Research Article

Aleksandra Telichowska*, Joanna Kobus-Cisowska, Piotr Szulc, Radosław Wilk, Dominik Szwajgier, Daria Szymanowska

Prunus padus L. bark as a functional promoting component in functional herbal infusions – cyclooxygenase-2 inhibitory, antioxidant, and antimicrobial effects

<https://doi.org/10.1515/chem-2021-0088>

received July 30, 2021; accepted September 5, 2021

Abstract: The study assessed the health-promoting properties and the content of minerals in the bark of bird cherry (*Prunus padus* L.), which was then used as an ingredient in functional teas. The infusions were made with the use of *Matricaria chamomilla* L., *Tilia cordata* Mill., and *Calendula officinalis* L., and then combined with the bark in various proportions. The prepared infusions were tested for antioxidant activity, ability to reduce copper ions and iron ions, as well as the ability to scavenge hydroxyl radicals. In the next stage, the antimicrobial activity and the ability to inhibit the enzyme cyclooxygenase-2 were assessed. Bird cherry bark contains a high potassium content of 19.457 ± 762 mg/kg d.m. In all the tests evaluating the antioxidant activity, infusions from the bark of bird cherry alone and with its 30% addition had the strongest properties. The analyzed infusions also have the ability to reduce Cu(II) ions; they are active to

reduce Fe(III) ions and scavenge hydroxyl radical. The highest antimicrobial activity was found for teas with 20 and 30% bark, especially against *Listeria monocytogenes* (25.0–27.0 mm) (± 3.0). The bark infusion was also found to have the highest inhibitory activity against cyclooxygenase-2 (COX-2) – 77.0%.

Keywords: bird cherry bark, infusions, antioxidant, inhibition of cyclooxygenase-2, antimicrobial

1 Introduction

The bird cherry (*Prunus padus* L.) is a tree or large shrub commonly found in Europe. Due to numerous health and healing properties, individual anatomical parts of bird cherry are often used as herbal raw materials [1]. Literature data indicate that the flowers can be chewed and the young leaves are eaten after cooking [2]. In Korea, the leaves are used as a cooked vegetable. On the other hand, ripening black cherry fruits, along with chemical changes in their composition, are enriched with substances with antioxidant properties [3]. The bark of the bird cherry has not been used in food technology so far, even though it contains many bioactive ingredients [4]. Additionally, literature data suggest that bird cherry bark contains many compounds which are of functional importance for human health. The literature of the subject includes studies indicating the significant antioxidant potential of *P. padus* – this applies to its fruit, leaves, bark, and flowers [5–8]. Its beneficial effects on the human body are due to such substances as malic acid, citric acid, and cinnamic acid derivatives, as well as phenolic compounds such as anthocyanins, flavanols, and quercetin and kaempferol derivatives [9].

It is well-known that oxidative stress is associated with the pathology of many human diseases. These

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

Joanna Kobus-Cisowska: Department of Gastronomy Sciences and Functional Food, Poznan University of Life Sciences, 60-637, Poznan, Poland

Piotr Szulc: Department of Agronomy, Poznan University of Life Sciences, 60-637, Poznan, Poland

Radosław Wilk: Department of Chemistry, Poznan University of Life Sciences, 60-637, Poznan, Poland

Dominik Szwajgier: Department of Biotechnology, Human Nutrition and Science of Food Commodities, University of Life Sciences in Lublin, 20-704, Lublin, Poland

Daria Szymanowska: Department of Biotechnology and Food Microbiology, Poznan University of Life Sciences, 60-638, Poznan, Poland; Department of Pharmacognosy, Poznan University of Medical Science, 60-781 Poznan, Poland

include diabetes, cancer, atherosclerosis, and neurodegenerative diseases. Antioxidants, on the other hand, are compounds found mainly in food that can counteract the negative effects of oxidative stress. The presence of polyphenols in the diet, especially in people who consume large amounts of tea, dark chocolate, coffee, or red wine, can be up to several hundred milligrams per day [10]. Teas and herbal infusions are especially rich in bioactive compounds. They can also support prophylaxis in the treatment of many diseases and can be used together with synthetic drugs as adjunctive therapies.

Herbal medicines are often consumed in the form of tea – an infusion of dried plant parts steeped in boiling water. Herbal teas have been gaining increasing popularity in recent years [11]. Therefore, to meet consumer expectations regarding new functional products, teas containing bird cherry (*P. padus*) bark were developed as part of this study. This study aimed to evaluate bird cherry bark's antioxidant properties, its ability to reduce iron and copper ions, and the degree of COX 2 inhibition, as well as its antimicrobial potential.

2 Materials and methods

2.1 Materials

The test sample was bird cherry (*P. padus*) bark from an orchard in Ozierany Małe in Podlasie region, Poland (53°13' 14.865'' N 23°51' 9.327'' E). The mean rainfall during the growing season was 317 mm 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 was stored frozen (temperature = −28°C) until the freeze-drying and extract preparation process. The freeze-drying was performed using a CHRIST 1–4 LSC freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) under constant conditions. The condensation temperature inside the freeze dryer was maintained at −28°C, whereas the shelf temperature was 20°C, and the product's temperature was −4°C. The entire process was conducted under reduced pressure for 24 h.

