacity, which for the analysed concentrations ranged from 20.43% to 89.91% (Table 2) and was stronger than the fruit extract, whose activity was determined in the range of 16.77-78.68 % (Table 2). When tested as a standard, acarbose was the most potent inhibitor. According to the study results, IC_{50} for acarbose was lower by more than 20-fold compared to the analysed extracts, which is equivalent to higher acarbose activity as an inhibitor.

Inhibition of glutathione reductase, inhibition of glutathione peroxidase and catalase

The enzymatic antioxidant barrier is formed by enzymes such as catalase, glutathione peroxidase, and glutathione reductase. Under physiological conditions, these enzymes interact with each other, and therefore inactivation of any of these enzymes weakens the body's antioxidant defense. The lowest ability to inhibit glutathione reductase was

demonstrated by water extract from bird cherry fruits (FW - 71.0^{a} ± 1.7%), and the highest ethanol extract from bird cherry bark (KE - 77.6^{a} ± 1.7%). In the case of the ability to inhibit glutathione peroxidase, the lowest value was determined for the acetone-water extract from fruit (FE - 58.3^{a} ± 0.7%) and acetone-water extract from bark (KA - 58.3^{a} ± 1.1%), and the water extract was the most active. from bark (KW - 63.8^{a} ± 2.0%). In the case of catalase inhibition studies, the lowest activity was demonstrated by the acetone-water extract from fruit (FA - 41.1^{a} ± 0.8%), and the highest by water extract from bird cherry fruit (FW - 60.2^{c} ± 0.9%) (table 3).

DISCUSSION

Currently, there are many different methods of antioxidant compound extraction from plant raw materials, of which solvent extraction is the most common technique for isolating polyphenols (Mišan et al., 2010). Active compounds are mainly secondary metabolites that include polyphenols. The solubility of polyphenols in extractants is related to the solvent polarity, the degree of depolymerization, the interaction of polyphenols with other components or the formation of insoluble complexes. This study shows that, depending on the solvent used, antioxidant activity assays obtained extracts with different activities. This is due to the amount and proportion of active compounds that dissolve in an extractant. Flavonoids in the form of glycosides are soluble in water as well as in ethyl and methyl alcohol, whereas aglycones are soluble in organic solvents. The extraction time can take up to 24 hours, depending on the specific nature and fineness of a plant material, and this time can be reduced by using, e.g., an ultrasonic bath. In this study, different extraction time was used for selected solvents. On the other hand, it is known that time extension is not always associated with an increase in the content of bioactive compounds. In this study, the 60 min extraction time, compared to previous studies (Telichowska, Kobus-cisowska, et al., 2020; Telichowska, Kobus-Cisowska, Ligaj, et al., 2020; Telichowska, Kobus-Cisowska, Stuper-Szablewska, et al., 2020) where the aqueous extraction time was 15 min, probably caused the degradation of bound forms and perhaps inactivation of some compounds. Thus, the extraction method determines the content of each compound. According to the presented studies, extracts of bird cherry fruit usually scavenged radicals to a greater extent, while extracts of bark had higher reducing and chelating activities. The final antioxidant effect of plant raw materials is thus difficult to predict.

Sile et al. (2021) examined the total phenolic content and DPPH antiradical activity of ethanolic extracts of *P.padus* flowers (Sile et al., 2021). The total phenolic content was 85.19 mg GAE/g of extract, and the EC_{50} value in the DPPH assay was 0.55 mg/ml (Sile et al., 2021). In this study, however, the highest antiradical activity measured by the DPPH assay was observed for the ethanolic extract of fruit (2.43^{d}±0.13 mM of Trolox/g DM). In another study, methanolic extracts of *P.padus* flowers and leaves

Table 2: Inhibition of alpha-glucosidase in extracts of bird cherry fruit and bark

Concentration (μg/mL)	*Prunus padus* L. fruit (%)	*Prunus padus* L. bark (%)	Acarbose
165	16.77^{a}±0.09	20.43^{b}±0.11	-
200	23.59^{a}±0.21	36.72^{b}±0.13	-
235	32.02^{a}±0.33	53.25^{b}±0.12	-
265	46.98^{a}±0.76	76.42^{b}±0.32	-
300	78.68^{a}±0.63	89.91^{b}±0.66	-
IC_{50} mg/mL	27.11^{b}±0.19	44.98^{c}±0.08	1.22^{a}±0.07

