

Szkoła Główna Gospodarstwa Wiejskiego
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**CHARAKTERYSTYKA CZEREMCHY
(*Prunus padus* L. i *Prunus serotina* L.)
JAKO ŹRÓDŁA SKŁADNIKÓW
AKTYWNYCH KSZTAŁTUJĄCYCH
WŁAŚCIWOŚCI FUNKCJONALNE
ŻYWNOŚCI**

Characteristic of *Prunus padus* L. and *Prunus serotina* L. as sources
of phytochemicals determining functional properties of food

Praca doktorska

Doctoral thesis

Praca wykonana pod kierunkiem
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Streszczenie

Charakterystyka czarernchy (*Prunus padus* L. i *Prunus serotina* L.) jako źródła składników aktywnych kształtujących właściwości funkcjonalne żywności

Celem pracy była charakterystyka czarernchy (*Prunus padus* L. i *Prunus serotina* L.) jako źródła składników aktywnych kształtujących właściwości funkcjonalne żywności. Wykazano, że rodzaj czarernchy ma wpływ na zawartość związków polifenolowych, potencjał przeciwutleniający oraz aktywność przeciwdrobnoustrojową, z czego odmiana *Prunus padus* ma większy potencjał jako surowiec o właściwościach funkcjonalnych do zastosowania w technologii żywności. Ponadto stwierdzono, że właściwości te zależą od części morfologicznej czarernchy zwyczajnej (*P. padus*), a kora może stanowić dobry surowiec do wytwarzania napojów funkcjonalnych. Wykazano, iż jej dodatek wpływa na właściwości przeciwutleniające naparów ziołowych oraz aktywność inhibicyjną wobec: cholinoesteraz, reduktazy i peroksydazy glutationowej, cyklooksygenazy, katalazy oraz dysmutazy ponadtlenkowej. Udowodniono, że dodatek kory wpływa na zintensyfikowanie smaku słodkiego i nie wpływa negatywnie na zapach, jak i smak naparów.

Słowa kluczowe: czarerncha, *Prunus padus*, *Prunus serotina*, polifenole, przeciwutleniacze, żywność funkcjonalna.

Summary

Characteristic of *Prunus padus* L. and *Prunus serotina* L. as sources of phytochemicals determining functional properties of food

The aim of the study was to characterize the bird cherry (*Prunus padus* L. and *Prunus serotina* L.) as a source of active ingredients shaping the functional properties of food. It has been shown that the type of bird cherry has an impact on the content of polyphenols, antioxidant potential, and antimicrobial activity, of which the *Prunus padus* variety has greater potential as a raw material with functional properties for use in food technology. Moreover, it was found that these properties depend on the morphological part of the bird cherry (*P. padus*) and the bark can be a good raw material for the production of functional drinks. Its addition has been shown to affect the antioxidant properties of herbal infusions and the inhibitory activity against cholinesterases, glutathione reductase and peroxidase, cyclooxygenase, catalase, and superoxide dismutase. The addition of bark has been shown to intensify the sweet taste and does not adversely affect both the smell and the taste of the infusions.

Keywords: bird cherry, *Prunus padus*, *Prunus serotina*, polyphenols, antioxidants, functional food.

PODZIĘKOWANIA

*Szczególne podziękowania składam mojemu Promotorowi,
Pani dr hab. Joannie Kobus-Cisowskiej, prof. UPP,
za nieocenione wsparcie podczas realizacji pracy doktorskiej,
poświęcony czas, zrozumienie i motywację do dalszych działań.
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a) Praca przeglądowa stanowiąca wprowadzenie do tematyki rozprawy doktorskiej

P I.:

Telichowska, A., Kobus-Cisowska, J., Szulc, P. (2020). Phytopharmacological possibilities of bird cherry *Prunus padus* L. and *Prunus serotina* L. species and their bioactive phytochemicals, *Nutrients*, 12(7), 1–21. <https://doi.org/10.3390/nu12071966>.

IF₂₀₂₁ 5.43

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b) Prace oryginalne stanowiące cykl wyników badań rozprawy doktorskiej

P II.:

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IF₂₀₂₁ 1.55

MEiN₂₀₂₁ 70

P III.:

Telichowska, A., Kobus-Cisowska, J., Stuper-Szablewska, K., Ligaj, M., Tichoniuk, M., Szymanowska, D., Szulc, P. (2020). Exploring antimicrobial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L., *International Journal of Food Properties*, 23(1), 2097–2109. <https://doi.org/10.1080/10942912.2020.1843486>.

IF₂₀₂₁ 2.77

MEiN₂₀₂₁ 70

P IV.:

Telichowska, A., Kobus-Cisowska, J., Szulc, P., Ligaj, M., Stuper-Szablewska, K., Szwajgier, D., 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(7), 3913. <https://doi.org/10.3390/su13073913>.

IF₂₀₂₁ 3.25

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P V.:

Telichowska, A., Kobus-Cisowska, J., Szulc, P., Wilk, R., Szwajgier, D., Szymanowska, D. (2021). *Prunus padus* L. bark as a functional promoting component in functional herbal infusions – cyclooxygenase-2 inhibitory, antioxidant, and antimicrobial effects, *Open Chemistry*, 19(1), 1052–106. <https://doi.org/10.1515/chem-2021-0088>.

IF₂₀₂₁ 1.55

MEiN₂₀₂₁ 70

P VI.:

Telichowska, A., Kobus-Cisowska, J., Cielecka-Piontek, J., Sip, Sz., Stuper-Szablewska, K., Szulc, P. (2022). *Prunus padus* L. as a source of functional compounds antioxidant activity and antidiabetic effect, *Emirates Journal of Food and Agriculture*, 34(2), 135-143. <https://doi.org/10.9755/ejfa.2022.v34.i2.2819>.

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1. WPROWADZENIE

1.1. Charakterystyka botaniczna *Prunus padus* L. oraz *Prunus serotina* L.

Czeremcha (*Prunus* L.) to rodzaj drzewa lub dużego krzewu zaliczany do podrodziny *Amygdaloideae* (= *Prunoideae*) w obrębie rodziny *Różowatych*. Rośliny z gatunku *Prunus* występują głównie na półkuli północnej oraz w strefie umiarkowanej, najczęściej w całej Europie, Azji Mniejszej i zachodniej Syberii (Shaw & Small, 2005). Gatunek ten skupia około dwudziestu rodzajów roślin, a najpopularniejsze są dwa z nich: czeremcha zwyczajna *Prunus padus* L. oraz czeremcha amerykańska *Prunus serotina* L.

Wysokość drzew (*P. padus*) może sięgać do 15 metrów (Mauri & Caudullo, 2016), natomiast krzewów oscyluje w granicach od 0,5 do 4 metrów. Drzewa czeremchy amerykańskiej (*P. serotina*) rosną do wysokości nawet 25 metrów (Szweykowski & Szweykowska, 2003; Telichowska et al., 2019). Charakteryzują się okrągłą lub jajowatą koroną wraz z opadającymi gałęziami oraz ciemnobrązowymi pędami, które tworzą formę krzewiastą (Leather, 1996; Rumińska & Ożarowski, 1990). Drzewa docelowe wymiary osiągają w wieku około 100 lat (Powell et al., 2011).

Odróżnienie czeremchy zwyczajnej (*P. padus*) od czeremchy amerykańskiej (*P. serotina*) możliwe jest dzięki kształtom liści i kwiatów. Czeremcha amerykańska charakteryzuje się podługnymi, lancetowatymi, zaostrozonymi liśćmi, które są ostro ząbkowane na brzegach, a z wierzchu błyszczące i ciemnozielone, natomiast od spodu mają barwę jasnozieloną (Auclair & Cottam, 1971). Odznaczają się słabo widoczną siatką nerwów. Liście czeremchy zwyczajnej są mniejsze, a jej brzegi drobniej ząbkowane. Ogonek liściowy jest zwykle czerwony, a same liście są bardziej matowe i skórzaste. Siatka nerwów jest gęsta, połączona na brzegu liścia (Leather, 1996). Czeremcha zwyczajna swoje kwitnienie rozpoczyna na przełomie kwietnia i maja, z kolei czeremcha amerykańska kwitnie dopiero w okolicach maja i czerwca. Kwiaty obydwu roślin są kremowo-białe i tworzą walcowate grona (Åström & Hæggström, 2018; Kulesza, 1952) jednak kwiaty czeremchy amerykańskiej nie wydzielają zapachu i są mniejsze niż kwiaty czeremchy zwyczajnej (Podbielkowski, 1980). Małe, kuliste, czarne pestkowce to owoce, które pojawiają się około sierpnia i września (Halarewicz, 2012).

Ekspansję ekologiczną czeremcha zawdzięcza wysokiej efektywności pomnażania wegetatywnego i rozmnażania generatywnego dzięki takim cechom, jak: duża zdolność i szybkość kiełkowania nasion, ich znaczna produkcja, daleka dyspersja, szybki wzrost

i rozwój, małe wymagania w stosunku do warunków siedliskowych oraz wysoka tolerancja na warunki klimatyczne (Starfinger et al., 2003). Czeremcha rośnie dobrze na stanowiskach częściowo zaciemnionych oraz bogatych w składniki mineralne glebach, a miejsce jej występowania nie ma wpływu na cechy drzew, takie jak liście, owoce czy kora (Karczmarczyk, 2012).

1.2. Porównanie cech botanicznych *Prunus padus* L. i *Prunus serotina* L.

W tabeli 1 zaprezentowano zestawienie cech botanicznych *Prunus padus* L. i *Prunus serotina* L.

Tabela 1. Porównanie cech botanicznych *Prunus padus* L. i *Prunus serotina* L.

Cecha botaniczna	<i>Prunus padus</i> L.	<i>Prunus serotina</i> L.
Pochodzenie	Gatunek rodzimy	Kenofit introdukowany z Ameryki Północnej – poza obszarem naturalnego występowania uważany jest za gatunek inwazyjny
Kraj pochodzenia	Europa, Azja Mniejsza	Ameryka Północna
Występowanie	Grądy, łęgi, żyzne i wilgotne lasy liściaste oraz mieszane	Bory i lasy mieszane
Wymagania siedliskowe	Eutroficzne gleby żyzne i wilgotne, dobrze rośnie w gorszych warunkach siedliskowych	Szeroka amplituda wymagań siedliskowych: od gleb ubogich i suchych do żyznych i zasobnych w wodę
Pokrój	Drzewo lub krzew osiąga około 15–17 m wysokości	Drzewo lub krzew osiąga do 20 m wysokości
Liście	– Długość 6–12 cm – Eliptyczne lub podługne odwrotnie jajowate, zaostrome, u nasady sercowate, na brzegu ostro piłkowane – Górna strona matowo-zielona, pomarszczona, dolna szaro-zielona, naga lub z kępkami włosków w kątach nerwów, ogonki krótkie, czerwone, z dwoma gruczołkami	– Długość 5–12 cm – Sztywne, podługne, jajowate lub lancetowate, zaostrome – Brzegi drobno, gruczołkowato piłkowane – Górna strona ciemnozielona i błyszcząca, dolna jaśniejsza, owłosiona na nerwie głównym, na ogonku występują niekiedy gruczołki
Użyłkowanie liścia	Gęsta	Słabo widoczna
Kwitnienie	Kwiecień / maj	Maj / czerwiec

Cecha botaniczna	<i>Prunus padus</i> L.	<i>Prunus serotina</i> L.
Kwiatostan	– Zwieszony lub rzadko wzniesiony – Płatki szeroko odwrotnie jajowate o długości 10-15 mm, prawie dwa razy dłuższe od pręcików	– Wzniesiony lub wznoszący się – Ząbki kielicha trwałe, pozostają na owocu – Owoce niedojrzałe, różowe lub czerwone, potem ciemnoczerwone do czarnych
Owoce	Aromatyczne, lekko cierpkie, gorzkawe, ciemnofioletowe	Aromatyczne, lekko cierpkie, słodkie, ciemnofioletowe
Kora	– Kora ma przyjemny zapach – Roztarte pączki mają zapach przypominający migdały	– Roztarta kora o charakterystycznym, ostrym, stosunkowo przyjemnym zapachu, podobnym do zapachu czarnej porzeczki
Znaczenie gospodarcze	– Roślina: miododajna, ozdobna; wykorzystywana do rekultywacji terenów po przemysłowych – Owoce i kwiaty wykorzystywane są jako surowiec farmaceutyczny i spożywczy	– Cenna domieszka biocenotyczna – Roślina miododajna, ozdobna

1.3. Wartość odżywcza i zawartość związków aktywnych w *Prunus padus* L. i *Prunus serotina* L.

Spożycie czeremchy miało miejsce już w czasach prehistorycznych. Świadczą o tym szczątki naczyń z neolitu i brązu, na których zdobienia obrazują czeremchę, które odkryto w palafitach (domach wzniesionych na palach) w Azji Południowo-Wschodniej. O jej użytkowaniu informuje także grecki historyk Herodot z Halikarnasu. Owoce konsumowano wówczas z dodatkiem soli, a niezależnie od tego wytwarzano też alkohol (Karczmareczuk, 2012). Aktualnie czeremcha zwyczajna (*P. padus*) oraz czeremcha amerykańska (*P. serotina*) nie znajdują szerokiego zastosowania w przemyśle farmaceutycznym ani spożywczym.