2.2 Infusion ingredients – plants

The bird cherry bark was ground for 15 s, at 500 rpm/min, and at 21°C to obtain a particle size of 0.5–0.9 mm, in a Grindomix GM 200 made by Retsch (Haan, Germany). Other ingredients, such as chamomile flowers, linden flowers, and marigold flower heads, were purchased from retail producers and included dried chamomile flowers (*Matricaria chamomilla* L.), dried linden flowers (*Tilia cordata* Mill.), and dried marigold flower heads (*Calendula officinalis* L.) (Figure 1).

2.3 Preparing teas and making tea infusions

A single extraction method was used to obtain the infusion. The teas were prepared by weighing out 2 g of each fraction according to the proportions. A total of 11 samples were prepared with 4 samples consisting of the single components (100% *Calendula officinalis* L. (flower) (C), 100% *Matricaria chamomilla* L. (flower) (Ch), 100% *Tilia cordata* Mill. (Flower) (T), and 100% *Prunus padus* L.

Figure 1: (a) bird cherry bark (b) base mix (*Matricaria chamomilla* L. + *Tilia cordata* Mill. + *Calendula officinalis* L.).

(bark) (P)). Base (B) was a mixture of three herbs mixed in equal proportions (*Calendula officinalis* L. (flower) 33.3% + *Matricaria chamomilla* L. (flower) 33.3% + *Tilia cordata* Mill. (Flower) 33.3%) and seven samples consisting of the base mixture (B) combined with the corresponding concentration of the *P. padus* bark (P) (Table 1). The samples were prepared as follows: BP5 (5% P + 31.66% C + 31.66% Ch + 31.66% T), BP10 (10% P + 30% C + 30% Ch + 30% T), BP15 (15% P + 28.33% C + 28.33% Ch + 28.33% T), BP20 (20% P + 26.66% C + 26.66% Ch + 26.66% T), BP25 (25% P + 25% C + 25% Ch + 25% T), and BP30 (30% P + 23.33% C + 23.33% Ch + 23.33% T).

The obtained mixtures were packaged into disposable paper tea bags (2 g); then, 200 mL of 80°C water was poured over them and they were extracted for 15 min. First, teas consisting of individual ingredients were weighed out and placed in disposable tea bags, and afterwards, ones containing multiple ingredients in appropriate proportions were prepared in the same way.

2.4 Minerals contained in the bird cherry bark

The evaluation of the bird cherry bark's (*P. padus*) mineral composition was performed using the ICP-OES (Inductively Coupled Plasma – Optical Emission Spectrometry) method. The C and N content analysis in the samples tested was performed using a Vario MACRO Cube CN analyzer (Elementar Analysensysteme GmbH, Germany). The elemental composition analysis was performed using ICP – OES iCAP 6500 Axial and Radial Vista (Thermo

Scientific, Waltham, Massachusetts, USA) per PN-EN ISO/IEC 17025:2005. Before the multielement analysis, samples (approximately 0.5 g of dry weight) were mineralized (5.0 mL of 69% HNO_3) in Teflon digestion bombs using a Milestone Start D Microwave Digestion System (Milestone S.r.l., Sorisole, Italy).

2.5 Assessment of antioxidant activity (Folin–Ciocalteu, hydroxyl radical antioxidant capacity (HORAC) method, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method)

The infusions were evaluated for compounds reacting with the Folin–Ciocalteu reagent according to the method described by Meda et al. [12]. A volume of 0.2 mL of the sample was mixed with acetonitrile (0.8 mL) and centrifuged (12,000g, 20 min). The centrifuged supernatant (0.01 mL) was mixed with DDI water (0.04 mL), F–C reagent (750 μL), and after 5 min, with 7.5% Na_2CO_3 water solution (750 μL). After 2 h, the absorbance was read at 760 nm against the reagent sample (containing water, F–C reagent, and Na_2CO_3) [12]. A calibration curve was prepared using gallic acid (0.34 mg) dissolved in DDI water (0.5 mL) and acetonitrile (0.5 mL). Fifteen solutions (1.12–24 μg gallic acid/50 μL) were prepared. To those solutions, of F–C reagent (750 μL), and after 5 min, 7.5% Na_2CO_3 water solution (750 μL) were added followed by the measurement.

Denev et al. HORAC method was used with some modifications. The sample (10 μL) was mixed with DDI (500 μL) and fluorescein solution (200 μL , 60 nM) followed by incubation at 37°C for 10 min. Then, 27.5 mM H_2O_2 solution (10 μL) and $\text{CoF}_2 \times 4\text{H}_2\text{O}$ solution (10 μL) (230 μM ; containing 1 mg of picolinic acid/mL) were added to the solution containing fluorescein and the tested sample. The fluorescence was read (excitation at 485 nm and emission at 520 nm) at start and every 1 min with constant shaking during the whole reaction until stabilization (typically 5–10 min). A blank sample containing phosphate buffer was run instead of the tested sample. Also, the background from samples was measured (a mixture containing the tested sample and DDI water only). The activity was expressed as gallic acid equivalents using 15 gallic acid solutions (equal to 12.6–573.2 μg of gallic acid/mL). Fluorescein (200 μL) was incubated with gallic acid (10 μL) standard

Table 1: Infusion sample descriptions and designations

Sample code	Raw material content (%)			
	<i>Prunus padus</i> L. (bark) %	<i>Matricaria chamomilla</i> L. (flower) %	<i>Calendula officinalis</i> L. (flower) %	<i>Tilia cordata</i> Mill. (flower) %
B	0	33.33	33.33	33.33
C	0	0	100	0
Ch	0	100	0	0
P	100	0	0	0
T	0	0	0	100
BP 5	5	31.66	31.66	31.66
BP 10	10	30	30	30
BP 15	15	28.33	28.33	28.33
BP 20	20	26.66	26.66	26.66
BP 25	25	25	25	25
BP 30	30	23.33	23.33	23.33

solution and DDI (40 μ L) and analyzed as described above [13]. The samples were run in at least four repeats.