Data represent the mean values from three repetitions of two series and standard deviation.
Mean values marked with different capital letters in the same row indicate significance of differences ($p \leq 0.05$)

Table 3: Inhibition of glutathione reductase, peroxidase and catalase

Sample (Inhibition %)	KW	KA	KE	FW	FA	FE
Glutathione Reductase	75.0^{a}±1.1	72.9^{a}±0.9	77.6^{a}±1.7	71.0^{a}±1.7	79.4^{b}±0.6	72.2^{a}±1.3
Glutathione Peroxidase	63.8^{a}±2.0	58.3^{a}±1.1	59.2^{a}±2.6	62.3^{a}±2.4	59.9^{a}±1.2	58.3^{a}±0.7
Catalase	47.4^{ab}±2.0	53.2^{b}±1.2	52.2^{b}±0.6	60.2^{c}±0.9	41.1^{a}±0.8	43.4^{a}±0.9

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($P \leq 0.05$)

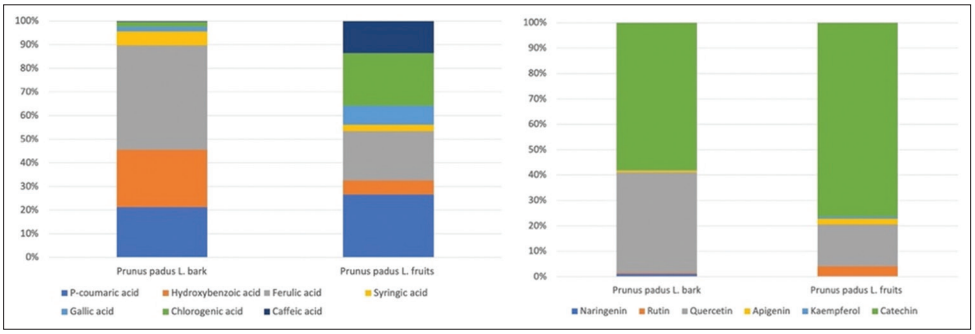

Fig 4. Polyphenols composition in *Prunus padus* L. bark and fruit aqueous extract.

were compared for antioxidant activity using the DPPH radical. The activity of leaves collected in May/June and fall leaves collected in July/August was evaluated. The highest antioxidant activity was found in leaves collected in autumn – 1.68 g g^{-1} of DPPH (Olszewska & Kwapisz, 2011). Other study investigated the antioxidant activity and polyphenol content of acetone-water extracts of *P.padus* fruit. The total polyphenol content was 11,053.3±491.28 mg GAE/kg of fruit weight (FW), and the antioxidant activity (FRAP) was 31.54±0.26 mM trolox/kg (Mikulic-Petkovsek et al., 2016). Other researchers investigated the polyphenol content of *Prunus serotina* L. fruit peel, the obtained results showed that it correlated with the antioxidant activity of fruit (r = 0.875 for DPPH and r = 0.959 for FRAP) (Luna-Vázquez et al., 2013). In this study, ethanolic extract of bird cherry bark had the highest reducing power, while the highest chelating activity was observed for acetone-water extract of bird cherry bark.

High contents of polyphenolic compounds such as catechin, quercetin, ferulic acid, p-coumaric acid and hydroxybenzoic acid were involved in the, specified in this study, activity of extracts of bird cherry bark and fruit. Sile et al. (2021) indicated that the main components of the ethanolic extract of *P.padus* flowers included diglycosides, quercetin, chlorogenic acid and spermidine N', N'-dicaffeoyl, N'-coumaroyl (Sile et al., 2021).

Other researchers indicated that caffeic acid, chlorogenic acid, coumaric acid, ferulic acid, dominant coumaric acid and 5-caffeolquinic acid 2 are mainly involved in the antioxidant activity of *P.padus* fruit (Donno et al., 2018; Kabara et al., 1972; Mikulic-Petkovsek et al., 2016). Moreover, the content of ellagic acid, gallic acid and vanillic acid is also of great importance (Donno et al., 2018). When it comes to flavonols, epicatechin and catechin as well as quercetin and its derivatives, hyperoside, kaempferol and isorhamnetin are of antioxidant importance (Donno et al., 2018).