Kuliste, ciemnofioletowe owoce czeremchy zawierają stosunkowo wysoką zawartość białka oraz węglowodanów (Luna-Vázquez et al., 2013). W ich składzie znajdują się cukry redukujące, takie jak glukoza czy fruktoza. W owocach *P. padus* występuje zarówno chlorofil *a*, jak i chlorofil *b*, jednakże zawartość chlorofilu *a* jest dwukrotnie wyższa. W składzie owoców czeremchy stwierdzono obecność karotenoidów, z których większą część stanowi beta-karoten oraz antocyjanów (Mikulic-Petkovsek et al., 2016). Dane

literaturowe wskazują, iż aktywność przeciwutleniająca cyjanidyny jest nawet o 4,4 razy wyższa w porównaniu do kwasu askorbinowego (Zuo et al., 2019). Występuje ona głównie w owocach o barwie czerwonej, niebieskiej i fioletowej (Mikulic-Petkovsek et al., 2016). Owoce czeremchy w swoim składzie zawierają również alfa-, beta- i gamma-tokoferol, przy czym alfa-tokoferol występuje w najwyższej ilości i stanowi aż 63% udziału wszystkich tokoferoli w owocach (Mikulic-Petkovsek et al., 2016). Kolejnym związkiem ważnym w profilaktyce wielu chorób, a obecnym w owocach czeremchy, jest witamina C, występująca w formie kwasu askorbinowego oraz kwasu dehydroaskorbinowego (Donno et al., 2018).

Głównym kwasem fenolowym, wpływającym na aktywność przeciwutleniającą liści czeremchy, jest kwas chlorogenowy, który występuje w liściach na poziomie 1.39–1.94%, co stwierdzono w badaniach przeprowadzonych przez Olszewską i Kwapisz (Olszewska & Kwapisz, 2011). W innych badaniach wskazano, że to w głównej mierze rutyna odpowiedzialna jest za właściwości antyoksydacyjne liści *P. serotina* (Luna-Vázquez et al., 2016). W literaturze przedmiotu wskazuje się również na aktywność przeciwdrobnoustrojową liści czeremchy (Hyun et al., 2015).

1.4. Surowce roślinne w profilaktyce chorób cywilizacyjnych

W ostatnim czasie wzrasta zainteresowanie naturalnymi związkami roślinnymi, które mogą oddziaływać na zdrowie. Do tej grupy należą polifenole, które są wtórnymi metabolitami roślin, charakteryzującymi się zróżnicowaną budową chemiczną. Liczba pierścieni aromatycznych oraz sposób ich połączenia dzieli je na poszczególne klasy. Do tych klas należą: flawonoidy, kwasy fenolowe, stilbeny oraz lignany. Polifenole zlokalizowane są w różnych częściach roślin, tj. występują w korzeniach, łodygach, liściach, kwiatach oraz owocach (Abbas et al., 2017).

Liczba i położenie grup hydroksylowych determinują właściwości antyoksydacyjne (w tym przeciwrodnikowe) tych związków. Głównymi rodnikami w układach biologicznych są reaktywne formy tlenu i reaktywne formy azotu. Ich nadprodukcja powoduje stres oksydacyjny odpowiedzialny za uszkodzenie makrocząsteczek komórkowych, w tym lipidów, białek, i DNA w organizmach (Lawson et al., 2018). Wolne rodniki przyczyniają się również do: uszkodzeń tkanek, śmierci komórkowej, inicjacji chorób nowotworowych, uszkodzeń centralnego układu nerwowego, starzenia się organizmu, zapalenia stawów, rozwoju miażdżycy tętnic, rozwoju chorób sercowo-

naczyniowych czy chorób niedokrwienych serca, otyłości oraz urazu wielu tkanek (Poprac et al., 2017). Dlatego ważne jest zapewnienie w diecie surowców, które zawierają przeciwutleniacze ograniczające następstwa stresu oksydacyjnego. Aktywność biologiczna związków polifenolowych zależy w dużej mierze od ich biodostępności (Quideau et al., 2011). Takie związki, jak tokoferole, kwas askorbinowy czy karetonoidy, mogą wzmacniać działanie polifenoli (Scalbert et al., 2005). Zawartość polifenoli i ich rozmieszczenie w owocach zależy w dużej mierze od tempa i sposobu dojrzewania owoców, uwarunkowań genetycznych oraz środowiskowych (Ferretti et al., 2010). W literaturze przedmiotu dostępne są wyniki badań wskazujące na wysoki potencjał przeciwutleniający owoców, jak również liści, kory oraz kwiatów *P. padus* i *P. serotina* (Donno et al., 2018; Hyun et al., 2015; Luna-Vázquez et al., 2013; Petkovsek et al., 2010).

Dane epidemiologiczne wskazują na bezpośrednią zależność między wielkością spożycia warzyw i owoców a redukcją ryzyka zapadalności na choroby neurodegeneracyjne, cukrzycę, nowotwory czy choroby układu krążenia. W literaturze znajdują się liczne doniesienia o pozytywnym wpływie polifenoli roślinnych. Korzystne działanie w profilaktyce i leczeniu nadciśnienia tętniczego stwierdzono w przypadku takich roślin, jak: nauclea (Akpanabiatu et al., 2005), herbata (Patil & Balaraman, 2011), nasion grejpfruta (Feringa et al., 2011), czosnku (Pérez-Torres et al., 2016) czy wiecznika kulistego (Arcanjo et al., 2011). Badania dotyczące wpływu spożycia poszczególnych części morfologicznych czeremchy przez osoby z nadciśnieniem tętniczym wykazały ich wysoką skuteczność, która wynikała głównie z obecności kwasu chlorogenowego jako związku o działaniu zwiotczającym mięśnie gładkie naczyń krwionośnych (Luna-Vázquez et al., 2013). Ekstrakty z owoców czeremchy zawierają polarne i niepolarne metabolity o działaniu rozszerzającym naczynia krwionośne (Luna-Vázquez et al., 2013). W owocach stwierdzono wysoką zawartość związków fenolowych, takich jak: kwas chlorogenowy, kwas galusowy, kwas kawowy, katechina, epikatechina czy kwercetyna, które są bezpośrednio związane z wysoką aktywnością przeciwutleniającą (Luna-Vázquez et al., 2013).

Wiele preparatów ziołowych stosowanych jest także w profilaktyce i we wspomaganiu leczenia cukrzycy (Salehi et al., 2019). Wykazano, że w cukrzycy typu II inhibitory alfa-glukozydazy pochodzenia naturalnego, takie jak akarboza, 1-deoksynojirimycyna i genisteina, korzystnie opóźniają wzrost poziomu glukozy we krwi (Ramdanis et al., 2012). Pozytywne działanie przeciwcukrzycowe zostało potwierdzone także dla ekstraktów

z gałęzi czeremchy, które badano pod kątem działania hamującego aktywność alfa-glukozydazy (Hyun et al., 2015).

Kolejnym poważnym problemem współczesnej medycyny jest rosnąca liczba zachorowań na nowotwory. Wyniki badań potwierdzają, że dieta bogata w polifenole może przyczynić się do zmniejszenia ryzyka zachorowania na niektóre typy nowotworów (Fantini et al., 2015; Fresco et al., 2009). Związki te chronią komórki przed peroksydacją lipidów oraz modulują wytwarzanie uczestniczących w procesie kancerogenezy leukotrienów oraz prostaglandyn, a także mogą wpływać na enzymy antyoksydacyjne, takie jak: katalaza, dysmutaza ponadtlenkowa, peroksydaza glutationowa czy reduktaza glutationowa. Stymulacja tych enzymów może przyczyniać się do obniżenia zdolności wytwarzania reaktywnych form tlenu oraz wolnych rodników (Fresco et al., 2006; Kaushal et al., 2018). Różnorodność procesów, na które wpływ mają roślinne polifenole, w organizmie człowieka jest wielokierunkowa. Oddziałują one zarówno na aktywność enzymów zaangażowanych w procesy detoksykacyjne ksenobiotyków, biorą udział w procesie zmiatania wolnych rodników oraz w procesie chelatowania metali, a także przyczyniają się do proliferacji komórek modulowania aktywności enzymatycznej, jak również posiadają właściwości immunomodulujące (Fresco et al., 2006; Niedzwiecki et al., 2016).

Proces zapalny jest ważnym mechanizmem obronnym w organizmie człowieka. Może on charakteryzować się zaczerwienieniem, obrzękiem, bólem, uczuciem ciepła i dysfunkcją tkanki oraz narządów. Procesy zapalne mogą przyczyniać się także do rozwoju wielu chorób neurodegeneracyjnych, takich jak choroba Parkinsona czy choroba Alzheimera (Gómez-Betancur et al., 2019; Kadi et al., 2019; Reis Simas et al., 2019; Serafini et al., 2010). Dostępne dane literaturowe wskazują, iż nadmierna produkcja azotu i prostaglandyny bierze udział w przebiegu chorób prozapalnych, w tym reumatoidalnym zapaleniu stawów, astmie czy nowotworach (Rakel & Rindfleisch, 2005). Ich wysokie poziomy są wynikiem aktywności enzymów, takich jak indukowalna syntaza tlenu azotu i cyklooksygenazy-2 (Jeong & Jeong, 2010). Dlatego też trwają liczne badania nad opracowaniem mechanizmu zahamowania ekspresji tych enzymów, co może okazać się skutecznym efektem terapeutycznym w leczeniu chorób zapalnych i neurodegeneracyjnych (Dinarello, 2010). Czeremcha posiada w swoim składzie związki, które mają istotne znaczenie we wspomaganiu leczenia stanów zapalnych (Olszewska & Kwapisz, 2011). Wykazują one działanie przeciwutleniające oraz właściwości przeciwdrobnoustrojowe (Choi et al., 2012; Kumarasamy et al., 2004).

2. CEL PRACY I HIPOTEZY BADAWCZE

Czeremcha (*Prunus*) jest gatunkiem rośliny popularnej zarówno w Polsce, jak i w Europie. Do tej pory nie znalazła jednak szerokiego zastosowania w technologii żywności. Aktualnie wzrasta zainteresowanie naturalnymi składnikami roślinnymi, których stosowanie mogłoby urozmaicić dietę i przyczynić się do poprawy zdrowia. Na rynku znajdują się nieliczne środki spożywcze zawierające czeremchę, dlatego też korzystne jest wskazanie możliwych kierunków zagospodarowania tej rośliny w żywności.

2.1. Cel pracy

Celem nadrzędnym pracy była charakterystyka czeremchy (*Prunus padus* L. i *Prunus serotina* L.) jako źródła składników aktywnych kształtujących właściwości funkcjonalne żywności.

Cel główny zrealizowano w oparciu o cele szczegółowe, które sprecyzowano następująco:

- Ocena przydatności czeremchy (*Prunus padus* L. i *Prunus serotina* L.) pozyskanej ze środowisk naturalnych jako źródła związków biologicznie aktywnych.
- Charakterystyka oraz ocena zawartości wybranych związków bioaktywnych w częściach morfologicznych czeremchy zwyczajnej (*Prunus padus* L.).
- Wpływ warunków ekstrakcji czeremchy (*Prunus padus* L.) na właściwości przeciwutleniające i inhibicyjne wobec wybranych enzymów.
- Ocena właściwości związków aktywnych kory czeremchy (*Prunus padus* L.) jako składnika funkcjonalnych mieszanek ziołowych.

2.2. Zadania badawcze przewidziane do realizacji

2.2.1. Ocena aktualnego stanu wiedzy w zakresie występowania, sposobu i warunków wzrostu oraz ekspansywności czeremchy. Analiza danych w zakresie składu i właściwości czeremchy zwyczajnej (*P. padus*) oraz czeremchy amerykańskiej (*P. serotina*). Ocena właściwości funkcjonalnych, użytkowych i dotychczasowego zastosowania w przemyśle.

2.2.2. Charakterystyka liści czeremchy zwyczajnej (*P. padus*) oraz czeremchy amerykańskiej (*P. serotina*) pod względem: zawartości polifenoli, potencjału przeciwutleniającego mierzonego metodami z kationorodnikiem ABTS i rodnikiem DPPH, chelatowania jonów metali, właściwości redukujących,

metodami elektrochemicznymi (SWV), oraz poprzez ocenę aktywności przeciwdrobnoustrojowej.

2.2.3. Ocena zawartości wybranych fitozwiązków w poszczególnych częściach morfologicznych czeremchy *P. padus* (kora, liście, owoce). Porównanie potencjału przeciwutleniającego oraz przeciwdrobnoustrojowego. Ocena zawartości związków aktywnych metodami elektrochemicznymi oraz ocena właściwości przeciwdrobnoustrojowych.

2.2.4. Opracowanie modelowych mieszanek ziołowych (herbatek) z wykorzystaniem kory czeremchy (*P. padus*) poprzedzone optymalizacją warunków ekstrakcji dla kory czeremchy metodą elektrochemiczną. Ocena zawartości związków aktywnych oraz potencjału przeciwutleniającego. Ocena wpływu czeremchy na stopień inhibicji enzymów: alfa-glukozydazy, cyklooksygenazy (COX-2), cholinoesterazy (acetylocholinoesterazy (AChE) oraz butyrylocholinoesterazy (BChE)), dysmutazy ponadtlenkowej (SOD), katalazy, reduktazy i peroksydazy glutationowej. Ocena opracowanych naparów pod względem aktywności przeciwdrobnoustrojowej oraz ocena sensoryczna.

2.2. Hipotezy badawcze

Na podstawie zaplanowanych zadań sformułowano następujące hipotezy badawcze:

H.1. Rodzaj czeremchy ma wpływ na zawartość związków polifenolowych, potencjał przeciwutleniający oraz aktywność przeciwdrobnoustrojową.

H.2. Zawartość związków aktywnych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa zależą od części morfologicznej czeremchy zwyczajnej (*P. padus*).

H.3. Właściwości funkcjonalne ekstraktów z kory i owoców czeremchy (*P. padus*) zależą od sposobu ekstrakcji.

H.4. Kora czeremchy zwyczajnej (*P. padus*), jako składnik mieszanki ziołowej, wpływa na właściwości przeciwutleniające naparów mierzone w układach wodnych i emulsyjnych oraz zwiększa aktywność inhibicyjną naparów wobec takich enzymów, jak: alfa-glukozydaza, cyklooksygenaza (COX-2), cholinoesterazy (AChE oraz BChE), dysmutaza ponadtlenkowa (SOD), katalaza, reduktaza i peroksydaza glutationowa.