The DPPH procedure was based on the reduction of DPPH solution absorbance (2,2-diphenyl-1-picrylhydrazyl) at 517 nm in the presence of free radicals [14]. The 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/g DW of extract.

The ABTS cation radical scavenging activity was measured according to Trolox equivalent antioxidant capacity assay in accordance with the methodology described by Kobus-Cisowska et al. [14]. The spectrophotometric measurement of the ability to scavenge $ABTS^{+}$ formed from ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)) by oxidation with potassium persulfate was carried out at 414 nm using the 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 Ability to reduce copper ions cupric reducing antioxidant capacity (CUPRAC) and iron ions ferric reducing antioxidant power (FRAP)

Öztürk et al. used CUPRAC method with minor modifications. A 40 μ L sample was mixed with ammonium acetate buffer (pH 7.0, 1 M) (900 μ L), neocuproine (700 μ L) (3.75 mM/L in DDI water:ACN 1:1), and 10 mM/L $CuCl_2$ (350 μ L). After 1 h, the absorbance was read at 450 nm against the blank sample (containing the corresponding tested solution (40 μ L) mixed with ammonium acetate buffer (1,950 μ L)). The reagent sample (without tested samples) was also measured and subtracted from the tested sample. Quercetin was used as an antioxidant standard: quercetin (0.23 mg) was dissolved in 0.5 mL and 0.5 mL of ACN. Then, 20 quercetin standard solutions (in the range of 0.0225–0.38 mmol/dm³) (40 μ L) were mixed with reagents as described above [15]. The samples were run in at least four repeats. Curve equation: $Y = 2.2237x$, $r^2 = 0.9992$.

Oyaizu's FRAP method was used with minor modifications. The sample (40 μ L) was mixed with 0.2 M sodium phosphate buffer (pH 6.6) (250 μ L) and 10 mg/mL potassium ferricyanide (250 μ L). The sample was incubated at 50°C for 20 min. Then, 100 mg/mL (w/v) trichloroacetic

acid (250 μ L) was added followed by centrifugation (2,000 g, 10 min). Moreover, the upper layer (500 μ L) was mixed with DDI water (500 μ L) and freshly prepared $FeCl_3$ solution (1 mg/mL) (100 μ L). The absorbance was read at 700 nm against a blank sample (DDI water instead of the sample). The background was measured as well (a mixture containing the tested sample and buffer only). A calibration curve was prepared using a Trolox solution: Trolox (0.38 mg) was dissolved in DDI water (1 mL). A volume of 40 μ L of 20 standard solutions (containing 0.44–7.11 μ g Trolox/mL) was studied as described above [16]. The samples were run in at least four repeats.

2.7 Herbal infusion antimicrobial activity evaluation

A bag containing 2 g of herbal tea was poured with water at 80°C. The tea's brewing lasted 15 min. The bag was then removed from the solution. The obtained solution was cooled to 20°C and further analyzed. Indicator microorganisms such as *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 7644, *Enterococcus faecalis* ATCC 29212, *Clostridium butyricum* ATCC 860, *Lactobacillus fermentum* ATCC 14932, *Lactococcus lactis* ATCC 11454, *Streptococcus thermophilus* ATCC 19258, *Bifidobacterium bifidum* ATCC 11863, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Salmonella typhimurium* ATCC 14028, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 31488, *Pseudomonas aeruginosa* ATCC 27853, *Alcaligenes faecalis* ATCC 35655, *Candida albicans* ATCC 10231, *Saccharomyces cerevisiae* ATCC 9776, *Fusarium* spp., *Aspergillus* spp., and *Mucor* spp. were transferred to test tubes containing Mueller–Hinton medium (for bacteria), yeast extract sucrose medium (for yeast), or Potato Dextrose medium (for mold). They were cultured at 37°C for 24–72 h. Subsequently, the liquefied agar medium was inoculated with 10% (v/v) 24 h indicator culture and poured into Petri dishes to obtain a distinct confluent layer. After solidification of the broth medium inoculated with the indicator microorganisms, wells were made with a cork borer. Each well was supplemented with a liquid extract cone medium (150 μ L). Next, the diameters of the growth inhibition or reduction zone of indicator microorganisms were measured. The inhibition of the growth of the indicator microorganism was manifested by complete lightening around the place where the liquid extract or slime was transferred. It indicated bactericidal activity of the bacterial strain. Bacteriostatic properties were determined by measuring the diameter of the growth inhibition zone (indicator strain growth limitation).

