Research Article

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Polyphenol content and antioxidant activities of *Prunus padus* L. and *Prunus serotina* L. leaves: Electrochemical and spectrophotometric approach and their antimicrobial properties

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Abstract: The aim of the study was to compare the content of selected phytochemicals as well as the antioxidant and antimicrobial potential of the leaves of *Prunus padus* L. and Prunus serotina L., as there is very little research on this subject in the literature. Therefore, it is used to deepen knowledge on this subject. In addition, an electrochemical test was also carried out, which was not yet available for the above plants. Antibacterial studies have also been deepened to include the analysis of new strains of bacteria and fungi, which has not been studied earlier. The water extracts of P. padus using the utra-performance liquid chromatography (UPLC) system showed a higher content of both phenolic acids and flavonols $(651.77^{b} \pm 18.12 \,\mathrm{mg}/100 \,\mathrm{g}\,\mathrm{dw}$ for acids and $3.85^{\rm b} \pm 0.08 \,\rm mg/100 \,\rm g$ dw for flavonols, respectively). Ferulic and *p*-coumaric acids were the dominant polyphenols in leaves. Extracts from P. padus showed higher activity against DPPH radical, which was $6.62^{b} \pm 0.06$ mg TE/1 g dw, as well as higher antioxidant capacity, measured using

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2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical (37.39 $^{\rm b}$ ± 3.81 mg TE/g dw). The higher antioxidant potential of *P. padus* was confirmed based on the oxidizing potentials of electroactive compounds present in them. Stronger inhibition against *Enterococcus faecium* and *Klebsiella pneumoniae* was found for *P. padus*, whereas *P. serotina* extract was more potent against *Enterococcus faecium* bacterium. It has been shown that *P. padus* can be an attractive raw material with antioxidant and antimicrobial properties that can be used on a much wider scale in food technology than its current application.

Keywords: *Prunus*, polyphenols, antioxidant, antibacterial, electrochemical

1 Introduction

Natural products of plant origin are gaining interest worldwide due to component contents that may affect health. Bird cherry (*P. padus*) and black cherry (*P. serotina*) are popular plants found in many regions in the world. They have fruits with a bitter aftertaste, which are most often used as raw material for making tinctures. For infusions, not only bird cherry bark is used, but also shoots, leaves, and leaf buds are used. In folk medicine, bird cherry was considered as a medicinal plant.

Bird cherry is a source of polyphenols. *P. padus* fruits contain mainly caffeic acid, chlorogenic acid, coumaric acid, ellagic acid, gallic acid, vanillic acid, and ferulic acid [1,2]. It has also been shown that the fruits of *P. padus* additionally contain organic acids, minerals, and vitamins, mainly vitamin C. The most important flavonols found in the fruits of *Prunus padus* L. were quercitrin and quercetin, epicatechin and catechin also dominated. In addition, quercetin derivatives, such as hyperoside, kaempferol, or isorhamnetin, were also determined. Kaempferol glycosides accounted for only 2%, and isoramnetine glycosides were detected in less than 1 mg/kg fw [1]. On the other hand, *Prunus* is considered as an invasive species. It contains

cyanogenic glycosides such as prunazine and amygdalin. Cyanogenic glycosides are plant components and may be toxic when consumed in large quantities due to the hydrolytic release of hydrocyanic acid [3,4].

It has been shown that the consumption of plant materials rich in polyphenols can have a health-promoting effect as well as a positive effect on biochemical processes in the body. Prunus padus L. has been demonstrated to be a raw material containing polyphenols with antioxidant and antimicrobial activities. The beneficial effects of Prunus padus L. seed extracts were confirmed against pathogenic bacteria such as Staphylococcus aureus, Staphylococcus hominis, and Proteus mirabilis [5]. The leaves and branches of bird cherry contain components that have a beneficial antidiabetic effect inhibiting alpha-glucosidase activity [6]. The positive effect of bird cherry on hypertension was also confirmed. This effectiveness is due to the presence of hyperoside and chlorogenic acid as compounds that relax the smooth muscles of blood vessels [7]. Extracts of P. serotina fruits contain polar and nonpolar metabolites with a vasodilating effect [7]. P. padus also contains anthocyanins, cyanogenic glycosides, flavonoids, and chlorogenic acid, which are important in the treatment of inflammation [8]. It has antibacterial and antifungal properties [5]. The beneficial antimicrobial effect was confirmed in extracts obtained from P. padus stems, indicating at the same time anti-inflammatory and analgesic effects. Strong anti-inflammatory properties result not only from the inhibition of inflammatory mediators but also from the properties that reduce inflammation edema [9]. Current scientific research results have confirmed selectively beneficial properties of extracts from various anatomical parts of both *P. serotina* and *P. padus*; however, there are no reports in the literature that would indicate to what extent these two types of Prunus differ in terms of polyphenol content and antioxidant activity measured spectroscopically and electrochemically. Therefore, the main aim of the work was to assess the antioxidant and antimicrobial properties of P. padus and P. serotina leaves and thus to present the possibility of creating values resulting from the application of bird cherry plant raw material as the source of polyphenols.