H.5. Mieszanki ziołowe z dodatkiem kory czeremchy (*P. padus*) mogą stanowić funkcjonalny produkt o atrakcyjnych cechach sensorycznych.

3. PRZEDMIOT I METODY BADAŃ

3.1. Materiał badany

Materiałem badanym były owoce, kora i liście *Prunus padus* L. oraz liście *Prunus serotina* L., pochodzące z gospodarstwa sadowniczego w Ozieranach Małych na Podlasiu (53° 13 '14,865 "N 23° 51' 9,327" E). Materiał badany przechowywano w stanie zamrożonym (temperatura = -28°C) a następnie liofilizowano i przygotowano ekstrakty.

3.2. Metody badań

Zbadano:

- Barwę i osmolalność ekstraktów (za pomocą spektrometru CM-5).
- Zawartość składników mineralnych (oznaczona techniką ICP-OES).
- Zawartość polifenoli ogółem (metoda spektrofotometryczna).
- Zawartość indywidualnych związków polifenolowych (wysokosprawna chromatografia cieczowa, HPLC).
- Zawartość niskocząsteczkowych kwasów organicznych (metoda chromatograficzna).
- Aktywność przeciwutleniającą (metoda spektrofotometryczna: zdolność do zmiatania rodników DPPH, zdolność do wygaszania kationorodników ABTS, zdolność do redukcjonowania jonów żelaza (FRAP), aktywność chelatującą, zdolność do redukcjonowania jonów miedzi (CUPRAC), zdolność antyoksydacyjna rodników hydroksylowych (HORAC), utlenianie beta karotenu w emulsji kwasu linolowego).
- Aktywność przeciwutleniającą (metoda elektrochemiczna, woltamperometria fali prostokątnej (SWV)).
- Właściwości przeciwdrobnoustrojowe (metoda studzienkowo-dyfuzyjna).
- Aktywność inhibicyjną wobec cyklooksygenazy-2 (COX-2) (metoda spektrofotometryczna).
- Aktywność inhibicyjną wobec cholinoesteraz (AChE, BChE) (metoda spektrofotometryczna).

- Aktywność inhibicyjną wobec dysmutazy ponadtlenkowej (SOD) (metoda spektrofotometryczna).
- Aktywność inhibicyjną wobec katalazy (metoda spektrofotometryczna).
- Aktywność inhibicyjną wobec oksydazy i peroksydazy glutationowej (metoda spektrofotometryczna).
- Aktywność inhibicyjną wobec alfa-glukozydazy (metoda spektrofotometryczna).

Ponadto wykonano:

- Analizę sensoryczną (metoda profilowania sensorycznego).
- Analizę statystyczną (program STATISTICATMPL 13.1 firmy StatSoft).

Szczegółowy opis poszczególnych metodyk został uwzględniony w publikacjach stanowiących osiągnięcie naukowe.

Model badań zilustrowano na rysunku nr 1.

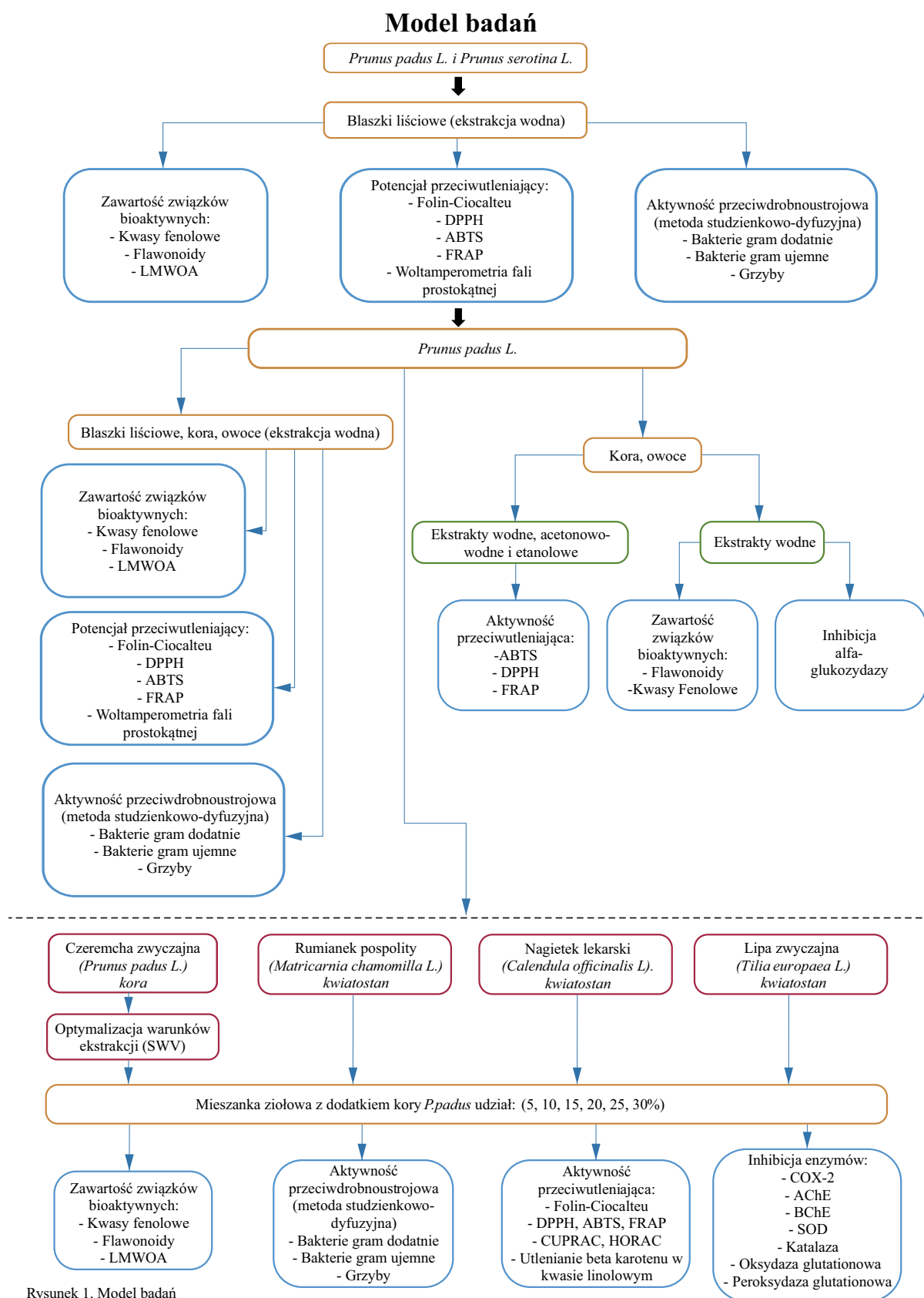
4. OMÓWIENIE WYNIKÓW

4.1. Ocena przydatności czeremchy (*Prunus padus* L. i *Prunus serotina* L.) pozyskanej ze środowisk naturalnych jako źródła związków biologicznie aktywnych

Celem pierwszego etapu badań realizowanych na potrzeby przygotowania pracy doktorskiej była ocena zawartości związków polifenolowych, potencjału przeciwutleniającego oraz aktywności przeciwdrobnoustrojowej w ekstraktach wodnych z liści dwóch rodzajów czeremchy: *P. padus* i *P. serotina*. Badania te miały ukierunkować dalsze postępowanie związane ze szczegółową charakterystyką poszczególnych części morfologicznych rośliny.

Materiał do badań stanowiły liście dwóch rodzajów czeremchy, tj. czeremchy zwyczajnej (*P. padus*) i czeremchy amerykańskiej (*P. serotina*). Wodne ekstrakty otrzymano w wyniku ekstrakcji w temperaturze 85°C przez 15 minut.

W uzyskanych ekstraktach oznaczono zawartość kwasów fenolowych i flawonoidów. W badanych ekstraktach wykazano różnice jakościowo-ilościowe analizowanych polifenoli. Wyższą zawartością polifenoli charakteryzował się ekstrakt z liści czeremchy zwyczajnej (*P. padus*). Spośród badanych kwasów dominował kwas ferulowy, kwas p-kumarowy i kwas synapowy, a spośród flawonoidów: katechina i rutyna.



Rysunek 1. Model badań

Wysoka zawartość polifenoli w *P. padus* determinowała wyższą aktywność przeciwutleniającą. W teście FRAP ekstrakt z liści *P. padus* wykazał o 30% wyższą aktywność w porównaniu z ekstraktem uzyskanym z liści *P. serotina*. Ocena aktywności przeciwutleniającej z rodnikiem DPPH potwierdziła wyższą o 22% (6.62 ± 0.06 mg TE/1 g s.m.) aktywność przeciwutleniającą ekstraktów z liści czeremchy zwyczajnej (*P. padus*). Analizy te potwierdziły również badania wykonane metodą z rodnikiem ABTS, gdzie aktywność ekstraktu z liści czeremchy zwyczajnej była także wyższa o około 22% w porównaniu do ekstraktu z liści czeremchy amerykańskiej. Aktywność przeciwutleniająca badanych ekstraktów została oznaczona także metodą woltamperometrii fali prostokątnej (SWV). Pomiary elektrochemiczne obrazujące zawartość związków redoks w badanych próbach wykazały, iż statystycznie istotnie wyższa zawartość tych związków oznaczona została w ekstraktach z liści *P. padus*.

W kolejnym etapie oceniono aktywność przeciwdrobnoustrojową wobec bakterii wskaźnikowych oraz wobec pleśni i grzybów. Najwyższą aktywność ekstraktów z liści *P. serotina* stwierdzono wobec bakterii *Enterococcus faecium* (24 mm) i *Listeria monocytogenes* (23 mm), natomiast w przypadku ekstraktów z liści *P. padus* wobec bakterii *Listeria monocytogenes* (24 mm).

PODSUMOWANIE

Na podstawie badań potwierdzono założoną hipotezę badawczą H.1: rodzaj czeremchy ma wpływ na zawartość związków polifenolowych, potencjał przeciwutleniający oraz aktywność przeciwdrobnoustrojową.

1. Stwierdzono, że zarówno liście *P. padus*, jak i *P. serotina* są bogatym źródłem związków aktywnych o działaniu przeciwutleniającym i przeciwdrobnoustrojowym.
2. Wyższą zawartość polifenoli oraz wyższy potencjał antyoksydacyjny stwierdzono w ekstraktach z liści *P. padus*.
3. Ze względu na wyższą zawartość związków polifenolowych oraz potencjał przeciwutleniający do następnego etapu badań wybrano czeremchę zwyczajną (*P. padus*).

Powyższe wyniki zostały opisane na podstawie następującej publikacji wchodzącej w skład wyodrębnionego osiągnięcia naukowego:

Telichowska, A., Kobus-Cisowska, J., Ligaj, M., Stuper, K., Szymanowska, D., Tichoniuk, M., 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 Chemistry*, 18(1), 1125–1135.

4.2. Charakterystyka oraz ocena zawartości wybranych związków bioaktywnych w częściach morfologicznych czeremchy zwyczajnej (*Prunus padus* L.)

W literaturze przedmiotu nie znaleziono szczegółowych wyników badań dotyczących czeremchy zwyczajnej (*P. padus*) oraz zawartości związków bioaktywnych zawartych w jej poszczególnych częściach morfologicznych. Nie znaleziono także informacji dotyczących bezpośredniego określenia ich wpływu na redukcję uszkodzeń oksydacyjnych. Dlatego celem drugiego etapu badań była ocena właściwości funkcjonalnych i charakterystyka składu ekstraktów z owoców, kory i liści czeremchy zwyczajnej *P. padus*.

Materiał badawczy stanowiły ekstrakty wodne pozyskane z liści, owoców i kory czeremchy zwyczajnej (*P. padus*), uzyskane w temperaturze 85°C w czasie 15 minut.

Najwyższą zawartością kwasów fenolowych charakteryzował się ekstrakt z liści (1063 ± 26 mg/100g s.m.), a w drugiej kolejności ekstrakt z kory czeremchy (978 ± 35 mg/100g s.m.). Dominującymi kwasami we wszystkich częściach morfologicznych badanej rośliny były: kwas ferulowy, kwas p-kumarowy, kwas galusowy i kwas chlorogenowy. Najwyższą zawartość kwasu chlorogenowego stwierdzono w owocach (109 ± 4 mg/100 g s.m.), a najniższą w korze. Najwyższą zawartość flawonoidów oznaczono w korze czeremchy (1530 ± 61 mg/100g s.m.), a najniższą w liściach (252 ± 11 mg/100 g s.m.). Dominującymi związkami była katechina oraz kwercetyna, których największą zawartość stwierdzono w ekstrakcie z kory (kwercetyna 599 ± 18 mg/100 g s.m. i katechina 879 ± 43 mg/100 g s.m.).

Oceniono także zawartość kwasów organicznych w ekstraktach z poszczególnych części morfologicznych *P. padus*. Stwierdzono, że ekstrakt z owoców zawierał ich najwyższy poziom (1658 ± 27 mg/100 g s.m.), na co wskazały też wyniki uzyskane wcześniej przez innych badaczy (Donno et al., 2018; Mikulic-Petkovsek et al., 2016).