2.8 Inhibition of cyclooxygenase-2

For the assay, reagents from Cayman COX Activity Assay Kit (No. 760151) were strictly prepared as suggested by the producer. They were combined with COX-2 enzyme (Human recombinant, Cayman No. 60122, pre-diluted 100-fold using 100 mM, pH 8.0 Tris buffer). A volume of 0.04 mL of the tested sample was mixed with Tris buffer (100 mM, pH 8.0) (0.12 mL), hemin (0.01 mL), shaken, and left for 5 min. at 25°C followed by the addition of a colorimetric substrate (0.02 mL) and of arachidonic acid solution (0.02 mL). To start the reaction, COX-2 solution was added (0.02 mL). The increase in the absorbance during the incubation at room temperature was recorded at 590 nm (Tecan microplate reader, Grödig, Austria). A negative (blank) sample (buffer instead of tested extract) and a positive one (COX-2 inhibitor DuP-697) were run simultaneously. The background of tested extracts (0.04 mL of the extract mixed with 0.19 mL buffer) was also measured and included in the calculations. Each sample was run in at least four repeats.

2.9 Statistical analysis

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

3 Results

3.1 The mineral content of bird cherry bark

The bird cherry bark was characterized in terms of macro- and micronutrients and trace elements that play an important role in plant development and nutrition and maybe of nutritional importance (Table 2). Bird cherry (*P. padus*) bark has been shown to contain a high potassium content

of $19,457 \pm 762$ mg/kg d.m. Significant calcium ($3,540 \pm 379$), magnesium ($3,202 \pm 71$), and phosphorus ($2,925 \pm 45$) contents have also been found.

3.2 Assessment of antioxidant activity (Folin–Ciocalteu, HORAC method, DPPH, and ABTS method)

The highest antioxidant activity in the Folin–Ciocalteu reagent test was observed in the case of the sample containing 20% of bird cherry bark BP 20 (102.3 ± 9.1^e µg gallic acid/mL), with slightly lower antioxidant activity in the case of the sample containing only bird cherry bark (P) (114.5 ± 8.3^f µg gallic acid/mL). Linden flower (T) also showed significant antioxidant activity (97.7 ± 1.6^e µg gallic acid/mL). Chamomile flower (Ch) contained the smallest amount of antioxidant compounds (55.0 ± 1.5^a µg gallic acid/mL).

The study was further expanded by the addition of the HORAC assay, which is based on the oxidation of fluorescein by hydroxyl radicals through the classical hydrogen atom transfer mechanism. The results obtained showed that both the tea consisting only of the base without added bark (B) (4.1 ± 0.2^b µg gallic acid/mL) and the tea with the highest 30% bird cherry bark content (BP30) (4.0 ± 0.2^b µg gallic acid/mL) exhibited the highest antioxidant activity against hydroxyl radicals.

In the DPPH radical scavenging assay, the highest scavenging potency occurred in the case of the extract containing only the bird cherry bark (P) 5.07 ± 0.03^b TE/g d.m.; this extract also showed the highest activity 6.62 ± 0.08^d TE/g d.m. in the ABTS radical scavenging assay (Table 3).

3.3 Ability to reduce copper ions (CUPRAC) and iron ions (FRAP)

It was found that the infusions tested were characterized by their ability to reduce Cu(II) copper ions. The highest activity occurred in the case of the sample which contained only cherry bark (P) (1083.9 ± 19.4^f µg quercetin/mL).

Table 2: Mineral composition of *P. padus* bark

Element mg/kg d.m.	Fe	Mo	B	Si	Zn	Cu	K	Ca	Mg	Ti	P	S	V	Mn	Al
Bark (<i>P. padus</i>)	142 ± 5.4	<0.01 01	4.7 ± 0.15	80 ± 19	171 ± 3	6 ± 0.1	19,457 ± 762	3,540 ± 379	3,202 ± 71	4 ± 1	2,925 ± 45	5,189 ± 62	2 ± 0.1	57 ± 8	34 ± 2

(mean $\pm$ SD; $N = 3$).

Table 3: The total content of Folin–Ciocalteu reagent-reactive compounds and antioxidant capacity against radicals (HORAC, DPPH, ABTS) in teas with added bird cherry bark

Sample	TPC ($\mu\text{g gallic acid/mL}$)	HORAC ($\mu\text{g gallic acid/mL}$)	DPPH (TE/g d.w.)	ABTS (TE/g d.w.)
B	79.4 ± 3.5^d	4.1 ± 0.2^b	3.32 ± 0.04^b	5.12 ± 0.11^c
C	80.1 ± 2.3^d	3.8 ± 0.0^b	2.76 ± 0.08^a	4.51 ± 0.12^b
Ch	55.0 ± 1.5^a	3.5 ± 0.1^a	2.62 ± 0.11^a	4.77 ± 0.08^b
P	114.5 ± 8.3^f	3.2 ± 0.2^a	5.07 ± 0.03^b	6.62 ± 0.08^d
T	97.7 ± 1.6^e	3.2 ± 0.3^a	4.87 ± 0.07^c	3.81 ± 0.03^a
BP5	74.8 ± 0.0^c	3.9 ± 0.1^b	3.87 ± 0.11^b	4.76 ± 0.15^b
BP10	80.9 ± 1.4^d	3.8 ± 0.1^b	3.55 ± 0.04^b	4.73 ± 0.08^b
BP15	93.1 ± 0.0^e	3.4 ± 0.1^a	4.11 ± 0.04^c	4.39 ± 0.03^b
BP20	102.3 ± 9.1^e	3.6 ± 0.2^{ab}	4.28 ± 0.06^c	4.98 ± 0.03^b
BP25	68.7 ± 0.0^b	3.9 ± 0.1^b	4.03 ± 0.04^c	5.37 ± 0.07^c
BP30	82.4 ± 0.0^d	4.0 ± 0.2^b	3.84 ± 0.02^b	5.34 ± 0.04^c

The results are mean values of three determinations $\pm$ standard deviation. The values sharing the same letter in a line were not significantly different ($P \leq 0.05$).