These compounds are not only important as antioxidants but it is also believed that they play a significant role in the prevention and treatment of diabetes. Synthetic alpha-glucosidase inhibitors continue to be prescribed to control blood sugar levels, lowering sugar levels by slowing or reducing the breakdown of carbohydrates in the intestine (Scheen, 2003). However, there has been a need to find natural formulations that can naturally inhibit the alpha-glucosidase activity. In this study, it was proved that an extract of bird cherry bark and fruit might have such a role.

Similarly, beneficial antidiabetic effects of *P.padus* have been confirmed by scientists in Korea for methanol extracts of leaves and branches. It was found that the branch extract showed higher activity as indicated by higher polyphenol content compared to the leaf extract (Hyun et al., 2015). In this study, the extract of bird cherry showed higher inhibitory activity against alpha-glucosidase than the extract of bird cherry fruit. The interest in plant raw materials stems from the possibility to reduce doses of synthetic antidiabetic drugs. The possibility of reducing the dose of acarbose when used together with gallic acid was proved by Oboh et al. (2016). The 1:1 mixture of both compounds was found to inhibit glucosidase activity in 65.7% – this mixture was only slightly weaker than acarbose alone (66.2%) and much stronger than pure gallic acid (43.9±0.7%) (Oboh et al., 2016). The mixture of acarbose and gallic acid also inhibited lipid peroxidation and exhibited antioxidant activity. According to the authors, the use of acarbose combined with gallic acid (1:1) in antidiabetic therapy may help reduce the side effects of acarbose. Hence, due to its high phenolic acid content, the use of bird cherry in supporting diabetes diet therapy may be a promising direction for further animal studies and clinical trials.

CONCLUSIONS

The study results consisted of an *in vitro* evaluation of the potential for antioxidant and antidiabetic activities of bird cherry *P.padus*, which is a herbal raw material with multidirectional effects. So far, many conventional raw materials are used in the diabetes diet therapy, and the activity of bird cherry in this area has not been specified yet. It was proved that the analysed extracts of bird cherry fruit and bark inhibit alpha-glucosidase activity and they may have a supporting role as antioxidants. However, the method of extraction determines the effect of an extract. Different extractants can result in different compositions of the extract and the way how its antioxidant activity works is difficult to predict.

Also, their anti-inflammatory effect determined by the presence of polyphenols and proved in our other studies is of great importance. Bird cherry bark and fruit may be ingredients in antidiabetic preparations that support pharmacological treatment. The effectiveness of the mixtures may be due to synergism of action between active compounds.

FUNDING

This research received no external funding by Department of Gastronomy Sciences and Functional Foods of Poznan University of Life Sciences, grant number 506.751.03.00.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Telichowska,A. and Kobus-Cisowska,J.; methodology, Kobus-Cisowska, J., Cielecka-Piontek, J., Sip, Sz., Stuper-Szablewska, K.; software, Telichowska, A., Szulc, P. Sip, Sz.; Stuper-Szablewska, K.; validation, Telichowska, A., Kobus-Cisowska, J., Sip, Sz.; formal analysis, Cielecka-Piontek, J., Stuper-Szablewska, K; investigation, Szulc, P., Telichowska, A.; Cielecka-Piontek,J.; resources, Kobus-Cisowska, J.; data curation, Telichowska, A. writing—original draft preparation, Telichowska, A.; writing—review and editing, Kobus-Cisowska, J.; visualization, Szulc, P.; supervision, Kobus-Cisowska, J.; project administration, Telichowska, A.; funding acquisition, Kobus-Cisowska, J. All authors have read and agreed to the published version of the manuscript.

REFERENCES

Boudina, S., Sena, S., Theobald, H., Sheng, X., Wright, J. J., Xia, X. H., Aziz, S., Johnson, J. I., Bugger, H., Zaha, V. G. and Abel, E. D. 2007. Mitochondrial energetics in the heart in obesity-related diabetes: Direct evidence for increased uncoupled respiration and activation of uncoupling proteins. Diabetes. 56: 2457-2466.