2 Materials and methods

2.1 Materials

The leaves of *P. padus* and *P. serotina* were harvested in September 2019 in the orchard farm in Ozierany Małe in

Podlasie, Poland (53° 13' 14.865" N 23° 51' 9.327" E). The soil in the orchard was characterized by an average abundance of macronutrients. The approximate value of pH for soil, marked in 1 M KCl, was 6.13, and the content of humus was 1.14%. The average amount of precipitation in the growing season was 317 mm per square meter, with an average daily temperature of 14.4°C. The leaves were stored in frozen conditions (temperature = -28°C) until lyophilization and the extracts were prepared. Lyophilization was performed in a CHRIST 1-4 LSC freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH. Osterode am Harz, Germany) under constant conditions. The condensation temperature in the freeze dryer was maintained at -28°C, the temperature on the freeze dryer shelf at -20°C, and the product temperature at -4°C. The entire process was carried out under reduced pressure for 24 h. The leaves were extracted after grinding in Grindomix GM 200 (Retsch, Haan, Germany) for 180 s at 1,700 \times g at 21°C.

2.2 Extraction

Extraction with solvents such as water or mixture of water and alcohol is widely used to assess the content of biologically active compounds in plant raw materials. Polyphenols, vitamins, and minerals are easily extracted with polar solvents, enabling extracts with high antioxidant activity. The water extract from P. padus (PPL) and P. serotina (PSL) was obtained using water at 85°C, and 1,000 mL of water was mixed with 50 g of raw material and extracted for 15 min. The extracts were filtered and centrifuged (800 \times g, 15 min) each time. The fractions were decanted and filtered (Whatman 1:11 µm). The prepared extracts were stored in dark tubes until examination at 4°C.

2.3 Color and osmolality of extract measurement

Color of leaves extract was measured. Color measurement was run in $L \times a \times b \times CEN$ unit system using spectrometer CM-5 (Konica Minolta, Japan) according to the methodology described by the device producer. As a source of light, D 65 was applied, and color temperature equaled 6,504 K. The observation angle of the standard colorimetric observer was 10°. Measurements for each sample was repeated fivefold. The instrument calibration was performed with the use of a black pattern.

2.4 UPLC determination of phenolic acids and flavonols

Phenolic compounds in water extract were analyzed after alkaline and acidic hydrolysis. The analysis was performed using an Acquity H class UPLC system equipped with a Waters Acquity PDA detector (Waters, USA), Chromatographic separation was performed on an Acquity UPLC® BEH C18 column (100 mm \times 2.1 mm, particle size 1.7 μ m) (Waters, Ireland). The elution was carried out gradient using following mobile phase composition: A, acetonitrile with 0.1% formic acid; B, 1% aqueous formic acid mixture (pH = 2). The eluent uptake rate was as follows: 0.4 mL/min. Concentrations of phenolic compounds were determined using an internal standard at wavelengths λ = 320 nm and 280 nm and the results were expressed as mg/ 100 g d.m of Prunus leaves. Compounds were identified based on a comparison of retention time of the analyzed peak with the retention amount of standard to the analyzed samples and a repeated analysis. Detection level is 1 ug/g. Retention times for phenolic acids were as follows: protocatechuic acid 1.56 min, gallic acid 4.85 min, p-coumaric acid 8.06 min, 2,5-dihydroxybenzoic acid 9.55 min, 4-hydroxybenzoic acid 9.89 min, chlorogenic acid 12.00 min, caffeic acid 15.20 min, syringic acid 15.60 min, sinapic acid 17.10 min, ferulic acid 19.00 min, salicylic 17.85 min, t-cinnamic acid 20.00 min, and vanillic acid 21.05 min. The retention time for flavonoids was as follows: apigenin 1.10 min, vitexin 8.00 min, kaempferol 11.00 min, luteolin 16.90 min, quercetin 17.00 min, narin-17.50 min. rutin 19.00 min. and catechin genin 19.50 min [10].

2.5 Antioxidative potential analysis by spectrophotometric method

The total phenolic content (TPC) of the obtained extracts was determined using the method described by Kulczyński et al. (2016) with minor modifications [11]. Aliquots of 100 μL diluted in 900 μL of 40% ethanol (Sigma-Aldrich, Germany) were mixed with 1 mL of Folin-Ciocalteu reagent (Sigma-Aldrich, Germany), followed by the addition of 1 mL of 35% sodium carbonate (POCH, Poland). Samples were vortexed for 5 s, and after incubation in darkness at room temperature for 90 min, the absorbance of the reaction mixture was measured at 765 nm against a blank. The TPC was expressed as milligram of gallic acid (Sigma-Aldrich, Germany)

equivalents (GAE) per 1g (mg/1g) of dry mass using the calibration curves of gallic acid.