Among all the teas tested, the one containing 20% bird cherry bark (BP20) showed the highest activity ($939.1 \pm 27.1^e \mu\text{g quercetin/mL}$). Chamomile flower (Ch), similarly to its antioxidant activity with the Folin–Ciocalteu reagent, also showed the lowest copper-ion-reducing activity ($549.0 \pm 44.2^a \mu\text{g quercetin/mL}$). In the ferric ion reducing antioxidant power assay, testing the ability to reduce Fe(III) ions, the strongest properties were once again demonstrated for the infusion containing only cherry bark (P), where the activity was $6.7 \pm 0.1^d \mu\text{g Trolox/mL}$. The tea with 20% added bird cherry bark (BP20) also had, as in the case of copper ion reduction, strong iron-ion-reducing properties ($4.6 \pm 0.1^b \mu\text{g Trolox/mL}$). Chamomile flower (Ch) activity confirmed the previous results and was once again the weakest, showing a reducing power of $3.3 \pm 0.2^a \mu\text{g Trolox/mL}$ (Table 4).

3.4 Herbal infusion antimicrobial activity evaluation

The effect of 11 herbal tea aqueous solutions on the growth of 20 indicator microbial species, including fungi and gram-positive and gram-negative bacteria, was investigated (Table 5). The effects of aqueous solutions of teas containing single plant materials, i.e., bird cherry bark, chamomile, marigold, and linden, were analyzed first. In this case, the highest antimicrobial activity was shown by the aqueous solution of bird cherry bark against molds of the genus *Fusarium* spp. (16 mm). The antimicrobial activity of bird cherry may be related to the fact that it contains lupeol, which has been shown to exert anti-inflammatory activity, targeting key molecular pathways including $\text{NF-}\kappa\text{B}$, cFLIP, Fas, Kras, phosphatidylinositol-3-kinase (PI3K)/Akt, and Wnt/beta-catenin in various cell types (SALEEM, 2009). Overall, marigold has shown the

weakest antimicrobial potential. The components of the aqueous solution of marigold showed no effect against *Enterococcus faecalis* ATTC 29,212, *Clostridium butyricum* ATTC 860, *Lactobacillus fermentum* ATCC 14932 and *Lactococcus lactis* ATCC 11454, and *Bacillus subtilis* ATCC 6633. The combination of marigold, linden, and chamomile resulted in the aqueous solution of all these herbs having increased antimicrobial properties. In this case, the highest activity was shown against molds of the genus *Fusarium* spp. (18 mm) and *Mucor* spp. (17 mm). Enriching the marigold–linden–chamomile mixture by adding bird cherry bark significantly increased the antimicrobial activity of the solution tested. The highest antimicrobial activity occurred when the bird cherry bark content was between 20–30%, particularly against *Listeria monocytogenes* bacteria.

Table 4: Content of compounds reducing copper ions (CUPRAC) and iron ions (FRAP) in teas with added bird cherry bark

Sample	CUPRAC activity ($\mu\text{g quercetin/mL}$)	FRAP activity ($\mu\text{g Trolox/mL}$)
B	786.7 ± 23.2^{bc}	4.5 ± 0.3^{bc}
C	712.4 ± 34.7^b	4.0 ± 0.4^b
Ch	549.0 ± 44.2^a	3.3 ± 0.2^a
P	1083.9 ± 19.4^f	6.7 ± 0.1^d
T	762.3 ± 20.3^{bc}	4.0 ± 0.3^b
BP5	755.1 ± 12.0^b	3.4 ± 0.0^a
BP10	803.5 ± 22.2^c	4.4 ± 0.2^b
BP15	892.9 ± 16.3^d	4.7 ± 0.2^{bc}
BP20	939.1 ± 27.1^e	4.6 ± 0.1^b
BP25	854.8 ± 30.2^{cd}	4.7 ± 0.3^{bc}
BP30	879.4 ± 13.4^d	4.9 ± 0.1^c

The results are mean values of three determinations $\pm$ standard deviation. The values sharing the same letter in a line were not significantly different ($P \leq 0.05$).