Potencjał antyoksydacyjny badanych części czeremchy zwyczajnej oceniono metodami sepektrofotometrycznymi oraz metodą elektrochemiczną. Wykazano statystycznie istotne różnice pomiędzy właściwościami badanych prób. Wszystkie ekstrakty zawierały związki reagujące z odczynnikiem Folina-Ciocalteu, jednak ich najwyższy poziom stwierdzono w ekstrakcie z kory (136 ± 9 mg GAE/g s.m.). Ocena potencjału antyoksydacyjnego metodą FRAP potwierdziła, że najwyższą aktywnością charakteryzował się ekstrakt z kory

czeremchy ($379 \pm 18 \mu\text{M FeSO}_4/1 \text{ g s.m.}$). Podobne wyniki uzyskano w doświadczeniu z rodnikiem DPPH ($9 \pm 0.03 \text{ mg TE/1 g s.m.}$). W badaniu z kationorodnikiem ABTS stwierdzono natomiast, że aktywność ekstraktu z kory była najniższa. Zawartość związków redoks w ekstraktach z *P. padus* oceniano także metodą woltamperometrii fali prostokątnej (SWV). Najwyższą aktywność przeciwutleniającą wykazano dla ekstraktu z liści, a aktywność dla ekstraktu z kory i owoców niższą odpowiednio o 77.6% i 9.3%.

Analiza składowych głównych (PCA) wykazała zróżnicowanie próbek pod względem zawartości składników aktywnych oraz stopnia wpływu poszczególnych związków na aktywność przeciwutleniającą.

Oprócz właściwości przeciwutleniających polifenole charakteryzują się działaniem bakteriobójczym i bakteriostatycznym. Rośliny wyższe zawierają metabolity wtórne, które wykazują aktywność hamującą wzrost bakterii i grzybów. Wyniki badań własnych wykazały, że poszczególne części morfologiczne rośliny *P. padus* mogą również wykazywać takie właściwości. Największą aktywność hamującą wobec bakterii Gram-ujemnych odnotowano dla ekstraktu z liści czeremchy. Ekstrakt z liści najbardziej aktywny był wobec bakterii *Klebsiella pneumoniae* (19 mm). Najniższą aktywność hamowania wzrostu bakterii Gram-ujemnych zaobserwowano dla ekstraktu z kory czeremchy wobec bakterii *Salmonella enteritidis* (1 mm).

Całkowity potencjał przeciwutleniający ekstraktów roślinnych trudny jest do oznaczenia. Niejednokrotnie badania prowadzone z użyciem różnych metod nie wskazują spójnych wyników. Jest to rezultat złożoności i różnorodności związków wchodzących w skład ekstraktów. Sumaryczny efekt antyoksydacyjny trudno przewidzieć, ponieważ związki te działają według różnych mechanizmów. W wyniku zrealizowanych badań wykazano, że ekstrakt z kory czeremchy zawiera związki, które determinują potencjał przeciwutleniający. Dlatego do dalszych etapów prac związanych z opracowaniem modelowego produktu funkcjonalnego wybrano korę czeremchy zwyczajnej *Prunus padus* L.

PODSUMOWANIE

Na podstawie badań potwierdzono założoną hipotezę badawczą H.2: zawartość związków aktywnych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa zależą od części morfologicznej czeremchy zwyczajnej (*P. padus*).

1. Wykazano, że spośród badanych części morfologicznych czarernchy zwyczajnej (*P. padus*) najwyższą zawartością związków polifenolowych charakteryzował się ekstrakt z kory.
5. Ekstrakt z kory czarernchy zwyczajnej (*P. padus*) wykazał najwyższą aktywność przeciwutleniającą zarówno w teście z użyciem odczynnika Folina-Ciocalteu, jak i w teście FRAP oraz w teście przeciworodnikowym z zastosowaniem rodnika DPPH.
6. Zrealizowane badania pozwoliły wskazać korę czarernchy zwyczajnej jako surowiec o wysokiej zawartości związków polifenolowych i o wysokiej aktywności przeciwutleniającej. Potencjał antyoksydacyjny i zawartość związków bioaktywnych kory czarernchy zwyczajnej stanowi kryterium wyboru jej do badań związanych z opracowaniem modelowego produktu funkcjonalnego.

Powyższe wyniki zostały opisane na podstawie następującej publikacji wchodzącej w skład wyodrębnionego osiągnięcia naukowego:

- Telichowska, A., Kobus-Cisowska, J., Stuper-Szablewska, K., Ligaj, M., Tichoniuk, M., Szymanowska, D., Szulc, P. (2020). Exploring antimicrobial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L., *International Journal of Food Properties*, 23(1), 2097–2109.

4.3. Wpływ warunków ekstrakcji na właściwości przeciwutleniające i inhibicyjne wobec wybranych enzymów

Metoda ekstrakcji i dobór odpowiednich parametrów oraz ekstrahenta są ważnym etapem w procesie pozyskania bioaktywnych składników z matrycy roślinnej. Wyekstrahowane składniki różnią się pod względem budowy chemicznej, a ich wzajemne interakcje są trudne do przewidzenia.

Materiałem badanym była kora oraz owoce czarernchy zwyczajnej (*P. padus*). Materiał ekstrahowano wodą destylowaną, mieszaniną aceton-woda i etanolem w stosunku 2 g rozdrobnionego materiału na 100 ml rozpuszczalnika.

Wszystkie badane ekstrakty wykazały aktywność przeciwutleniającą zależną od rodzaju surowca i ekstrahenta. Najwyższą aktywność mierzoną testem ABTS wykazano dla ekstraktu etanolowego i acetonowo-wodnego z owoców czarernchy (1.23 ± 0.01 mM Trolox/g s.m. i 1.22 ± 0.02 mM Trolox/g s.m.). Ekstrakt acetonowo-wodny z kory czarernchy (1.22 ± 0.04 mM Trolox/g s.m.), a także ekstrakt wodny z kory (0.94 ± 0.02 mM Trolox /g s.m.) wykazały porównywalną aktywność. Najwyższą aktywność wobec rodnika

DPPH stwierdzono dla ekstraktu etanolowego z owoców, acetonowo-wodnego z owoców oraz wodnego z kory czeremchy (2.43 ± 0.13 mM Trolox/g s.m., 2.32 ± 0.04 mM Trolox/g s.m. oraz 2.01 ± 0.01 mM Trolox/g s.m.).

Badane ekstrakty scharakteryzowano pod kątem zdolności do redukcji jonów żelaza. Wszystkie ekstrakty wykazywały aktywność redukującą zależną od stężenia ekstraktu, jednak najwyższą zdolność do redukcji jonów żelaza stwierdzono dla etanolowego ekstraktu z kory. Ekstrakty z owoców i kory czeremchy wykazywały aktywność do chelatowania jonów żelaza, co zależało zarówno od zastosowanego do ekstrakcji rozpuszczalnika, jak i od rodzaju materiału, z którego uzyskano ekstrakt. Najwyższą aktywność chelatującą stwierdzono dla ekstraktu acetonowo-wodnego z kory czeremchy: 44,87% przy stężeniu 800 ppm.

Inhibitory alfa-glukozydazy hamują rozpad wiązań alfa węglowodanów, zmniejszając wchłanianie glukozy z przewodu pokarmowego do krwi, co ostatecznie przyczynia się do obniżenia glikemii poposiłkowej (Laar, 2008). Obecnie w leczeniu cukrzycy stosuje się trzy inhibitorowe związki lecznicze: akarbozę, woglibozę i miglitol.

Ekstrakty analizowanych surowców badano w stężeniach 165–300 µg/mL. Ponadto zmierzono IC_{50} dla tych ekstraktów i roztworu akarbozy. Wykazano, że ekstrakty z kory charakteryzowały się wysoką aktywnością inhibicyjną wobec alfa-glukozydazy, która dla analizowanych stężeń ekstraktów z kory mieściła się w zakresie od 20.43% do 89.91%, a dla ekstraktu z owoców 16.77–78.68%.

Najwyższą aktywność inhibicyjną wobec enzymu katalazy wykazano dla ekstraktu wodnego z owoców czeremchy ($60.2 \pm 0.9\%$) oraz ekstraktu etanolowego z kory czeremchy ($52.2 \pm 0.6\%$), a najniższą aktywność, wobec tego enzymu dla ekstraktu acetonowo-wodnego z owoców ($41.1 \pm 0.8\%$). Najwyższe działanie inhibicyjne wszystkich badanych prób stwierdzono wobec reduktazy glutationowej. Aktywność ta mieściła się w zakresie od $79.4 \pm 0.6\%$ w próbie z owoców czeremchy na bazie acetonu i wody do $71.0 \pm 1.7\%$ w wodnym ekstrakcie z owoców czeremchy. Wodny ekstrakt z kory czeremchy wykazał najwyższą aktywność inhibicyjną wobec peroksydazy glutationowej ($63.8 \pm 2.0\%$), natomiast dla ekstraktu acetonowo-wodnego z kory czeremchy oraz ekstraktu etanolowego z owoców aktywność ta była najniższa.

PODSUMOWANIE

Na podstawie badań potwierdzono założoną hipotezę badawczą H.3: właściwości funkcjonalne ekstraktów z kory i owoców czeremchy (*P. padus*) zależą od sposobu ekstrakcji.

1. Ekstrakty wodne, acetonowo-wodne i etanolowe z owoców i kory czeremchy *P. padus* wykazywały aktywność redukującą i chelatującą zależną od stężenia ekstraktu. Ekstrakty z owoców czeremchy w większym stopniu wymiatają wolne rodniki, natomiast ekstrakty z kory wykazywały większą aktywność redukującą i chelatującą.
2. Aktywność inhibicyjna związków czeremchy *P. padus* wobec enzymów: katalazy, oksydazy i peroksydazy glutationowej zależy od sposobu ekstrakcji. Najwyższą aktywność inhibicyjną wobec enzymu katalazy stwierdzono dla ekstraktu wodnego z owoców czeremchy, natomiast wobec reduktazy glutationowej dla ekstraktu acetonowo-wodnego z owoców. Wodny ekstrakt z kory wykazał najwyższą aktywność inhibicyjną wobec peroksydazy glutationowej. Ponadto stwierdzono wysoką aktywność inhibicyjną ekstraktu wodnego z kory czeremchy wobec alfa-glukozydazy.
3. Do dalszego etapu badań związanego z oceną właściwości czeremchy *P. padus* wybrano proces ekstrakcji wodnej z uwagi na wysoką zawartość związków polifenolowych w otrzymanych preparatach oraz właściwości funkcjonalne, a także na jej praktyczne możliwości zastosowania przez konsumenta.

Powyższe wyniki zostały opisane na podstawie następującej publikacji wchodzącej w skład wyodrębnionego osiągnięcia naukowego:

- Telichowska, A., Kobus-Cisowska, J., Cielecka-Piontek, J., Sip, Sz., Stuper-Szablewska, K., Szulc, P. (2022). *Prunus padus* L. as a source of functional compounds antioxidant activity and antidiabetic effect, *Emirates Journal of Food and Agriculture*, 34(2), 135-143.

4.4. Ocena właściwości związków aktywnych kory czeremchy (*Prunus padus* L.) jako składnika funkcjonalnych mieszanek zielonych

Korę czeremchy (*P. padus*) scharakteryzowano pod względem zawartości makro- i mikroelementów oraz pierwiastków śladowych. W jej składzie oznaczono wysoką zawartość potasu (19.45 mg/kg s.m.), stwierdzono również znaczną zawartość wapnia (3.54 mg/kg s.m.), magnezu (3.20 mg/kg s.m.) i fosforu (2.92 mg/kg s.m.).

Etapem, który determinuje zawartość związków fenolowych, a także aktywność antyoksydacyjną naparów, jest proces ekstrakcji. Co więcej, istotnym elementem jest również wybór czasu i temperatury. Aktywność przeciwutleniającą metodą elektrochemiczną ekstraktów z kory *P. padus* badano w próbach otrzymanych w temperaturze 80°C i 100°C w czasie 15, 30 i 45 minut. Najwyższą aktywnością antyoksydacyjną mierzona metodą elektrochemiczną charakteryzował się ekstrakt przygotowany w czasie 45 min i w temperaturze 80°C (całkowita powierzchnia pików $3.933 \text{ V} \times \mu\text{A}$). Wydłużenie czasu procesu ekstrakcji z 15 do 45 min zwiększyło badaną aktywność elektrochemiczną o 170%, jednak ze względu na bardzo długi czas procesu do dalszych badań wybrano czas wynoszący 15 min w temperaturze 80°C, co uzasadniono praktycznym zastosowaniem w warunkach komercyjnych.

W celu określenia przydatności kory czeremchy jako składnika funkcjonalnych mieszanek ziołowych zaproponowano dodatkowe składniki recepturowe modelowych mieszanek ziołowych do sporządzania naparów. Składnikami recepturowymi mieszanek oprócz kory czeremchy (*Prunus padus* L.) były kwiat lipy (*Tilia europaea* L.), kwiat rumianku (*Matricaria Chamomilla* L.) oraz nagietek lekarski (*Calendula officinalis* L.). Mieszanki ziołowe przygotowano poprzez zmieszanie suchych składników i odważenie 2-gramowych porcji, które zapakowano w jednorazowe bibułkowe torebki.

Przygotowano jedenaście prób, z których cztery zawierały tylko jeden składnik: P (100% kora *P. padus*), Ch (100% *Matricaria chamomilla* L.), C (100% *Calendula officinalis* L.) i T (100% *Tilia europaea* L.). Kolejną próbę stanowiła mieszanka podstawowa ziół tzw. baza herbatek B (33,33% Ch + 33,33% C + 33,33% T), natomiast sześć pozostałych prób przeprowadzono na bazie wcześniej przygotowanej bazy oraz kory czeremchy (P) w różnych proporcjach, odpowiednio: BP5 (5% P + 95% B), BP10 (10% P + 90% B), BP15 (15% P + 85% B), BP20 (20% P + 80% B), BP25 (25% P + 75% B), BP30 (30% P + 70% B). 200-mililitrowe napary uzyskano poprzez ekstrakcję wodną w temperaturze 80°C w czasie 15 min.