The DPPH procedure was based on the reduction of DPPH solution absorbance (2,2-diphenyl-1-picrylhydrazyl) at wavelength 517 nm in the presence of free radicals [12]. Measurements were performed using the SP-830 Plus apparatus (Metertech, Taiwan). The percentage of DPPH radical scavenging was evaluated based on the standard curve for y = 321.54x + 21.54 ($R^2 = 0.986$) and presented as mg TE/1 g dw of extract.

The ABTS cation radical scavenging activity was measured according to the Trolox Equivalent Antioxidant Capacity test according to the methodology described by Kobus-Cisowska et al. (2020) [12]. Spectrophotometric measurement of the ability to scavenge ABTS+ formed from ABTS (2,20-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) by oxidation with potassium persulfate was carried out at a wavelength of 414 nm using SP-830 Plus apparatus (Metertech, Taiwan). The percentage rate of ABTS⁺ scavenging was calculated from the standard curve for y = 121.63x + 26.33 ($R^2 = 0.96$) and expressed as mg TE/ g dw of extract.

2.6 Ferric reducing

The antioxidant properties of the extracts were determined using a ferric reducing/antioxidant power assay (FRAP method) according to the procedure described by O'Sullivan et al. [13]. FRAP reagent (2 mL; 0.01 mol TPTZ [2,4,6-tripyridyl-s-triazine] in 0.04 mol HCl, 0.02 mol FeCl₃·6H₂O and 0.3 mol acetate buffer) was added to 1 µL of each sample diluted in 999 µL distilled H₂O. A calibration curve was constructed using FeSO₄·7H₂O. Samples were incubated for 30 min, and the absorbance was measured at 593 nm (Metertech SP880, Taiwan). Data were expressed as µM FeSO₄/ 1 g dw of extract.

2.7 Antioxidative potential analysis by electrochemical assay

The content of redox compounds in Prunus leaves extracts was determined using square wave voltammetry (SWV). Voltammetric measurements were performed using potentiostat PGSTAT12 with the GPES 4.9 control software (EcoChemie, The Netherlands). A threeelectrode measuring system consisting of a reference electrode Ag/AgCl (3 M KCl) (Mineral, Poland), platinum as an auxiliary electrode (Mineral, Poland), and carbon paste as a working electrode (CPE) was used for the measurements. The CPE was developed according to a described procedure [14]. Carbon paste was made by mixing graphite powder (Sigma) with mineral oil (Sigma) in the ratio of 70:30 (w/w). The surface of the CPE was renewed before use by removing the outer layer of carbon paste on filter paper, application of fresh paste, and polishing it to a smooth finish on a frosted glass microscope slide. Before electrochemical measurement, the surface of CPE was treated with 0.05 M phosphate buffer mixed with 0.01 M KCl (pH 7.0) at a potential of +1.7 V for 60 s. After that, the electrodes were immersed for 120 s in the solution containing extract dissolved in phosphate buffer in the ratio 1:1 (v/v), whether the SWV measurement in the range from -0.3 V to +1.4 V was made. Applied SWV parameters were as follows: step potential of 5 mV, frequency of 50 Hz, and amplitude of 40 mV. Three repetitions of SWV measurement for each extract were performed. SWV voltammograms were smoothed using Sa the vitzky-Golay's method [15]. From SWV voltammograms, the baselines determined with moving average procedure were subtracted and finally were determined the data including peak potential, peak height (current), peak area for each signal, and the total peak areas. Based on our results for Cornus mas extracts [16] an electrochemical index (EI) describing the electrochemical activity of tested extracts, expressed as the total area of all redox signals, in relation to 1 g dry matter of examined plant material was also determined. With respect to the tested samples, 1 mL of the extract was prepared from 0.063 g of leaves, which after dilution in the buffer gave a final 0.03125 g dry matter content of plant material in the tested sample.