Table 5: Evaluation of the effect of herbal teas on indicator microbes

Sample	P	Ch	C	T	B	BP5	BP10	BP15	BP20	BP25	BP30
Zone of inhibition (mm)											
Gram-positive bacteria											
<i>Staphylococcus aureus</i> ATCC 25923	8 ± 1	7 ± 1	5 ± 0	9 ± 1	12 ± 2	13 ± 2	15 ± 2	17 ± 3.0	19 ± 3	21 ± 3	24 ± 3
<i>Listeria monocytogenes</i> ATCC 7644	2 ± 0	4 ± 1	2 ± 0	6 ± 1	16 ± 2	18 ± 3	20 ± 3	22 ± 3	25 ± 3	27 ± 3	27 ± 3
<i>Enterococcus faecalis</i> ATCC 29212	11 ± 2	3 ± 0	0 ± 0	7 ± 1	14 ± 2	16 ± 2	16 ± 2	18 ± 2	19 ± 3	20 ± 3	22 ± 3
<i>Clostridium butyricum</i> ATCC 860	8 ± 1	0 ± 0	0 ± 0	7 ± 1	12 ± 2	17 ± 2	19 ± 2	21 ± 3	23 ± 3	25 ± 3	24 ± 3
<i>Lactobacillus fermentum</i> ATCC 14932	2 ± 0	2 ± 0	0 ± 0	9 ± 1	12 ± 2	14 ± 2	16 ± 2	18 ± 2	15 ± 2	17 ± 2	18 ± 2
<i>Lactococcus lactis</i> ATCC 11454	2 ± 0	2 ± 0	0 ± 0	11 ± 2	13 ± 2	15 ± 2	17 ± 2	19 ± 2	19 ± 2	20 ± 3	24 ± 3
<i>Streptococcus thermophilus</i> ATCC 19258	3 ± 0	3 ± 0	2 ± 0	9 ± 1	14 ± 2	16 ± 2	18 ± 2	19 ± 2	20 ± 3	22 ± 3	24 ± 3
<i>Bifidobacterium bifidum</i> ATCC 11863	3 ± 1	1 ± 0	3 ± 0	7 ± 1	10 ± 2	12 ± 2	15 ± 2	16 ± 2	15 ± 2	16 ± 2	18 ± 2
<i>Bacillus subtilis</i> ATCC 6633	9 ± 1	1 ± 0	0 ± 0	9 ± 1	13 ± 2	14 ± 2	16 ± 2	17 ± 2	18 ± 2	19 ± 2	21 ± 3
Gram-negative bacteria											
<i>Escherichia coli</i> ATCC 25922	5 ± 1	4 ± 0	3 ± 0	4 ± 0	10 ± 2	7 ± 1	9 ± 1	9 ± 1	10 ± 2	11 ± 2	12 ± 2
<i>Salmonella typhimurium</i> ATCC 14028	3 ± 1	4 ± 0	4 ± 0	3 ± 0	9 ± 1	8 ± 1	9 ± 1	11 ± 2	12 ± 2	12 ± 2	13 ± 2
<i>Proteus mirabilis</i> ATCC 12453	3 ± 1	4 ± 0	4 ± 0	3 ± 0	10 ± 2	9 ± 1	8 ± 1	9 ± 1	9 ± 1	10 ± 2	12 ± 2
<i>Klebsiella pneumoniae</i> ATCC 31488	9 ± 1	3 ± 0	4 ± 0	4 ± 0	8 ± 1	9 ± 1	10 ± 2	12 ± 2	15 ± 2	18 ± 2	19 ± 2
<i>Pseudomonas aeruginosa</i> ATCC 27853	8 ± 1	4 ± 0	4 ± 0	4 ± 0	8 ± 1	7 ± 1	8 ± 1	9 ± 1	9 ± 1	10 ± 2	13 ± 2
<i>Alcaligenes faecalis</i> ATCC 35655	6 ± 1	3 ± 0	2 ± 0	5 ± 0	7 ± 1	7 ± 1	7 ± 1	9 ± 1	11 ± 2	12 ± 2	13 ± 2
Fungi											
<i>Candida albicans</i> ATCC 10231	13 ± 1	4 ± 0	3 ± 0	8 ± 1	14 ± 2	15 ± 2	12 ± 2	14 ± 2	17 ± 2	18 ± 2	19 ± 2
<i>Saccharomyces cerevisia</i> ATCC 9776	11 ± 2	3 ± 0	4 ± 0	9 ± 1	16 ± 2	16 ± 2	12 ± 2	14 ± 2	15 ± 2	16 ± 2	18 ± 2
<i>Fusarium</i> spp.	16 ± 2	3 ± 0	4 ± 0	9 ± 1	18 ± 2	15 ± 2	16 ± 2	17 ± 2	17 ± 2	18 ± 2	19 ± 2
<i>Aspergillus</i> spp.	12 ± 2	3 ± 0	2 ± 0	9 ± 1	12 ± 2	9 ± 1	11 ± 2	14 ± 2	16 ± 2	17 ± 2	18 ± 2
<i>Mucor</i> spp.	14 ± 2	2 ± 0	3 ± 0	9 ± 2	17 ± 2	13 ± 2	14 ± 2	16 ± 2	17 ± 2	17 ± 2	18 ± 2

The size of the clear zone ranged between 25 and 27 mm (± 3) (Table 5).

3.5 Inhibition of cyclooxygenase-2

The cherry bird bark was also found to have the highest inhibitory activity against cyclooxygenase-2 (COX-2) – 77.0%. Therefore, using it as an ingredient in infusions is reasonable because it can enhance the effects of functional infusions that are a mixture of herbs. Among the proposed mixture formulations, the infusion with the highest proportion of bark (BP30) ($63.0 \pm 1.1^{\text{e}}$ %) had the highest activity in this regard. The weakest COX-2 inhibitory properties were demonstrated for the infusion of chamomile flower ($42.1 \pm 2.5^{\text{a}}$ %) and the base mixture (B) – $43.7 \pm 1.8^{\text{a}}$ % (Figure 2).