Spośród badanych naparów najwyższą zawartością polifenoli charakteryzował się ekstrakt z kory czeremchy zwyczajnej ($7939.8 \pm 106.6 \text{ mg}/100\text{g s.m.}$). Napary otrzymane z mieszanek ziołowych z udziałem kory *P. padus* zawierały związki polifenolowe, których zawartość mieściła się w zakresie od $5759.6 \pm 86.8 \text{ mg}/100 \text{ g}$ do $7509.2 \pm 189.6 \text{ mg}/100\text{g s.m.}$

W następnym etapie herbatki oceniono pod kątem aktywności przeciwutleniającej. W teście HORAC, opierającym się na pomiarze hamowania utleniania fluoresceiny przez rodniki hydroksylowe, wykazano, że herbatka składająca się z ziołowych składników

podstawowych – bazy oraz herbatka z największym udziałem kory, tj. 30-procentowym, wykazywały najwyższą aktywność w tym zakresie, to jest odpowiednio: równoważnik 4.1 ± 0.2 µg kwasu galusowego/mL i równoważnik 4.0 ± 0.2 µg kwasu galusowego/mL. Z kolei w teście zmiatania rodników DPPH spośród mieszanek ziołowych najwyższą aktywność uzyskano dla próby z 20-procentowym udziałem kory (równoważnik 4.28 ± 0.06 µg kwasu galusowego/mL).

W badaniu aktywności przeciwutleniającej metodą pomiaru stopnia utleniania kwasu linolowego najwyższą aktywność, spośród naparów z dodatkiem kory czeremchy, stwierdzono w herbatce z 10-procentowym dodatkiem (115.0 ± 18.4 µg kwasu askorbinowego /mL), a najniższą w herbatce z jej 25-procentową zawartością (100.4 ± 16.1 µg kwasu askorbinowego/mL). Ocena potencjału przeciwutleniającego metodą z beta-karotenem wykazała najwyższą aktywność dla próby z 30-procentowym dodatkiem kory ($12.7 \pm 0.2\%$) oraz z 15-procentowym dodatkiem kory czeremchy ($12.5 \pm 0.0\%$). W teście z oznaczaniem zdolności do redukcji jonów żelaza Fe(III) najwyższą aktywność wykazano dla naparu z kory czeremchy (25.3 ± 0.9 µg Trolox/mL) oraz naparu z 20-procentowym dodatkiem kory (22.1 ± 0.3 µg Trolox/mL).

Herbatki badano również pod kątem aktywności redukującej jony miedzi Cu(II), gdzie stwierdzono, że najwyższą aktywność wykazał napar z kory czeremchy (1083.9 ± 19.4 µg kwercetyny/mL). Wysokie wartości uzyskano także dla naparu z 20- i 15-procentowym dodatkiem kory (939.1 ± 27.1 µg kwercetyny/mL oraz 892.9 ± 16.3 µg kwercetyny/mL).

Napary z przygotowanych mieszanek ziołowych oceniono również pod względem aktywności przeciwdrobnoustrojowej wobec 20 wskaźnikowych gatunków drobnoustrojów, w tym grzybów oraz bakterii Gram-dodatnich i Gram-ujemnych. Stwierdzono, że wzbogacenie mieszanki ziołowej w korę z czeremchy znacznie zwiększyło aktywność przeciwdrobnoustrojową (najwyższą aktywność wobec *Listeria monocytogenes* (od 25.0 do 27.0 mm) zaobserwowano dla 20- i 30-procentowego dodatku kory).

W kolejnej części oceniono właściwości funkcjonalne opracowanych mieszanek ziołowych. Najwyższą aktywnością inhibicyjną wobec cholinoesteraz charakteryzował się napar z kory czeremchy, tj. 15.8 ± 1.1 µg neostygminy/mL w przypadku AChE oraz 21.2 ± 1.0 µg neostygminy/mL w przypadku BChE. Napary z 20-procentowym dodatkiem surowca również wykazały znaczną aktywność: 12.1 ± 0.1 µg neostygminy/mL dla AChE oraz 18.5 ± 1.5 µg neostygminy/mL dla BChE. Najwyższą aktywność inhibicyjną wobec katalazy oraz SOD spośród mieszanek z korą czeremchy stwierdzono w przypadku herbatek z 30-procentowym dodatkiem. Ustalono także wysoką aktywność inhibicyjną ekstraktu

z kory czeremchy wobec cyklooksygenazy-2 (COX-2). Jej dodatek zwiększał aktywność podstawowej mieszanki ziołowej. Mieszanka z 30-procentowym dodatkiem kory charakteryzowała się najwyższą aktywnością w tym zakresie ($63 \pm 1.1\%$).

Badane napary oceniono pod kątem zdolności do hamowania reduktazy glutationowej oraz peroksydazy glutationowej. Spośród badanych herbatek najwyższa aktywność została stwierdzona dla naparów z 25-procentowym dodatkiem kory.

Sporządzone napary zostały poddane ocenie sensorycznej przy uwzględnieniu następujących wyróżników jakości: barwa, smak, zapach oraz ogólna pożądalność. Dominującą barwą badanych herbatek zawierających korę czeremchy dla większości próbek była barwa brązowa. Dodatek kory do mieszanek ziołowych wpływał na zwiększenie intensywności smaku kwaśnego i trawiastego. Ponadto próby z udziałem kory oceniono jako bardziej słodkie. Dodatek kory do mieszanek wpłynął na zintensyfikowanie zapachu. Nie stwierdzono pojawienia się zapachu i smaku obcego lub niepożądanego.

PODSUMOWANIE

Na podstawie wyników badań potwierdzono założone hipotezy badawcze H.4 i H.5: kora czeremchy zwyczajnej (*P. padus*) jako składnik mieszanki ziołowej wpływa na właściwości przeciwutleniające naparów mierzone w układach wodnych i emulsyjnych oraz zwiększa aktywność inhibicyjną naparów wobec takich enzymów, jak: alfa-glukozydaza, cyklooksygenaza (COX-2), cholinoesterazy (AChE oraz BChE), dysmutaza ponadtlenkowa (SOD), katalaza, reduktaza i peroksydaza glutationowa oraz mieszanki ziołowe z dodatkiem kory czeremchy (*P. padus*) mogą stanowić alternatywny produkt w diecie o atrakcyjnych cechach sensorycznych.

1. Ocena wpływu sposobu ekstrakcji kory czeremchy metodą elektrochemiczną wykazała wysoki potencjał naparów parzonych w czasie 45 minut w temperaturze 80°C. Z uwagi na zbyt długi czas parzenia do dalszych etapów badań wybrano najbardziej praktyczny z punktu widzenia konsumenta czas, tj. 15 minut oraz temperaturę 80°C.
2. Dodatek kory czeremchy do mieszanek ziołowych wpływał na wyższą aktywność inhibicyjną wobec enzymów, takich jak: reduktaza glutationowa, peroksydaza glutationowa, acetylocholinoesteraza, katalaza czy SOD. Stwierdzono, że 20- i 30-procentowy dodatek kory czeremchy do naparów znacznie zwiększył ich potencjał funkcjonalny, w szczególności wobec takich enzymów, jak katalaza i peroksydaza glutationowa.

3. Dominującą barwą badanych herbatek zawierających korę czeremchy dla większości prób była barwa brązowa. Dodatek kory do mieszanek ziołowych wpływał na zwiększenie intensywności smaku kwaśnego i trawiastego. Ponadto próby z udziałem kory oceniono jako bardziej słodkie. Dodatek kory do mieszanek wpłynął na zintensyfikowanie zapachu. Nie stwierdzono pojawienia się zapachu i smaku obcego lub niepożądanego.

Powyższe wyniki zostały opisane na podstawie następujących publikacji wchodzących w skład wyodrębnionego osiągnięcia naukowego:

- Telichowska, A., Kobus-Cisowska, J., Szulc, P., Ligaj, M., Stuper-Szablewska, K., Szwajgier, D., 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(7), 3913.
- Telichowska, A., Kobus-Cisowska, J., Szulc, P., Wilk, R., Szwajgier, D., Szymanowska, D. (2021). *Prunus padus* L. bark as a functional promoting component in functional herbal infusions – cyclooxygenase-2 inhibitory, antioxidant, and antimicrobial effects, *Open Chemistry*, 19(1), 1052–106.

4. STWIERDZENIA I WNIOSKI

Wyniki przeprowadzonych badań pozwoliły na wysunięcie następujących stwierdzeń i wniosków:

- Liście *P. padus* i *P. serotina* są źródłem związków o działaniu przeciwutleniającym i przeciwdrobnoustrojowym. Rodzaj czeremchy determinuje ilościową i jakościową ich zawartość oraz potencjał przeciwutleniający, jak też aktywność przeciwdrobnoustrojową. Wyższą zawartość polifenoli oraz wyższy potencjał antyoksydacyjny mierzony metodami z rodnikami DPPH i ABTS oraz metodą elektrochemiczną stwierdzono w ekstraktach z liści *P. padus*. Ekstrakty z liści *P. padus* wykazały wyższą aktywność przeciwdrobnoustrojową wobec bakterii: *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Bacillus coagulans* oraz *Pseudomonas aeruginosa*.
- Części morfologiczne czeremchy zwyczajnej (*P. padus*) charakteryzują się różną zawartością związków polifenolowych oraz wykazują odmienną aktywność

przeciwutleniającą i przeciwdrobnoustrojową. Spośród badanych części – kora, liście i owoce – najwyższą zawartość związków polifenolowych stwierdzono w ekstrakcie z kory. Ten ostatni wykazał najwyższą aktywność przeciwutleniającą mierzoną metodą FRAP oraz metodą z rodnikiem DPPH. Ekstrakty z kory *P. padus* wykazały najwyższą aktywność przeciwdrobnoustrojową wobec bakterii *Pseudomonas aeruginosa*.

- Aktywność przeciwutleniająca kory i owoców czeremchy zależy od sposobu ekstrakcji. Ekstrakty wodne, acetonowo-wodne i etanolowe wykazują aktywność redukującą i chelatującą, która wzrasta wraz ze wzrostem stężenia ekstraktu. Ekstrakty z kory czeremchy wykazywały wyższą aktywność redukującą i chelatującą, natomiast ekstrakty z owoców w większym stopniu zmiatały rodniki. Wyższą aktywnością inhibicyjną wobec alfa-glukozydazy charakteryzowały się ekstrakty z kory o stężeniu 300 µg/mL. Aktywność inhibicyjna wobec katalazy, oksydazy i peroksydazy glutationowej zależy od sposobu ekstrakcji. Najwyższą aktywność inhibicyjną wobec peroksydazy glutationowej stwierdzono dla wodnego ekstraktu z kory czeremchy, natomiast najniższą dla ekstraktu acetonowo-wodnego z owoców. Natomiast najwyższą zdolność do hamowania enzymu katalazy wykazał ekstrakt wodny z owoców czeremchy, a wobec hamowania enzymu reduktazy glutationowej ekstrakt acetonowo-wodny z owoców. Do dalszego etapu badań wybrano ekstrakcję wodną z uwagi na wysoką zawartość związków polifenolowych oraz właściwości funkcjonalne.
- Wykazano, iż kora czeremchy zwyczajnej jako składnik mieszanki ziołowej wpływa na właściwości przeciwutleniające naparów mierzone w układach wodnych i emulsyjnych oraz zwiększa aktywność inhibicyjną naparów wobec takich enzymów, jak: acetylocholinoesteraza, reduktaza i peroksydaza glutationowa, cyklooksygenaza (COX-2), katalaza oraz dysmutaza ponadtlenkowa (SOD). Dodatek kory do mieszanek wpłynął na zintensyfikowanie zapachu intensywnego, ponadto próby z udziałem kory oceniono jako bardziej słodkie. Nie stwierdzono pojawienia się zapachu i smaku obcego lub niepożądanego.
- Napary ziołowe w ostatnich latach zyskują coraz większą popularność, dlatego, wychodząc naprzeciw oczekiwaniom konsumentów w zakresie nowych funkcjonalnych produktów, w ramach pracy opracowano modelowe mieszanki ziołowe wzbogacone w korę czeremchy (*P. padus*). Stwierdzono, że często trudne jest porównanie poszczególnych aktywności za pomocą różnych metod. Badania potwierdziły tym samym różnorodność mechanizmów działania przeciwutleniaczy zawartych

w *P. padus*, a wyniki oceny sensorycznej wskazały, że mieszanki ziołowe z dodatkiem kory czeremchy (*P. padus*) mogą stanowić interesujący produkt w diecie o atrakcyjnych cechach sensorycznych.

Potwierdzono założone hipotezy badawcze:

- H.1. Rodzaj czeremchy ma wpływ na zawartość związków polifenolowych, potencjał przeciwutleniający oraz aktywność przeciwdrobnoustrojową.
- H.2. Zawartość związków aktywnych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa zależą od części morfologicznej czeremchy zwyczajnej (*P. padus*).
- H.3. Właściwości funkcjonalne ekstraktów z kory i owoców czeremchy (*P. padus*) zależą od sposobu ekstrakcji.
- H.4. Kora czeremchy zwyczajnej (*P. padus*), jako składnik mieszanki ziołowej, wpływa na właściwości przeciwutleniające naparów mierzone w układach wodnych i emulsjach oraz zwiększa aktywność inhibicyjną naparów wobec takich enzymów, jak: alfa-glukozydaza, cyklooksygenaza (COX-2), cholinoesteraza (AChE), dysmutaza ponadtlenkowa (SOD), katalaza, reduktaza i peroksydaza glutationowa.
- H.5. Mieszanki ziołowe z dodatkiem kory czeremchy (*P. padus*) mogą stanowić funkcjonalny produkt o atrakcyjnych cechach sensorycznych.