2.8 Antimicrobial activity testing using the well-diffusion method

Indicator microorganisms such as Gram-negative bacteria: Klebsiella pneumoniae (ATCC 31488), Salmonella enteritidis (ATCC 860), Pseudomonas aeruginosa (ATCC 27853), and Acinetobacter baumannii (ATCC 19606) and Gram-positive bacteria: Enterococcus faecium (ATCC 27270), Enterococcus faecium (ATCC 27270), Staphylococcus aureus (ATCC 25923), Lactobacillus fermentum (ATCC 14932), Clostridium butyricum

(ATCC 13076), Listeria monocytogenes (ATCC 19115), and Bacillus coagulans (GBI-30, 6086) as well as fungi of the species Candida utillis (ATCC 9950), Aspergillus sp. and Fusarium sp. were propagated in Muller-Hinton medium (Oxoid, UK) at 30°C (yeast) or 37°C (bacteria) for 24 h. Subsequently, to obtain a clear bacterial layer, the liquid Mueller-Hinton agar medium was inoculated with a 10% 24 h indicator culture with an optical density of 0.5 on McFarland scale and poured into Petri dishes. A well was drilled in the surface of the solid medium inoculated with indicator microorganisms, to which 50 microliters of the extract was added. Plates were incubated under conditions suitable for a given group of microorganisms for 24-48 h. Then, the growth inhibition zone of indicator microorganisms was measured (clearing around the application site of the sample).

2.9 Statistical analysis

Statistical analysis of all results was performed using Microsoft Excel 2013 software (USA) and Statistica 13 software (StatSoft, Poland). The electrochemical results were treated as an additional factor to the model based on standard analytical techniques. The p values for Levene's test of independent variables were calculated.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results

3.1 Characteristics of P. padus and P. serotina extracts

Prunus extracts were physically and chemically characterized (Table 1). It was shown that the color of the tested extracts differed in terms of assessed parameters. Parameter L^* determining the brightness was 26.06 \pm 0.29 in the PPL sample and 34.59 \pm 1.96 in the PSL sample. Parameter a^* , responsible for the color change in the range from green to red, was 9.95 \pm 0.04 for PPL and 14.69 \pm 0.19 for PSL, whereas parameter b^* responsible for the color change in the range from blue to yellow had lower values for PPL (1.80 \pm 0.12) and higher for PSL (11.22 \pm 0.22).

Table 1: Characteristics of the tested P. padus and P. serotina leaves, given in CIE $L^*a^*b^*$ units and osmolality

Sample	PSL	PPL
Osmolality (mOsm/kg H ₂ O)	$0.171^{b} \pm 0.01$	$0.156^{a} \pm 0.01$
Freezing temperature (°C)	$-0.289^a \pm 0.01$	$-0.289^a \pm 0.01$
L*	$34.59^{b} \pm 1.96$	$26.06^a \pm 0.29$
a*	$14.69^{b} \pm 0.19$	$9.95^{a} \pm 0.04$
b*	$11.22^{b} \pm 0.22$	$1.80^{a} \pm 0.12$
Color		

Abbreviation: PPL, water extract from Prunus padus L, leaves, PSL, water extract from Prunus serotina L. leaves, results are mean values of three determinations \pm standard deviation. Values sharing the same letter in a line are not significantly different $(P \le 0.05).$

The osmolality of the extracts indicates the freezing point of the extract and its differences relative to the freezing of water, which is a measure of the osmotic pressure of the tested extract. Extracts' osmolality was 0.156 mOsm/kg H₂O for the PPL extract and 0.171 mOsm/ kg H₂O for the PSL extract.

3.2 Phenolic acid and flavonoid contents

The content of phenolic acids and flavonols was determined in the obtained extracts (Table 2). Qualitative and quantitative characteristics of individual polyphenols in the extracts differed between the samples. Higher phenolic acid contents were found in the PPL extract, which was $651.77 \pm 18.12 \,\mathrm{mg}/100 \,\mathrm{g}\,\mathrm{dw}$. The dominant acids were p-coumaric acid 157.6 \pm 8.33 mg/100 g dw, ferulic acid 195.6 \pm 5.64 mg/100 g dw, and sinapic acid $147.5 \pm 2.21 \,\text{mg}/100 \,\text{g}$ dw. The lowest amounts among the tested acids were detected for vanillic acid 2.6 \pm 0.11 mg/ 100 g dw and syringic acid 8.95 ± 0.04 mg/100 g dw. The water extract of PSL leaves contained the highest content of ferulic acid (185.3 \pm 6.72 mg/100 g dw) and p-coumaric acid (103.6 \pm 0.21 mg/100 g dw). The lowest levels were determined for vanillic acid $(1.24 \pm 0.06 \,\mathrm{mg}/100 \,\mathrm{g}\,\mathrm{dw})$ and syringic acid (5.62 ± 0.12 mg/100 g dw). PPL leaf extract contained a higher concentration of flavonols $(3.85 \pm 0.08 \,\mathrm{mg}/100 \,\mathrm{g}\,\mathrm{dw})$ than PSL extract. PPL leaves contained naringenin, rutin, quercetin, and dominant catechin (2.07 \pm 0.02 mg/100 g dw). The extract from PSL