4 Discussion

Many research authors described the composition and properties of bird cherry; however, no direction was indicated for the use of the bark for nutritional purposes. The presented study shows that the bark may be a source

of calcium and magnesium, which may be important for deficiencies found in populations. This is also confirmed by other studies, where it was proved that bird cherry contains 192.30 ± 0.58 mg/100 g of calcium and $249.15 \pm 0.34^{\text{a}}$ mg/100 g of magnesium [17]. Those components are soluble in water, thus obtaining infusions from mixtures containing bark can enrich them in the above-mentioned components.

P. padus bark, leaves and fruit are also known for their anti-inflammatory, antimicrobial, and antioxidant properties [8]. The known use of the fruit or the leaves will not affect the limitation of bird cherry expansion in the environment. The bark also contains plant metabolites, particularly polyphenols, which play an important role in protecting the body against the adverse effects of free radicals involved in oxidative stress [18]. The high antioxidant potential of the bark was also presented by other authors who analyzed methanolic extracts of bird cherry bark for DPPH radical scavenging activity. They found that the bark has high antioxidant potential ($85.82 \pm 4.42^{\text{b}}$ % EDA – electron-donating ability) [19]. In the presented study using hydroxyl radical, the addition of bark to the herbal mixture increased the antioxidant activity by 52% compared to the control tea. Likely, the compounds found in bird cherry bark acted synergistically

Figure 2: COX 2 inhibition in infusions with the bark of bird cherry.

with the compounds extracted from linden flower which showed higher activity by 146% compared to that of the base infusion.

Therefore, the use of the bark may have beneficial antioxidant and supporting functions of the raw materials known and used as infusion ingredients. Furthermore, it should be pointed out that the results indicating the highest antioxidant potential of the bark – compared to the results of our previous studies concerning bird cherry leaves and fruit – further confirmed the validity of using the bark as an ingredient of functional mixtures [20]. The above-mentioned effect of the bark was also pointed out by other authors who assessed the antioxidant activity using the DPPH-free radical scavenging assay. The antioxidative effect of *P. padus* bark water extract was 71% at 350 µg/mL and higher than 70% at all concentrations [21]. The effects of bark components may be multidirectional.

The compounds that belong to the group of antioxidants and are extracted from the bark can act according to different mechanisms. According to the presented study, the bark components can enhance the antioxidant activity of infusions by increasing the activity to reduce Fe(III) ions to Fe(II) and Cu(II) ions to Cu(I).

The literature review shows that antioxidant properties of plant-derived products determined by the FRAP method tend to be in strong correlation with ascorbic acid content and with polyphenolic compound content. Hence, in the FRAP assay, the brewer containing bird cherry bark alone showed in the presented study a 48% higher activity than the infusion containing the base mixture. We determined the same correlation in the CUPRAC assay, where the mixture containing the bark alone showed a higher 37% activity to reduce copper ions than the base mixture alone. The infusion with 20% added bird cherry bark enhanced the tea activity by 20%.

Functional effects may include inhibition of COX-2, the enzyme involved in inflammation. It was proved that the bird cherry bark infusion and the infusion with the highest concentrations of bird cherry bark had the highest activity. Choi et al. demonstrated that methanolic extracts of *P. padus* stems possessed potent anti-inflammatory properties and not only suppressed various inflammatory mediators *in vitro*, but also reduced inflammatory swelling *in vivo*. It has also been shown to have potent antinociceptive effects, acting as a partial opioid agonist through central and peripheral mechanisms [8].

Although numerous new antibiotics have been developed in recent years, many human pathogens gained increased resistance to them due to the mass commercial use of antibiotics [22]. Therefore, alternative antibiotics – including plant extracts and phytochemicals – are needed to develop new treatments for infectious diseases. Bird cherry bark teas can also supplement a bacteriostatic diet. The highest antimicrobial activity occurred when the bird cherry bark content was between 20–30%, particularly against *Listeria monocytogenes* bacteria. The effect of *P. padus* bird cherry bark was also evaluated in another study. In this case, the methanolic extract of bird cherry bark showed antimicrobial activity against most of the gram-positive bacteria tested, while for gram-negative bacteria, it showed the highest activity against *Kocuria rhizophila* (Minimum inhibitory concentration = 125 µg/mL) [23].

5 Conclusion

The bark of the bird cherry is characterized by a high content of biologically active compounds that have a

beneficial effect on the human body, as well as high anti-oxidant activity. Therefore, it can effectively reduce the occurrence of oxidative stress, which contributes to the emergence of civilization and neurodegenerative diseases. At the same time, it should be noted that the bark of the bird cherry is characterized by a diversified profile of bioactive compounds and a different antioxidant activity. This means that the best health-promoting effect can be obtained by using the bark of the bird cherry as an ingredient of functional food, e.g., herbal infusions with its addition. The research showed that the addition of bird cherry bark to herbal infusions can beneficially enrich the health-promoting properties of teas. Therefore, they can play an important role in the prevention of neurodegenerative diseases, thanks to their properties resulting from their inhibitory activity against key enzymes in health prophylaxis.