5. LITERATURA

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Jan Chomda Aleksander

(czytelny podpis autora pracy)

Review

Phytopharmacological Possibilities of Bird Cherry *Prunus padus* L. and *Prunus serotina* L. Species and Their Bioactive Phytochemicals

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Abstract: Wild cherry is a plant observed in the form of trees or shrubs. This species comprises about twenty kinds of plants and the most popular are two, *Prunus padus* L. and *Prunus serotina* L., whose properties and content of phytochemical compounds are subject to studies. Wild cherry contains many active compounds, including tocopherols, vitamins, polyphenols and terpenes, which can have beneficial effects on health. On the other hand, wild cherry contains cyanogenic glycosides. Nevertheless, current research results indicate pro-health properties associated with both *P. serotina* and *P. padus*. The aim of this study was to collect and present the current state of knowledge about wild cherry and to review available in vitro and in vivo studies concerning its antioxidant, anti-inflammatory, antibacterial and antidiabetic activity. Moreover, the current work presents and characterizes phytochemical content in the leaves, bark and fruits of *P. padus* and *P. serotina* and compiles data that indicate their health-promoting and functional properties and possibilities of using them to improve health. We find that the anatomical parts of *P. padus* and *P. serotina* can be a valuable raw material used in the food, pharmaceutical and cosmetic industries as a source of bioactive compounds with multi-directional action.

Keywords: *Prunus padus*; *Prunus serotina*; bird cherry; phytochemical compounds; cyanogenic glycosides; antioxidant; antibacterial; anti-inflammatory

1. Introduction

Currently, there is a growing interest in the possibilities of an application of plant raw materials in medicine as well as in functional food designing. The biological activity of raw materials depends on the content of individual phytochemicals, which are found in different anatomical parts of plants: fruits, shoots, buds, leaves, roots and bark [1,2].

One of these plants is wild cherry. The species comprises about twenty kinds of plants, however, two of them are the most popular: European bird cherry (*P. padus* L. syn. *Prunus avium* L.) and American black cherry (*P. serotina* L.) [3] The ecological expansion of the wild cherry tree results from its high efficiency of vegetative multiplication and generative propagation due to features such as its high capacity and speed of seed germination, high seed production, long dispersion, rapid growth and development, low requirements in relation to habitat conditions and high tolerance to climatic conditions [4].

The wild cherry is mistakenly considered inedible. The fruit seeds contain cyanogenic glycosides, the excessive consumption of which, without thermal treatment, may have adverse health effects [5]. The consumption of wild cherry already took place in prehistoric times. This is evidenced by the remains of Neolithic and bronze vessels, the decorations of which depict the wild cherry, which were discovered in palafittes (houses erected on stilts) in South-East Asia. The Greek historian Herodotus of Halicarnassus also informs about its use. At that time, the fruit was consumed with the addition of salt, and regardless of this, alcohol was also produced from it [6].

Currently, the European bird cherry (*P. padus*) and the American black cherry (*P. serotina*) are not widely used in pharmaceutical or food industries. The aim of this study is to characterize the phytochemical compounds present in wild cherries, their biological properties and the possibility of their use in the pharmaceutical, food and cosmetics industries on the basis of available literature data. This review encompassed research conducted at the in vitro (outside of the normal biological context, commonly called test-tube experiments) and in vivo (experiments developed using the whole organism) levels. Additionally, the authors present the data from all available papers, in a comprehensive way, providing complete information about the main analytical parameters. Although many researchers have reported the effectiveness of wild cherry in in vitro and in vivo studies, more work needs to be done to clinically prove their effectiveness.

2. Data Collections

All data presented in this review were summarized from the references, including scientific journals, book chapters or dissertations. These references were systematically searched against electronic databases: PubMed, CNKI (<http://new.oversea.cnki.net/index/>), Web of Science, Scopus and Google Scholar and SciELO with a keyword “*Prunus padus*” and “*Prunus serotina*”. To search for maximum relative references, the keyword was set as “bird cherry” without any other restrictions. Subsequently, references closely related to chemical compositions, traditional uses and pharmacological properties, including in vitro and in vivo investigations, were screened for further data extraction. In addition, to survey the taxon, phenotypes and geographical distributions of *Prunus* species, several online taxonomic databases, including <http://theplantlist.org/>, <http://www.worldfloraonline.org/>, <https://www.gbif.org/> and <http://www.iplant.cn/foc/>, were also explored.

3. Habitats and Classification

Wild cherry (*Prunus* L.) is a type of tree or large shrub belonging to the subfamily *Amygdaloideae* (*Prunoideae*) within the *Rosaceae* family (Table 1). This subfamily also includes three other genera which are distinguished by their drupe-shaped fruit [7,8].

Table 1. Systematics of the species belonging to genus *Prunus* L.

Domain	Eukaryota
Kingdom	Plantae
Clade	Vascular plants
Clade	Seed plants
Class	Angiosperms
Clade	Rosids
Order	Rosales
Family	Rosaceae
Genus	<i>Prunus</i>

The classification of the species *Prunus* L. has always been controversial and has been presented differently by many authors. In 1700, the French botanist Joseph Pitton de Tournefort was the first to classify the distinguished species into six genera, distinguishing them on the basis of differences in fruit structure: *Amygdalus*, *Armeniaca*, *Cerasus*, *Laurocerasus*, *Persica* and *Prunus*. This classification was

adopted in 1754 by the Swedish naturalist Carl Linnaeus, who distinguished four genera: *Amygdalus*, *Cerasus*, *Prunus* and *Padus*.

The idea of merging all the species was first published by George Bentham and Joseph Dalton Hooker in 1865 [7,9]. Rehder's classification from 1940 of five subgenres within *Prunus* gained the widest acceptance: *Amygdalus*, *Cerasus*, *Laurocerasus*, *Padus* and *Prunus* [7,10].

Recent DNA tests have shown that *Prunus* is monophylic and derived from some Eurasian ancestors [8]. Plants of the *Prunus* species mainly occur in the northern hemisphere and the temperate zone, most often in Europe, Asia Minor and Western Siberia [11]. Wild cherry grows well in partially darkened sites and mineral-rich soils, and its location does not affect the characteristics of trees such as leaves, fruits or bark [6].

4. Botanical Characteristics of *P. padus* and *P. serotina*

The height of trees of the bird cherry tree (*P. padus*) can reach up to 15 m [12], while shrubs range from 0.5 to 4 m (Table 2). American black cherry trees (*P. serotina*) can reach a height of up to 25 m [13]. Its structure consists of a round or egg-shaped crown with falling branches and dark brown shoots, which form a shrubby form [14,15]. The trees reach their target dimensions at the age of 100 years [16].

The distinction between the bird cherry *P. padus* and the American black cherry *P. serotina* is possible due to the shape of the leaves and flowers. The American black cherry is characterized by elongated, lancet, pointed leaves, which are sharply serrated at the edges and are shiny and dark green on the surface. They are light green on the underside [17,18]. They are characterized by a poorly visible vein network. The leaves of the bird cherry are smaller and its edges are finely serrated. The petiole is usually red and the leaves themselves are more matt and leathery. The vein network is dense, connected at the edge of the leaf [15].

Table 2. Comparison of botanical characteristics of *P. padus* and *P. serotina* [6].

	<i>P. padus</i>	<i>P. serotina</i>
Country of origin	Europe, Asia Minor	North America
Height of trees	up to 15 m	up to 25 m
Leaves	Smaller, finely serrated	Elongated, sharpened, more serrated
Veins network (leaves)	Dense	Poorly visible
Fruits	Bitter	Sweeter
Flowering	April/May	May/June
Inflorescence	Suspended or rarely elevated	Elevated or ascending
Fruits	Petals broadly inversely egg-shaped, 10–15 mm long, almost twice as long as stamens. Hairy flower bottom.	The serrated edges of the calyx are permanent and remain on the fruit. The fruit is unripe pink or reddish, then dark red to black. Smooth pip
Bark, aroma	The bark smells good. Torn buds smell like almonds.	Torn bark with a characteristic, sharp, relatively pleasant smell, similar to that of blackcurrant.

The bird cherry begins to flower at the end of April and the beginning of May, while the American black cherry only flowers around May and June. The flowers are creamy white and form cylindrical clusters [19,20]. The flowers of American black cherry do not have an aroma and are smaller than those of the bird cherry [21]. Small, spherical black drupes are fruits that appear around August and September [22,23].

5. Nutrition Value and Mineral Content of *P. padus* and *P. serotina*

Natural products, mainly of plant origin, are gaining interest all over the world. Some of them are already used as a substitute or complementary to unconventional forms of drugs due to the toxicity and side effects of synthetic drugs [24–29].

The analysis of the chemical composition of leaves, bark, flowers and fruits of wild cherry trees has been the subject of many studies concerning selected traits or species of wild cherry (Table 3).

Table 3. Nutrition value and mineral content in *P. padus* i *P. serotina*.

Class	Component	[30]	[31]	[32]	
		<i>P. padus</i>	<i>P. serotina</i>	<i>P. serotina</i>	
		Fruits	Fruits	Seeds Raw	Seeds Toasted
Moisture			81.18 ± 0.081	8.92 ± 0.42	10.75 ± 0.35
Ash			0.86 ± 0.11%	3.19 ± 0.18	2.72 ± 0.21
Mineral	Ca		12.90 ± 1.90	192.30 ± 0.58	127.11 ± 17.51
	Fe			9.49 ± 0.3	1.21 ± 0.003
	Mg		21.20 ± 0.20	249.15 ± 0.34	216.68 ± 18.75
	P		28.10 ± 0.40	439.0 ± 0.16	323.40 ± 0.14
	K		184.30 ± 3.50	873.22 ± 12.64	454.82 ± 0.41
	Zn			3.40 ± 0.10	2.96 ± 0.24
	Na		22.40 ± 1.60	82.98 ± 0.90	23.59 ± 0.8
			2.10 ± 0.01	37 ± 0.16	36.55 ± 0.22
Protein Aminoacid	Asp			112.29 mg/g	116.97 mg/g
	Glu			256.84 mg/g	27.73 mg/g
	Ser			32.84 mg/g	42.11 mg/g
	His			21.60 mg/g	21.29 mg/g
	Gly			37.43 mg/g	38.82 mg/g
	Thr			52.85 mg/g	59.16 mg/g
	Arg			84.24 mg/g	87.42 mg/g
	Ala			41.47 mg/g	44.06 mg/g
	Tyr			48.75 mg/g	60.99 mg/g
	Met			8.93 mg/g	9.83 mg/g
	Val			45.48 mg/g	45.62 mg/g
	Phe			48.64 mg/g	52.00 mg/g
	Ile			39.17 mg/g	40.33 mg/g
	Leu			75.10 mg/g	82.11 mg/g
	Lys			8.85 mg/g	11.17 mg/g
Fat	Total		0.05 ± 0.01	40.37 ± 0.73	39.97 ± 0.20
Carbohydrates		129.28 ± 3.47	12.23 ± 0.79	7.76 ± 2.24	8.65 ± 4.28
Sugars	Glucose	62.19 ± 1.92			
	Fructose	33.34 ± 1.32			
	Sorbitol	33.73 ± 1.51			
Crude Fiber			3.58 ± 0.03	10.73 ± 1.49	12.12 ± 4.06

ND—not detected.

Table 3 shows the nutritional value of the fruits of the *P. padus* and *P. serotina* cherries. Fruits of *P. serotina* contain a relatively high content of protein and a high content of carbohydrates [31]. The protein content in fruits of *P. serotina* is higher than reported in the literature concerning plums ($0.9\% \pm 0.03\%$), apricots (*Prunus armeniaca* L.) ($1.4\% \pm 0.33\%$), peaches (*Prunus persica* L. Batsch) ($0.9\% \pm 0.01\%$) or grapes ($0.72\% \pm 0.03\%$) [33].

A higher carbohydrate content was noted in the fruit of *P. padus* [30,32]. It was also found to contain reducing sugars such as glucose, fructose and sorbitol [30]. Sorbitol is also found in other fruits besides wild cherries and is present in relatively high amounts in apples, peaches, apricots and in small amounts in gooseberries, currants, bananas, pineapples, but also in rowanberries [34–37]. The mineral components found in the seeds of *P. serotina* are mainly: iron, magnesium, potassium, zinc, phosphorus and sodium. The content of magnesium in raw and roasted seeds of *P. serotina* was not affected by heat treatment, but the content of Ca, Fe, P, K, Zn and Na decreased after the seeds were roasted. Raw wild cherry seeds also contain a relatively high protein content ($37\% \pm 0.16\%$). It was shown that the content of amino acids in roasted seeds increased [32]. The content of fiber in the seeds depended on the heat treatment and its highest content was found in the roasted seeds of *P. serotina* [32].

6. Phytocompounds Content in *P. padus* and *P. serotina*

6.1. Chlorophyll and Anthocyanins

Chlorophyll absorbs energy from light, which is used to convert carbon dioxide into carbohydrates [38]. Chlorophyll occurs in several different forms [39,40]. The main types are chlorophylls A and B. In the fruit of *P. padus* there is both chlorophyll A and chlorophyll B, but there are twice as many chlorophyll A (Table 4).

Table 4. Phytocompounds content in *P. padus* and *P. serotina*.