Table 2: Content of polyphenolic compounds in P. serotina and P. nadus leaves

	PSL (mg/100 g dw leaves)	PPL (mg/100 g dw leaves)
Phenolic acids		
Gallic acid	$19.56^a \pm 0.64$	$22.3^{b} \pm 0.64$
2,5-	$14.52^{a} \pm 0.44$	$16.52^{b} \pm 0.24$
Dihydroxobenzoic acid		
4-Dihydroxobenzoic acid	$23.6^{a} \pm 0.12$	$29.45^{b} \pm 0.13$
Caffeic acid	$11.45^a \pm 0.61$	$13.65^{b} \pm 0.09$
Syringic acid	$5.62^{a} \pm 0.12$	$8.95^{b} \pm 0.04$
p-Coumaric acid	$103.6^a \pm 0.21$	$157.6^{b} \pm 8.33$
Ferulic acid	$185.3^a \pm 6.72$	$195.6^{b} \pm 5.64$
Chlorogenic acid	$29.5^a \pm 0.09$	$36.8^{b} \pm 0.24$
Sinapic acid	$97.68^a \pm 0.39$	$147.5^{b} \pm 2.21$
t-Cinnamic acid	$13.4^a\pm0.08$	$20.8^{b} \pm 0.05$
Vanillic acid	$1.24^a\pm0.06$	$2.6^{b} \pm 0.11$
Salicylic acid	ND	ND
Total phenolic acids	$505.47^a \pm 9.48$	$651.77^{b} \pm 18.12$
Flavonoids		
Naringenina	$0.13^a\pm0.00$	$0.62^{b} \pm 0.02$
Vitexin	ND	ND
Rutin	ND	1.03 ± 0.02
Quercetin	$0.17^{b} \pm 0.01$	$0.13^{a} \pm 0.02$
Apigenin	ND	ND
Kaempferol	ND	ND
Luteolin	ND	ND
Catechin	$1.01^{a} \pm 0.01$	$2.07^{b} \pm 0.02$
Total flavonoids	$1.31^a\pm0.02$	$3.85^{b} \pm 0.08$

Abbreviation: as in Table 1, ND, not detected, results are mean values of three determinations \pm standard deviation. Values sharing the same letter in a line are not significantly different $(P \le 0.05).$

leaves contained the highest content of catechins (1.01 \pm 0.01 mg/100 g dw) among the flavonols tested.

3.3 Antioxidant potential analysis by spectrophotometric method

The analyzed extracts were evaluated for their antioxidant potential by spectroscopic methods (Table 3). It was found that extracts prepared from leaves of black cherry PSL and bird cherry PPL had different properties. The higher content of these compounds was found in the extract of PPL leaves $(37.39 \pm 3.81 \,\mathrm{mg}\,\mathrm{GAE/g}\,\mathrm{dw})$. In the FRAP test, PPL leaf extract also showed a 30% higher activity when compared with PSL. Test results were also complemented by determining the effect of the extracts using the DPPH radical test. PPL extract was shown to scavenge radicals at 6.62 \pm 0.06 mg TE/1 g dw, while DPPH anti-radical activity for PSL

Table 3: Chemical structure of compounds analyzed

Phenolic acids		R1	R2	R3	R4	R5
ОУОН	4-Hydroxobenzoic acid 2,5-Dihydroxobenzoic acid	–H –H	–H –OH	–H –H	–ОН –Н	–H –OH
R^{5} R^{1}	Gallic acid	-H	-OH	-OH	-OH	-H
ĭ Ť	t-Cinnamic acid	-H	-H	-H	-H	-H
R ⁴ R ²	Salicylic acid	-OH	-H	-H	-H	-H
R ³	Syringic acid	–Н	−OCH ₃	-OH	−OCH ₃	-H
R	Vanillic acid	-H	-OCH ₃	-OH	-H	-H
0	Ferulic acid	-H	-H	-OH	−OCH ₃	-H
Д ОН	Caffeic acid	-H	-H	-OH	-OH	-H
	<i>p</i> -Coumaric acid	-H	-H	-OH	-OH	-H
	Sinapic acid	-H	-OCH₃	-OH	−OCH ₃	-H
R^5 R^1						
R ⁴						
R ³						
HO ₂ CO₂H						
0						
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HO,,,		Chlorogenic aci	d			
ŌН						
311	`∕ОН					
	о́н					
Flavonoids						
HO		Naringenin				
OH O	ОН	Namgemii				
Glu(C)	OH					
HO		Vitexin				
OH O						
OH Ö R2		R1	R2			
OH	Apigenin	–H	-H			
OH	Kaempferol	-OH	-H			
но	Quercetin	-OH	-OH			
\vee		-H	-OH			
	Luteolin	11				
RI	Catechin	-OH	-H			

extract was slightly lower and amounted to 5.43 \pm 0.07 mg TE/1 g dw. These analyses were also confirmed by the tests carried out using the ABTS radical method, which also showed higher activity of the solution from PPL leaves, where the value of aqueous extracts PPL was 9.65 \pm 0.09 mg TE/g dw and was higher than for PSL extracts, where it was 8.55 \pm 0.08 mg TE/g dw.