De Boer, I. H., Bangalore, S., Benetos, A., Davis, A. M., Michos, E. D., Muntner, P., Rossing, P., Zoungas, S. and Bakris, G. 2017. Diabetes and hypertension: A position statement by the American diabetes association. Diabetes Care. 40: 1273-1284.

Donno, D., Mellano, M. G., De Biaggi, M., Riondato, I., Rakotoniaina, E. N. and Beccaro, G. L. 2018. New findings in *Prunus padus* L. fruits as a source of natural compounds: Characterization of metabolite profiles and preliminary evaluation of antioxidant activity. Molecules. 23: 725.

Harmon, J. S., Bogdani, M., Parazzoli, S. D., Mak, S. S. M., Oseid, E. A., Berghmans, M., LeBoeuf, R. C. and Robertson, R. P. 2009. β-cell-specific overexpression of glutathione peroxidase preserves intranuclear MafA and reverses diabetes in db/db mice. Endocrinology. 150: 4855-4862.

Higdon, J. V. and Frei, B. 2003. Tea catechins and polyphenols: Health effects, metabolism, and antioxidant functions. Crit. Rev. Food Sci. Nutr. 43: 89-143.

Højlund, K., Wrzesinski, K., Larsen, P. M., Fey, S. J., Roepstorff, P., Handberg, A., Dela, F., Vinten, J., McCormack, J. G., Reynet, C. and Beck-Nielsen, H. 2003. Proteome analysis reveals phosphorylation of ATP synthase β-subunit in human skeletal muscle and proteins with potential roles in Type 2 diabetes. J. Biol. Chem. 278: 10436-10442.

Hyun, T. K., Kim, H. C. and Kim, J. S. 2015. *In vitro* screening for antioxidant, antimicrobial, and antidiabetic properties of some Korean native plants on Mt. Halla, Jeju Island. Indian J. Pharm. Sci. 77: 668-674.

Kabara, J. J., Swieczkowski, D. M., Conley, A. J. and Truant, J. P. 1972. Fatty acids and derivatives as antimicrobial agents. Antimicrob. Agents Chemother. 2: 23-28.

Kim, K. Y., Nam, K. A., Kurihara, H. and Kim, S. M. 2008. Potent α-glucosidase inhibitors purified from the red alga *Grateloupia elliptica*. Phytochemistry. 69: 2820-2825.

Kobus-Cisowska, J., Szulc, P., Szczepaniak, O., Dziedziński, M., Szymanowska, D., Szymandera-Buszka, K., Goryńska-Goldmann, E., Gazdecki, M., Telichowska, A. and Ligaj, M. 2020. Variability of *Hordeum vulgare* L. cultivars in yield, antioxidant potential, and cholinesterase inhibitory activity. Sustainability. 12: 1938.

Luna-Vázquez, F. J., Ibarra-Alvarado, C., Rojas-Molina, A., Rojas-Molina, J. I., Yahia, E. M., Rivera-Pastrana, D. M., Rojas-Molina, A. and Zavala-Sánchez, M. Á. 2013. Nutraceutical value of black cherry *Prunus serotina* Ehrh. fruits: Antioxidant and antihypertensive properties. Molecules. 18: 14597-14612.

McClung, J. P., Roneker, C. A., Mu, W., Lisk, D. J., Langlais, P., Liu, F. and Lei, X. G. 2004. Development of insulin resistance and obesity in mice overexpressing cellular glutathione peroxidase. Proc. Natl. Acad. Sci. U. S. A. 101: 8852-8857.

Mikulic-Petkovsek, M., Stampar, F., Veberic, R. and Sircelj, H. 2016. Wild prunus fruit species as a rich source of bioactive compounds. J. Food Sci. 81: C1928-C1937.

Mišan, A. Č., Mimica-Dukić, N. M., Mandić, A. I., Sakač, M. B., Milovanović, I. L. and Sedej, I. J. 2010. Development of a rapid resolution HPLC method for the separation and determination of 17 phenolic compounds in crude plant extracts. Central Eur. J. Chem. 9: 133-142.