Class	Component	Result	Species	Plant Part	Reference
Chlorophyll	Chlorophyll A	42.58 ± 1.92 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Chlorophyll B	22.43 ± 1.30 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Alfa-Caroten	0.05 ± 0.01 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Beta-Caroten	3.06 ± 0.17 mg/kg FW	<i>P. padus</i>	Fruits	[30]
Anthocyanins	Cyanidin-2-galactoside	33.69 ± 2.52 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Cyanidin-3-glucoside	1501.53 ± 61.32 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		272 mg/100 g	<i>P. serotina</i>	Fruits	[41]
	Cyanidin-3-rutinoside	623.68 ± 46.97 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Cyanidin rhamnosyl hexoside	13.09 ± 1.00 mg/kg FW	<i>P. padus</i>	Fruits	[30]
Tocopherols	Tocopherol alfa	6.51 ± 0.50 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Tocopherol gama	1.35 ± 0.12 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Tocopherol delta	2.48 ± 0.22 mg/kg FW	<i>P. padus</i>	Fruits	[30]
Vitamin C	Ascorbic acid	25.20 ± 3.48 mg/100 FW	<i>P. padus</i>	Fruits	[30]
	Dehydroascorbic acid	50.87 ± 16.23 mg/100 FW	<i>P. padus</i>	Fruits	[30]
Terpen	Limonene	31.40 ± 5.65 mg/100 FW	<i>P. padus</i>	Fruits	[30]
	Phellandrene	8.51 ± 2.69 mg/100 FW	<i>P. padus</i>	Fruits	[30]
	Sabinene	1.21 ± 0.18 mg/100 FW	<i>P. padus</i>	Fruits	[30]
	γ-terpinene	65.52 ± 6.25 mg/100 FW	<i>P. padus</i>	Fruits	[30]
	Ursolic acid	X	<i>P. serotina</i>	Fruits	[31]
	Uvaol	X	<i>P. serotina</i>	Fruits	[31]
	Cis-Linalool oxide	0.7%	<i>P. padus</i>	Flowers	[42]
	Trans s-Linalool oxide	0.1%	<i>P. padus</i>	Flowers	[42]
	(Z)-8-Hydroxylinalool	30.4%	<i>P. padus</i>	Flowers	[42]
	(E)-β-Farnesene	0.2%	<i>P. padus</i>	Flowers	[42]
	(E, E)-α-Farnesene	0.1%	<i>P. padus</i>	Flowers	[42]

X—component is present in the sample.

Wild cherries also contain carotenoids—most of them are beta-carotene [30]. Recently, there has also been a significant increase in interest in possible pro-health effects associated with the consumption of anthocyanins. It is suggested that fruits rich in anthocyanins or their extracts show a wide range of protective effects [43,44]. The consumption of anthocyanins prevents the risk of cardiovascular diseases, oxidative stress and diabetes [43–46]. The antioxidant activity of cyanidin is 4.4 times higher than that of ascorbic acid. Among edible plants, berries of red, blue or purple color, such as wild cherries, are one of the most important sources of anthocyanins in the diet [5,47–50]. Anthocyanins are mainly present in the outer layers of subcutaneous tissue.

In *P. padus* fruits, the total content of anthocyanins was 2071.16 ± 91.02 mg/kg FW [30]. The main compounds in this group were mainly: cyanidin-3-glucoside, cyanidin-3-rutinoside as well as cyanidin-3-galactoside and cyanidin rhamnosyl hexoside [30].

6.2. Vitamin: Tocopherols and Vitamin C

Tocopherols are commonly found in cereals, vegetable oils and eggs [51,52]. These include alpha-tocopherol, beta-tocopherol, gamma-tocopherol and delta-tocopherol. However, alpha-tocopherol with antioxidant properties has the greatest nutritional importance [53]. Alpha, beta and gamma-tocopherol have been found in *P. padus* fruits, where alpha-tocopherol is dominant and constitutes as much as 63% of all tocopherols (Table 4) [30]. In raw seeds, alpha-tocopherol is present at a level of 3.916 mg / 100 g, while it is absent in roasted seeds [32,54].

The fruits of *P. padus* also contain vitamin C, in the form of ascorbic acid at a level of 25.20 ± 3.48 mg/100 FW and in the form of dehydroascorbic acid at a level of 50.87 ± 16.23 mg/100 FW [55,56]. Ascorbic acid is a saccharide derivative. It is produced from D-glucose in organisms of animals capable of synthesizing it [57]. In plants it is produced from D-glucose or D-galactose. Vitamin C is biologically active [58]. It takes part in many reactions and transformations, stimulating various biochemical processes in the body. Because of good solubility of vitamin C and active transport, about 80% is absorbed by the organism [59,60]. The structure of ascorbic acid, which contains two adjacent groups, hydroxyl and carbonyl, makes this molecule an excellent hydrogen or electron donor, which determines the antioxidant activity. Ascorbic acid can be a donor of two electrons and therefore takes part as a cofactor in many enzymatic reactions taking place in the body.

6.3. Terpens

Non-glycosylated pentacyclic triterpenoids such as ursolic acid (3b-hydroxyurs-12-en-28-ic acid), corosolic acid (2a, 3b-dihydroxyurs-12-en-28-ic acid) and oleanolic acid (3b-hydroxyolean-12-en-28-ic acid) are lipophilic constituents, which have recently aroused great interest in the context of phytotherapy because of their comprehensive biological properties, including anti-inflammatory, anti-ulcer, antioxidant, hepatoprotective, anticancer, antiatherosclerotic and antidiabetic ones [61–66].

These acids are widely distributed in various medicinal plants and plants of the *Prunus* species (*Rosaceae*). It was found that leaves and whole inflorescences of *P. padus* were the richest source of triterpenic acids (Table 4). It was shown that during the entire vegetation season, the highest levels of triterpenes were accumulated in the leaves between September and October, which are therefore recommended as the optimal time to harvest high quality plant material [67]. Terpenes such as limonene, phellandrene, sabinene, γ -terpinene, ursolic acid, uvaol, cis-linalool oxide, trans s-linalool oxide, (Z)-8-hydroxylinalool, E)- β -Farnesene or (E,E)- α -Farnesene were also found in wild cherry fruits [42].

6.4. Organic Acids

The plant raw materials also contain malic acid, citric acid and tartaric acid [68–70]. In the fruits they are mostly observed in free form, unlike in case of vegetables, where organic acids are bound. Malic and citric acid were found to be the main organic acids in wild cherry fruits (*P. padus*) (Table 5). In the fruit of *P. padus*, the highest content among the determined organic acids is noted for quinic acid and citric acid [55] and the lowest for shikimic acid [30]. There are also such organic acids as oxalic acid, malic acid or fumaric acid [30].

Table 5. Polyphenolic compounds in wild cherries (*P. padus* and *P. serotina*).

Class	Component	Result	Species	Plant Part	Reference
Cinnamic acid derivatives	Caffeic acid	6.61 ± 1.35 mg/100g FW	<i>P. padus</i>	Fruits	[55]
	Chlorogenic acid	10.48 ± 0.28 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		1.39–1.94% DW	<i>P. padus</i>	Flowers	[71]
	Coumaric acid	12.20 ± 3.07 mg/100g FW	<i>P. padus</i>	Fruits	[55]
	Ferulic acid	10.45 ± 3.65 mg/100g FW	<i>P. padus</i>	Fruits	[55]
	5- <i>p</i> -Coumaroylquinic acid 1	16.14 ± 1.11 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	5- <i>p</i> -Coumaroylquinic acid 2	2.25 ± 0.05 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Caffeic acid hexoside 1	11.71 ± 1.45 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	p-Coumaric acid hexoside 1	10.25 ± 1.00 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	5-Caffeoylquinic acid 1	357.30 ± 14.08 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	5-Caffeoylquinic acid 2	48.25 ± 1.70 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		X	<i>P. serotina</i>	Fruits	[31]
	Dicaffeoylquinic acid	15.40 ± 1.08 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Ellagic acid	11.41 ± 1.25 mg/100g FW	<i>P. padus</i>	Fruits	[55]
Hydroxy-benzoic Acid derivatives	Gallic acid	3.54 ± 0.81 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		X	<i>P. serotina</i>	Fruits	[31]
	Vanillic acid	X	<i>P. serotina</i>	Fruits	[31]
Flavones	Apigenin rhamnoside	24.99 ± 1.61 mg/kg FW	<i>P. padus</i>	Fruits	[30]
Flavonols	Hyperoside	7.38 ± 0.41 mg/100g FW	<i>P. padus</i>	Fruits	[55]

Table 5. Cont.

Class	Component	Result	Species	Plant Part	Reference
	Quercetin	0.15–0.23% DW	<i>P. padus</i>	Flowers	[71]
		11.86 ± 2.36 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		1.37–1.56% DW	<i>P. padus</i>	Flowers	[71]
	Quercitrin	16.37 ± 3.51 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		X	<i>P. serotina</i>	Fruits	[31]
		2.67 ± 1.02 mg/100g FW	<i>P. padus</i>	Fruits	[55]
	Rutin	X	<i>P. serotina</i>	Fruits	[31]
		2.48 ± 0.09 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		7.49 ± 0.54 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Quercetin hexosyl pentoside 3	223.45 ± 10.20 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		X	<i>P. serotina</i>	Fruits	[31]
		52.80 ± 1.41 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Quercetin-3-galactoside	21.40 ± 1.34 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Quercetin-3-rhamnoside	1.98 ± 0.38 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Quercetin-3-rutinoside	64.70 ± 21.18 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Quercetin 3-O-β-galactopyranoside	X	<i>P. padus</i>	Flowers	[71]
	Quercetin diglycosides	1.74–1.91% DW	<i>P. padus</i>	Spring leaves	[71]
		1.67–1.78% DW	<i>P. padus</i>	Flowers	[71]
		1.22 ± 0.03 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Isorhamnetin diglycoside	0.36–0.59% DW	<i>P. padus</i>	Flowers	[71]
	Kaempferol hexoside pentoside	0.72 ± 0.03 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		X	<i>P. serotina</i>	Fruits	[31]
		1.06 ± 0.14 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Kaempferol hexoside 2 (glu)	X	<i>P. serotina</i>	Fruits	[31]
		1.43 ± 0.05 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		X	<i>P. serotina</i>	Fruits	[31]
	Kaempferol-3-rutinoside	2.81 ± 0.29 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		25.43 ± 3.16 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		95.22 ± 10.60 mg/kg FW	<i>P. padus</i>	Fruits	[30]
Catechins	Epicatechin	56.66 ± 16.88 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		53.95 ± 8.90 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		26.66 ± 5.97 mg/100g FW	<i>P. padus</i>	Fruits	[55]
Tannins	Vescalagin	217.24 ± 14.95 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		24.76 ± 1.32 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		12.16 ± 2.19 mg/100g FW	<i>P. padus</i>	Fruits	[55]
Organic acids	Citric acid	324.48 ± 57.21 mg/100g FW	<i>P. padus</i>	Fruits	[55]
		6.45 ± 0.25 mg/kg FW	<i>P. padus</i>	Fruits	[30]
		18.71 ± 0.81 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Malic acid	2.66 ± 0.16 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Shikimic acid	2.66 ± 0.16 mg/kg FW	<i>P. padus</i>	Fruits	[30]
	Fumaric acid	80.08 ± 3.19 mg/kg FW	<i>P. padus</i>	Fruits	[30]

X—component is present in the sample

6.5. Polyphenolic Compounds in Wild Cherries (*P. padus* and *P. serotina*)

Phytochemical analysis showed that the main components of fruits (*P. padus*) are organic acids (48.62 ± 2.31%), polyphenols (35.34 ± 1.80%), monoterpenes (9.36 ± 0.64%) and vitamin C (6.68 ± 0.22%) (Table 5). Polyphenols present in *Prunus* are mainly: cinnamic acid, flavonols, benzoic acids, catechins and tannins [55]. The most important flavonols determined in the fruit were quercitrin (16.37 ± 3.51 mg/100 g FW) and quercetin (11.86 ± 2.36 mg/100 g FW).

Research on the influence of dietary polyphenols on human health has developed significantly in the last 10 years. They have been shown to be effective in the prevention of degenerative diseases, especially cardiovascular diseases and cancer [72,73]. The antioxidant properties of polyphenols have been extensively studied, but the health effects of polyphenols depend on the amount consumed and their bioavailability [74].

In fruits of *P. padus*, the presence of mainly caffeic acid, chlorogenic acid, coumaric acid and ferulic acid was found, of which coumaric acid was dominant [55,75] as well as 5-caffeolquinic acid 2 [30]. The content of ellagic acid, gallic acid and vanillic acid was also determined [55].

Flavonols, in turn, are among the most numerous plant polyphenols [76]. Their group is extremely diverse. The main share among flavonols in berries constitute quercetin derivatives [30]. It was demonstrated that epicatechin and catechin dominate among flavonols in *P. padus*. The content of flavanols in the fruits of *P. padus* also consist of quercetin and its derivatives, hyperoside, kaempferol and isorhamnetin. Kaempferol glycosides accounted for only 2%, and isorhamnetin glycosides were detected in amounts below 1 mg/kg FW [55].

According to Hertog et al., 1992 [77], a proper diet should provide plant polyphenols. The fruits of *P. padus* were shown to have a high flavonol content of 382 mg/kg FW compared to other vegetables and fruits [77]. *P. padus* can therefore be considered as a source of flavonols and used as a food ingredient or raw material for the production of supplements. Other studies demonstrated that flavones, like flavonols, are of significant biological importance [78]. Among flavones, apigenin rhamnoside in amounts of 24.99 ± 1.61 mg/kg FW was identified in the fruits of *P. padus* [30].

Another ingredient found in wild cherry are tannins. The content of condensed tannins in the fruit is mainly influenced by the environmental conditions of plant growth and the degree of fruit ripeness. Fruits of *P. padus* are characterized by a high tannin content of 5356 mg/kg FW [30]. It was demonstrated that tannins in the diet support the treatment of diabetes and have an anti-inflammatory effect [79].

7. Cyanogenic Glycosides: Amygdalin and Prunazine

The *P. serotina* plant is considered an invasive species. It contains cyanogenic glycosides such as prunazine and amygdalin. Their presence in the plant is considered to protect it from herbivores and pathogens [80]. Cyanogenic glycosides are produced by plants and are potentially toxic to herbivores due to the hydrolytic release of hydrocyanic acid [81].