3.4 Antioxidant potential analysis by an electrochemical assay

The electrochemical activity of the extracts was determined using SWV. Electrochemical measurements showing the content of redox compounds in the extracts demonstrated the presence of two signals on

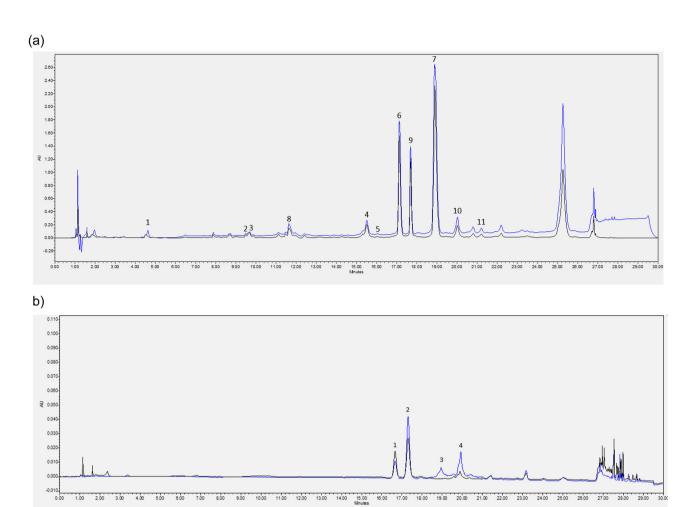


Figure 1: Comparison of chromatograms of phenolic acids for P. serotina and P. padus (a), comparision of chromatograms of flavonoids for P. serotina and P. padus (b). (a) Comparison of chromatograms of phenolic acids for P. serotina (black line) and P. padus (blue line) leaves: 1 - gallic acid, 2 - 2,5-dihydroxobenzoic acid, 3 - 4-dihydroxobenzoic acid, 4 - caffeic acid, 5 - syringic acid, 6 - p-coumaric acid, 7 - ferulic acid, 8 - chlorogenic acid, 9 - sinapic acid, 10 - t-cinnamic acid, 11 - vanillic acid. (b) Comparison of chromatograms of flavonoids for P. serotina (black line) and P. padus (blue line) leaves: 1 - quercetin, 2 - naringenin, 3 - rutin, 4 - catechin.

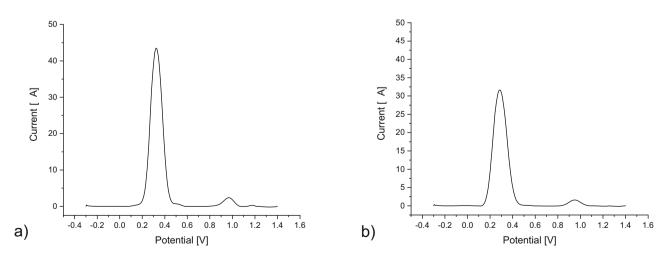


Figure 2: SWV signals for P. padus (a) and P. serotina (b) leaf extracts.

Table 4: P. padus and P. serotina leaf extracts with FRAP, DPPH, ABTS radicals and total polyphenol content

Sample/activity	PSL	PPL
TPC (mg GAE/g dw) DPPH (mg TE/1 g dw)	$21.54^{a} \pm 3.34$ $5.43^{a} + 0.07$	$37.39^{b} \pm 3.81$ $6.62^{b} + 0.06$
ABTS (mg TE/g dw)	$8.55^{a} \pm 0.08$	$9.65^{b} \pm 0.09$
FRAP (μ M FeSO ₄ /1 g dw)	$179.24^a \pm 3.66$	$233.20^{b} \pm 5.97$

Abbreviation: as in Table 1.

voltamperograms (Figure 1). In the PSL leaf extract, they were located at $+0.325 \pm 0.000$ and $+0.967 \pm 0.010$ V, while for PPL, these signals were slightly shifted toward the negative potential, which was $+0.285 \pm 0.000$ and $+0.955 \pm 0.000$ V, respectively. The dominant signal was the first one registered in both cases, located in the +0.3 V region (Figure 2).

Table 4 summarizes electrochemical parameters determined for the analyzed extracts. The extract of PPL leaves exhibited higher electrochemical activity, expressed as the total area of redox signals, and the EI determined on its basis. The total signal area was 4.451 \pm $0.117 \text{ V} \times \mu\text{A}$, while EI reached the value of 142.417 \pm 3.737. These values for PSL leaf extract were 3.602 \pm $0.439 \text{ V} \times \mu\text{A}$ and 115.267 ± 14.043 , respectively.