Moreira, P. R., Maioli, M. A., Medeiros, H. C. D., Guelfi, M., Pereira, F. T. V. and Mingatto, F. E. 2014. Protective effect of bixin on carbon tetrachloride-induced hepatotoxicity in rats. Biol. Res. 47: 1-7.

O'Sullivan, A. M., O'Callaghan, Y. C., O'Connor, T. P. and O'Brien, N. M. 2013. Comparison of the antioxidant activity of commercial honeys, before and after *in-vitro* digestion. Polish J. Food Nutr. Sci. 63: 167-171.

Oboh, G., Ogunsuyi, O. B., Ogunbadejo, M. D. and Adefegha, S. A. 2016. Influence of gallic acid on α-amylase and α-glucosidase inhibitory properties of acarbose. J. Food Drug Anal. 24: 627-634.

Olszewska, M. A. and Kwapisz, A. 2011. Metabolite profiling and antioxidant activity of *Prunus padus* L. flowers and leaves. Nat. Prod. Res. 25: 1115-1131.

Scheen, A. J. 2003. Pathophysiology of Type 2 diabetes. Acta Clin. Belg. 58: 335-341.

Sile, I., Videja, M., Makrecka-Kuka, M., Tirzite, D., Pajuste, K., Shubin, K., Krizhanovska, V., Grinberga, S., Pugovics, O. and Dambrova, M. 2021. Chemical composition of *Prunus padus* L. flower extract and its anti-inflammatory activities in primary bone marrow-derived macrophages. J. Ethnopharmacol. 268: 113678.

Singh, R. P., Padmavathi, B. and Rao, A. R. 2000. Modulatory influence of Adhatoda vesica (*Justicia adhatoda*) leaf extract on the enzymes of xenobiotic metabolism, antioxidant status and lipid peroxidation in mice. Mol. Cell. Biochem. 213: 99-109.

Tang, Z., Hasegawa, M., Hasegawa, M., Nagai, Y., Saito, M. and Kawazoe, Y. 2002. First-principles calculation of coincidence Doppler broadening of positron annihilation radiation. Phys. Rev. B Condens. Matter Mater. Phys. 65: 045108.

Telagari, M. and Hullatti, K. 2015. *In-vitro* α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. Indian J. Pharmacol. 47: 425-429.

Telichowska, A., Kobus-Cisowska, J., Ligaj, M., Stuper-Szablewska, K., Szymanowska, D., Tichoniuk, M. and Szulc, P. 2020. Polyphenol content and antioxidant activities of *Prunus padus* L. and *Prunus serotina* L. leaves: Electrochemical and spectrophotometric approach and their antimicrobial properties. Open Chem. 18: 1125-1135.

Telichowska, A., Kobus-Cisowska, J., Stuper-Szablewska, K., Ligaj, M., Tichoniuk, M., Szymanowska, D. and Szulc, P.

2020. Exploring antimicrobial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L. Int. J. Food Prop. 23: 2097-2109.

Telichowska, A., Kobus-cisowska, J. and Szulc, P. 2020. Phytopharmacological possibilities of bird cherry *Prunus padus* L. and *Prunus serotina* L. species and their bioactive phytochemicals. Nutrients. 12: 1-21.

Telichowska, A., Kobus-Cisowska, J., Szulc, P., Ligaj, M., Stuper-Szablewska, K., Szwajgier, D. and Bujak, H. 2021. Comparative analysis of infusions with the addition *P. padus* Bark: Assessment of the antioxidant potential and their inhibitory effect on enzymes associated with oxidative stress. Sustainability. 13: 3913.

Truscheit, E., Frommer, W., Junge, B., Müller, L., Schmidt, D. D. and Wingender, W. 1981. Chemistry and biochemistry of microbial α-glucosidase inhibitors. Angew. Chem. 20: 744-761.

Watanabe, M., de Moura Neiva, L. B., da Costa Santos, C. X., Laurindo, F. R. M. and de Fátima Fernandes Vattimo, M. 2007. Isoflavone and the heme oxygenase system in ischemic acute kidney injury in rats. Food Chem. Toxicol. 45: 2366-2371.