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

Author contributions: A.T., J.K.C. – conceptualization; P.S., R.W. – data curation; A.T., D.S. – formal analysis; J.K.C. – funding acquisition; A.T., J.K.C., D.S. – investigation; J.K.C., R.W., D.S., D.S. – methodology; A.T. – project administration; A.T., P.S. – resources; D.S., R.W. – software; J.K.C., P.S. – supervision; A.T., D.S. – validation; D.S. – visualization; A.T. – writing – original draft; J.K.C., P.S. – writing – review & editing

Conflict of interest: The authors declare no conflicts of interest.

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

Data availability statement: All data generated or analyzed during this study are included in this published article.

References

- [1] Gordon AG, Rowe DCF. Seed manual for ornamental trees and shrubs. UK For Comm Bull. 1982;59.
- [2] Wickens GE, Kunkel G. Plants for human consumption. Kew Bull. 1987;42(1):273. doi: 10.2307/4109919.
- [3] Schultes RE. Cornucopia: a source book of edible plants. J Ethnopharmacol. 1991;34:291–2.
- [4] Telichowska A, Kobus-Cisowska J, Stuper-Szablewska K, Ligaj M, Tichoniuk M, Szymanowska D, et al. Exploring antimicrobial and antioxidant properties of phytochemicals from different anatomical parts of *Prunus padus* L. Int J Food Prop. 2020;23:2097–109.
- [5] Telichowska A, Kobus-cisowska J, Szulc P. Phytopharmacological possibilities of bird cherry *prunus padus* L. and *Prunus serotina* L. species and their bioactive phytochemicals. Nutrients. 2020;12:1–21.
- [6] Telichowska A, Kobus-Cisowska J, Stuper-Szablewska K, Ligaj M, Tichoniuk M, Szymanowska D, et al. Exploring antimicrobial and antioxidant properties of phytochemicals from different anatomical parts of *Prunus padus* L. Int J Food Prop. 2020;23:2097–109.
- [7] Telichowska A, Kobus-Cisowska J, Ligaj M, Stuper-Szablewska K, Szymanowska D, Tichoniuk M, et al. Polyphenol content and antioxidant activities of *Prunus padus* L. and *Prunus serotina* L. leaves: Electrochemical and spectrophotometric approach and their antimicrobial properties. Open Chem. 2020;18:1125–35.
- [8] Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of *Prunus padus*. J Ethnopharmacol. 2012;144:379–86.
- [9] Mikulic-Petkovsek M, Stampar F, Veberic R, Sircelj H. Wild *Prunus* fruit species as a rich source of bioactive compounds. J Food Sci. 2016;81:C1928–37.
- [10] Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J. Nutr. 2000;130(8):2073–85. American Institute of Nutrition.
- [11] Chan EWC, Lim YY, Chong KL, Tan JBL, Wong SK. Antioxidant properties of tropical and temperate herbal teas. J Food Compos Anal. 2010;23:185–9.
- [12] Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005;91:571–7.
- [13] Denev P, Kratchanova M, Ciz M, et al. Biological activities of selected polyphenol-rich fruits related to immunity and gastrointestinal health. Food Chem. 2014;157:37–44.
- [14] Kobus-Cisowska J, Szulc P, Szczepaniak O, Dziedzinski M, Szymanowska D, Szymandera-Buszk K, et al. Variability of *Hordeum vulgare* L. Cultivars in yield, antioxidant potential, and cholinesterase inhibitory activity. Sustainability. 2020;12:1938.
- [15] Öztürk M, Duru ME, Kivrak Ş, Mercan-Doğan N, Türkoglu A, Özler MA. In vitro antioxidant, anticholinesterase and antimicrobial activity studies on three *Agaricus* species with fatty acid compositions and iron contents: a comparative study on the three most edible mushrooms. Food Chem Toxicol. 2011;49:1353–60.
- [16] Oyaizu M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Japanese J Nutr Diet. 1986;44:307–15.
- [17] García-Aguilar L, Rojas-Molina A, Ibarra-Alvarado C, Rojas-Molina JI, Vázquez-Landaverde PA, Luna-Vázquez FJ, et al. Nutritional value and volatile compounds of black cherry (*prunus serotina*) seeds. Molecules. 2015;20:3479–95.
- [18] Telichowska A, Kobus-Cisowska J, Szulc P, Ligaj M, Stuper-Szablewska K, Szwajgier D, et al. 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. *Sustain.* 2021;13:3913.
- [19] Hyun TK, Kim HC, Kim JS. In vitro screening for antioxidant, anti-microbial, and antidiabetic properties of some Korean native plants on Mt. Halla, Jeju Island. *Indian J Pharm Sci.* 2015;77:668–74.
- [20] Telichowska A, Kobus-Cisowska J, Stuper-Szablewska K, Ligaj M, Tichoniuk M, Szymanowska D, et al. Exploring anti-microbial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L. *Int J Food Prop.* 2020;23:2097–109.
- [21] Hwang D, Kim H, Shin H, Jeong H, Kim J, Kim D. Cosmetic effects of *Prunus padus* bark extract. *Korean J Chem Eng.* 2014;31:2280–5.
- [22] Pattanayak S. Alternative to antibiotics from herbal origin - outline of a comprehensive research project. *Curr Pharmacogenomics Person Med.* 2018;16:9–62.
- [23] Hyun TK, Kim HC, Kim JS. In vitro screening for antioxidant, antimicrobial, and antidiabetic properties of some Korean native plants on Mt. Halla, Jeju Island. *Indian J Pharm Sci.* 2015;77:668–74.