3.5 Antimicrobial activity

The influence of ground PSL and PPL leaves against indicator microorganisms of both Gram-positive and Gram-negative bacteria, as well as molds and fungi, was analyzed (Table 5).

The highest antimicrobial activity was demonstrated for PSL leaves against Enterococcus faecium (ATCC 27270) (24 mm) and PPL leaves against Listeria monocytogenes (ATCC 19115) (24 mm). The lowest activity of PSL leaf extract was demonstrated for bacteria from the Gram-negative group Acinetobacter baumannii (ATCC 19606), and it was 8 mm. The extract also showed low activity against fungi, similarly as PPL leaf extract.

4 Discussion

Less known plants or under-utilized species have recently been the subject of great research interest due to the presence of compounds exhibiting beneficial health properties. There are studies available in the

Table 5: The electrochemical parameters determined for extracts from P. padus and P. serotina leaves

SWV parameters			
Sample	PSL	PPL	
Peak potential E_p [V]	0.325 ± 0.000	0.285 ± 0.000	
Peak current / [µA]	0.967 ± 0.010 27.102 + 0.871	0.950 ± 0.000 29.065 + 1.081	
reak current / [µA]	1.267 ± 0.087	1.419 ± 0.225	
Peak area $E_P \times I [V \times \mu A]$	3.469 ± 0.425	4.280 ± 0.089	
	0.134 ± 0.014	0.171 ± 0.027	
Total peak current / [μΑ]	$28.379^a \pm 0.957$	$30.484^{b} \pm 1.306$	
Total peak area $[V \times \mu A]$	$3.602^a \pm 0.439$	$4.451^{b} \pm 0.117$	
EI $[V \times \mu A/1 g dw]$	$115.267^a \pm 14.043$	142.417 ^b ± 3.737	

Abbreviation: as in Table 1.

literature, which, like in the current work, have indicated the antioxidant potential of P. padus and P. serotina leaves, but also of fruits, bark, and flowers [1–7]. As in the present study, other authors have pointed out polyphenols as compounds that affect antioxidant potential. Current studies, however, do not indicate differences in the content of specific biologically active compounds in P. padus and P. serotina leaves, and most importantly, the work to date did not include both spectroscopic and electrochemical studies, allowing the assessment of antioxidant activity. The use of electrochemical methods to assess the antioxidant potential is new in the field of bird cherry research. The aim of this study was also to compare selected phytochemicals as well as to investigate the antioxidant and antimicrobial activity of bird cherry leaves, because there is very little research on the subject in the literature. Antimicrobial activity studies have been deepened to include previously unknown strains of gram-positive and gramnegative bacteria as well as fungi. The main phenolic acid is chlorogenic acid, whose content in PPL was determined in earlier studies at the level of 1.39-1.94% dw [8]. Chlorogenic acid was also present in P. padus fruits at the level of $10.48 \pm 0.28 \,\text{mg}/100 \,\text{g}$ fw [1]. Its presence in the fruits of P. serotina was also confirmed by ref. [7]. Other authors determined the content of individual polyphenols in one Prunus variety. These leaves were not compared. Quercetin was determined in P. serotina [7] but also in P. padus fruits (11.86 \pm 2.36 mg/ 100 g fw) [1], indicating its importance in total antioxidant potential. Other studies demonstrated that rutin was primarily responsible for the properties of *P. serotina* leaves [17] and fruits of P. padus (2.67 \pm 1.02 mg/ 100 g fw) [1]. It has been repeatedly suggested in the literature that polyphenol content determines the

Table 6: Micorbiological activity of *P. padus* and *P. serotina* leaves extract

Indicator microorganisms	PSL Growth inhibition zone (mm)	PPL
Gram-negative bacteria		
Klebsiella pneumoniae (ATCC 31488)	18 ± 30	19 ± 30
Salmonella enteritidis (ATCC 13076)	16 ± 30	14 ± 20
Pseudomonas aeruginosa (ATCC 27853)	15 ± 20	17 ± 30
Acinetobacter baumannii (ATCC 19606)	8 ± 10	15 ± 30
Gram-positive bacteria		
Enterococcus faecium (ATCC 27270)	24 ± 40	22 ± 30
Staphylococcus aureus (ATCC 25923)	20 ± 30	15 ± 30
Lactobacillus fermentum (ATCC 14932)	18 ± 30	17 ± 30
Clostridium butyricum (ATCC 860)	19 ± 30	16 ± 30
Listeria monocytogenes (ATCC 19115)	23 ± 40	24 ± 40
Bacillus coagulans (GBI-30, 6086)	17 ± 30	19 ± 30
Fungi		
Candida utillis (ATCC 9950)	8 ± 10	7 ± 10
Aspergillus sp.	5 ± 10	3 ± 10
Fusarium sp.	3 ± 00	4 ± 10

Abbreviation: PPL, water extract from *Prunus padus* L. leaves, PSL, water extract from *Prunus serotina* L. leaves.

antioxidant properties, whose mechanism of action can be multidirectional. Polyphenols can inhibit the formation of free radicals; they can scavenge them and increase the catalytic activity of endogenous enzymes involved in free radical neutralization. Studies on bird cherry and the compounds it contains can be found in the literature. However, there is no information on the effect of bird cherry on reducing the degree of oxidative damage caused by the OH radical using an electrochemical DNA biosensor. The electrochemical method shows a significant advantage over other methods enabling the determination of 8-oxoguanine level, because it allows direct testing of DNA sample, without the need for its hydrolysis, which is necessary for other highly sensitive methods [18]. The performed voltammetric measurement allowed determining the degree of damage based on the observed changes in the signals of DNA bases. Such changes in the guanine signal level are commonly considered in this type of analysis, or less frequently the appearance of nitrogen base-derived signals, including 8-oxodG [19]. The OH hydroxyl

radical is one of the most reactive forms of ROS, which can be generated by the Fenton reaction from H₂O₂ in the presence of transition metal cations such as Fe²⁺. Natural plant sources are also sought for in the context of the bactericidal and bacteriostatic activities since the overuse of antibiotics can have adverse effects. It has been demonstrated that many substances present in rhizomes, fruits, leaves, or bark can exert a biocidal effect [20]. The current study assessed the influence of leaf compounds of P. serotina against indicator microorganisms of both Gram-positive and Gram-negative bacteria, as well as molds and fungi (Table 6). The highest antimicrobial activity was demonstrated for P. serotina (PSL) leaves against Enterococcus faecium (ATCC 27270) (24 mm). Kumarasamy et al. (2004) [5] also evaluated the activity of *P. padus* seed dichloromethane extracts against Enterococcus faecalis and showed weak growth inhibition of these bacterial strains as well as Staphylococcus hominis. In the same study, the highest antibacterial activity was demonstrated for methanol extract from P. padus seeds against Staphylococcus aureus (ATCC 25923) (1.0 \times 10⁻⁴ mg/mL). In addition, the extracts also showed the activity against methicillinresistant bacterial strains Staphylococcus aureus (ATCC 25923), Staphylococcus hominis, and Proteus mirabilis [5]. The tested water extracts from PPL showed the highest activity against Listeria monocytogenes (ATCC 19115) (24 mm) and the lowest against fungi. Another study evaluated the antimicrobial effect using methanol extracts from the leaves and branches of P. padus. Both leaf and branch extract showed antimicrobial activity against most Gram-positive bacteria tested; however, only the branch extract exhibited any activity against Gram-negative bacteria. The extract from P. padus branches was most active against Kocuria rhizophila (MIC = $125 \mu g/mL$) [6]. The lowest activity of P. serotina leaf extract (PSL) was demonstrated for bacteria from the Acinetobacter baumannii (ATCC 19606) Gram-negative group, and it was 8 mm. Low bird cherry activity has been shown against fungi. The antioxidant properties of bird cherry leaves depend on the content of biologically active compounds, and their measurement may be different depending on the methods used (spectroscopic, electrochemical). The variation in the result of activity is most likely due to the affinity of the extracted compounds for the reagents in the given methods and specificity of action. Not only the composition and the total content of individual compounds, but above all the mutual proportions, where, as the literature indicates, individual compounds may exhibit synergistic or antagonistic effects, which is important for both antioxidant and antimicrobial activities. Therefore, it is necessary to test each raw material, because predicting activity for most raw materials is difficult or impossible. It has been shown that *P. padus* can be an attractive raw material with antioxidant and antimicrobial properties that can be used on a much wider scale in food technology than its current application.

5 Conclusions

In recent years, there has been a very high interest in plantderived products and their health-promoting properties. Bird cherry (P. padus and P. serotina) is a new raw material that can gain significance not only as an innovation in the development of functional food. It is common in many parts of the world and does not require special cultivation conditions. It can be used to design innovative dietary supplements or functional foods. Food should not only provide basic nutrients but should also be considered in terms of health benefits that can be obtained from it. Bird cherry leaves are a good source of polyphenolic compounds with high antioxidant activity. Polyphenols showed a protecting effect on cell structures against damage caused by free radicals, which contribute to faster aging of the body. Current scientific research results confirm the beneficial properties of both P. serotina and P. padus extracts. It has been demonstrated that bird cherry leaf extracts containing polyphenolic compounds have antioxidant and antibacterial properties. Thus, P. padus and P. serotina preparations can be a valuable raw material used in the food, pharmaceutical, and cosmetics industries as a source of bioactive compounds with multidirectional antioxidant activity.

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