The content of prunazine and amygdalin was determined in the fruits of wild cherry and its amount was compared to other raw materials (Table 6). It was shown that the leaves of the American black cherry (*P. serotina*) contain both these glycosides in amounts of 59.49 ± 1.31 mg/g $\pm$ SD prunazine and 20.95 ± 0.25 mg/g $\pm$ SD amygdalin [82]. These concentrations are high in comparison with other raw materials where the amygdalin content is lower, e.g., in *P. amygdalus* Batsch fruit oil, was 6.37 mg/g [83], in *Eriobotrya japonica* Lindl. flowers it was 50.76 ± 0.92 μ g/mL [84]. In other fruits and vegetables the amygdalin content is as follows: green plum 17.49 ± 0.26 mg/g, apricot 14.37 ± 0.28 mg/g, black plum 10.00 ± 0.14 mg/g, peach 6.81 ± 0.02 mg/g, red cherry 3.89 ± 0.31 mg/g, black cherry 2.68 ± 0.02 mg/g, nectarine 0.12 ± 0.01 mg/g, courgette 0.21 ± 0.13 mg/g, cucumber 0.07 ± 0.02 mg/g and marrow 0.06 ± 0.01 mg/g [85].

Table 6. Cyanogenic glycosides: amygdalin content in different species.

Species	Value	Reference
<i>P. serotina</i> (leaves)	20.95 ± 0.25 mg/g	[82]
<i>Prunus amygdalus</i> Batsch. (fruit)	6.37 mg/g (oil)	[83]
<i>Prunus amygdalus</i> L. (fruit)	0.12 ± 0.06 mg/g	[85]
<i>Eriobotrya japonica</i> Lindl. (flower)	50.76 ± 0.92 μ g/mL	[84]
<i>Prunus armeniaca</i> L. (raw seeds)	118 (29) μ mol HCN equivalents/g DW	[86]
<i>Amygdalus communis</i> L. (nectar)	6.7 (ppm)	[87]
<i>Prunus mume</i> L. (fruit)	17.49 ± 0.26 mg/g	[85]
<i>Prunus domestica</i> L. (fruit)	10.00 ± 0.14 mg/g	[85]
<i>Prunus persica</i> L. (peach, fruit)	6.81 ± 0.02 mg/g	[85]
<i>Prunus avium</i> L. (red, fruit)	3.89 ± 0.31 mg/g	[85]
<i>Prunus avium</i> L. (black, fruit)	2.68 ± 0.02 mg/g	[85]
<i>Prunus persica</i> L. (nectarine, fruit)	0.12 ± 0.01 mg/g	[85]

HCN—Hydrogen Cyanide, DW—dry weight.

8. Pharmacological Activity, Health-Promoting Properties

8.1. Antioxidant Activity

There are many studies available in the literature that indicate the high antioxidant potential of *P. padus* and *P. serotina* plants (Table 7). This concerns fruit as well as leaves, bark and flowers [31,55,87,88]. The studies were carried out in this range, where methanol extracts from the bark and leaves of *P. padus* were made and significant concentration-dependent antioxidant activity was demonstrated [88].

Table 7. In vitro and in vivo activity of *P. padus* and *P. serotina*.

Activity	Method	Extract	Species	Plant Part	Organism	Result/Observed Effect	Reference
Antioxidant Activity	FRAP	Acetone	<i>P. padus</i>	Frozen Fruits		31.54 ± 0.26 mM trolox/kg	[30]
	FRAP	Methanol	<i>P. padus</i>	Fruits		17.78 ± 0.84 mmol Fe ²⁺ kg ⁻¹	[55]
	FRAP	Methanol	<i>P. padus</i>	Leaves		100 µg/mL (concentration) 0.34 ± 0.04 µg/mL	[88]
	FRAP	Methanol	<i>P. padus</i>	Leaves		200 µg/mL (concentration) 0.62 ± 0.01 µg/mL	[88]
	FRAP	Methanol	<i>P. padus</i>	Leaves		300 µg/mL (concentration) 0.88 ± 0.00 µg/mL	[88]
	FRAP	Methanol	<i>P. padus</i>	Branch		100 µg/mL (concentration) 0.51 ± 0.01	[88]
		Methanol	<i>P. padus</i>	Branch		200 µg/mL (concentration) 0.74 ± 0.01	[88]
		Methanol	<i>P. padus</i>	Branch		300 µg/mL (concentration) 0.99 ± 0.00	[88]
	FRAP	Water	<i>P. serotina</i>	Fresh Fruits		1455.2 ± 92.5 µmol TE/100 g of FW	[31]
	FRAP	Water	<i>P. serotina</i>	Flesh Fruits		1100.7 ± 35.4 µmol TE/100 g of FW	[31]
	FRAP	Water	<i>P. serotina</i>	Peel Fruits		1991.4 ± 40.1 µmol TE/100 g of FW	[31]
	DPPH	Methanol	<i>P. padus</i>	Flowers		1.43–1.49 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Autumn Leaves		1.68 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Spring Leaves		2.10–2.29 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Summer Leaves		1.81–1.93 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Flowers		1.78–1.84 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Autumn Leaves		2.49 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Spring Leaves		4.27–4.92 g g ⁻¹	[71]
	DPPH	Methanol	<i>P. padus</i>	Summer Leaves		3.12–3.95 g g ⁻¹	[71]
	DPPH	Water	<i>P. serotina</i>	Fresh Fruits		2056.7 ± 108.0 µmol TE/100 g of FW	[31]
	DPPH	Water	<i>P. serotina</i>	Flesh Fruits		1764.6 ± 170.4 µmol TE/100 g of FW	[31]
	DPPH	Water	<i>P. serotina</i>	Peel Fruits		2681.6 ± 180.0 µmol TE/100 g of FW	[31]
Antimicrobial	MIC	Methanol	<i>P. padus</i>	Branch	<i>Bacillus Atrophaeus</i>	250 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Bacillus Atrophaeus</i>	500 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Kocuria rhizophila</i>	125 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Kocuria rhizophila</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Micrococcus luteus</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Micrococcus luteus</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Staphylococcus epidermidis</i>	250 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Staphylococcus epidermidis</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Bacillus subtilis</i>	500 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Bacillus subtilis</i>	500 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Klebsiella pneumoniae</i>	1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Klebsiella pneumoniae</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Enterobacter cloacae</i>	500 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Enterobacter cloacae</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Salmonella enterica</i>	500 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Salmonella enterica</i>	>1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Branch	<i>Pseudomonas aeruginosa</i>	1000 µg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Leaves	<i>Pseudomonas aeruginosa</i>	1000 µg/mL	[89]
		Dichloromethane	<i>P. padus</i>	Seeds	<i>Enterococcus faecalis</i>	1.0 mg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Seeds	<i>L. plantarum</i>	1.0 × 10 ⁻² mg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Seeds	<i>P. mirabilis</i>	1.0 × 10 ⁻² mg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Seeds	<i>S. aureus</i>	1.0 × 10 ⁻⁴ mg/mL	[89]

Table 7. Cont.

Activity	Method	Extract	Species	Plant Part	Organism	Result/Observed Effect	Reference
Antidiabetic	MIC	Dichloromethane	<i>P. padus</i>	Seeds	<i>S. hominis</i>	1.0 mg/mL	[89]
	MIC	Methanol	<i>P. padus</i>	Seeds	<i>S. hominis</i>	1.0×10^{-2} mg/mL	[89]
	The α -glucosidase inhibitory effect	Methanol	<i>P. padus</i>	Leaves		1.0 ± 0.1 μ g/mL	[88]
	The α -glucosidase inhibitory effect	Methanol	<i>P. padus</i>	Branch		82.7 ± 4.2 μ g/mL	[88]
Cardiovascular activity	NOS Enzymatic activity assay	Dichloromethane	<i>P. serotina</i>	Fruits	Wistar male rats 250–300 g	Ursolic acid ($EC_{50} = 21.5 \pm 3.5$ μg/mL; $E_{max} = 97.7\% \pm 3.9\%$); Uvaol ($EC_{50} = 19.3 \pm 2.5$ μg/mL; $E_{max} = 93.4\% \pm 5.1\%$) caused a significant concentration-dependent relaxation of the aorta. ACh ($EC_{50} = 8.7 \pm 0.8$ μg/mL; $E_{max} = 69.5\% \pm 5.7\%$), used as positive control, was more potent than ursolic acid and uvaol, but showed lower efficacy than both triterpenes. The nitrite concentration increased, when aortic tissue was incubated with ursolic acid ($(NO_2') = 7.95 \pm 0.22\mu$M) and uvaol ($(NO_2') = 7.55 \pm 0.17\mu$M); both triterpenes induced a higher nitrite concentration than that of ACh ($(NO_2') = 5.5 \pm 0.47\mu$M). Similarly, quantification of H_2S showed that stimulation of aortic tissue with ursolic acid ($(H_2S) = 234 \pm 12.7\mu$M) and uvaol ($(H_2S) = 253 \pm 6.8\mu$M) increased four times the H_2S concentration with respect to the control. In the presence of ACh, H_2S levels were only three times higher than those of the control; the vasodilator effect produced by ursolic acid involves activation of the NO/cGMP and H_2S/KATP channel pathways, possibly through direct activation of NOS and CSE. Tested material delayed reaction times to a nociceptive stimulus 60 min after oral administration (38.61% at 250 mg/kg and 68.51% at 500 mg/kg, $po0.001$). The anti-nociceptive effects of MPP (250, 500 mg/kg) occurred between 30 and 90 min and maximum analgesia was reached at 60 min (37.98%, after 0.01 and 62.18%, after 0.001 respectively). The treatment with MPP induced a significant decrease in the number of writhing motions dose dependently (52.5% at 250 mg/kg, $po0.001$ and 72.8% at 500 mg/kg, $po0.001$). <i>P. padus</i> shown anti-inflammatory properties not only by suppressing various inflammatory mediators in vitro but also by reducing inflammatory swelling in vivo. A strong anti-nociceptive effect through the central and peripheral mechanism acting as a partial opioid agonist was also demonstrated.	[90]
Anti-inflammatory-nociceptive properties	Measurement of iNOS enzyme activity Western blot analysis Trypsin-induced paw edema Acute toxicity test Tail immersion test Hot plate test Acetic acid-induced writhing test Formalin test	Methanol	<i>P. padus</i>	Dried stem	ICR mice (6 weeks old) weighing 20–25 g and C57BL/6 mice (5 weeks old) weighing 18–22 g		[91]

DPPH—antioxidant activity with DPPH radicals (2,2-diphenyl-1-picrylhydrazyl), FRAP—ferric reducing antioxidant power, MIC—antimicrobial test (minimum inhibitory concentration assay). TE—Trolox equivalent, FW—fresh weight, NOS—inhibitor of NO synthase, CSE—inhibitor of cystathionine- γ -lyase, ICR—Institute of Cancer Research.

This activity was confirmed by 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging methods as well as by a reducing force. A positive correlation between antioxidant activity and flavonoid content was shown. Higher levels of flavonoid content were found in *P. padus* leaves ($210.8 \pm 2.6 \mu\text{g QUE/g}$) compared to bark extract [88]. High antioxidant activity of aqueous extracts from fruits, flesh and peel of *P. serotina* was also shown by other authors. Higher activity of extracts prepared from the peel was also positively correlated with the presence of polyphenols [31]. These results are consistent with previous reports, where it was shown that *Prunus* fruit peels have at least a 20% higher polyphenol content than flesh [92,93]. This relationship also applies to other fruits such as apples and grapes [94,95]. Analyzing the data presented, it can be concluded that the antioxidant activity measured by DPPH and FRAP tests for whole fruits of *P. serotina* was higher than that obtained for plums or grapes by at least 15% [96,97].

These results indicate that wild cherry fruits can be a raw material with similar antioxidant activity as those considered as good sources of antioxidants. The content of polyphenols in the fruit peel of *P. serotina* correlated with the antioxidant activity of the fruits ($r = 0.875$ for DPPH and $r = 0.959$ for FRAP) [31]. However, such dependencies are not unequivocal. The content of polyphenols and their distribution in fruit depends largely on the ripening process and the genetic and environmental conditions [98].

Twelve phenolic compounds were found in the fruit peel of *P. serotina* which contribute to their strong antioxidant properties. The HPLC-MS analysis showed that cyanidin-3-O-rutinoside, chlorogenic acid, hyperoside and quercetin pentoside are present in the greatest amount in the *P. serotina* peel. However, the main phenolic compounds in the fruit flesh are cyanidin-3-O-rutinoside, chlorogenic acid, procyanidin B, hyperoside and quercetin malonilglucoside [31].

In another study, the antioxidant activity and polyphenol content in acetone and aqueous extracts from fruits of *Prunus padus* L was examined. The total polyphenol content was $11053.3 \pm 491.28 \text{ mg GAE/kg FW}$ fruit weight and the antioxidant activity (FRAP) $31.54 \pm 0.26 \text{ mM trolox/kg}$ [30]. Another study on fresh fruits of *P. padus* confirmed antioxidant properties and high content of polyphenols [55]. Extracts from fresh fruit were characterized by their ability to chelate metals at the level of $17.78 \pm 0.84 \text{ mmol Fe}^{2+} / \text{kg FW}$. In another study, methanol extracts of flowers and leaves of *P. padus* were compared for antioxidant activity using DPPH radical. The aim of the comparison was to assess the activity of leaves collected at the turn of May and June and autumn leaves collected at the turn of July and August. It was shown that the highest antioxidant activity was observed in flowers, whose antioxidant activity was $1.43\text{--}1.49 \text{ g g}^{-1} \text{ DPPH}$, as well as in leaves harvested in autumn, giving a value of $1.68 \text{ g g}^{-1} \text{ DPPH}$ [71].

8.2. Antimicrobial Activity

The abuse of antibiotic therapy can have adverse effects [99–102]. Side effects of antibiotics such as immunosuppression, allergic reactions and hypersensitivity are known. Therefore, natural sources of bactericidal and bacteriostatic compounds in plants are sought [103]. Many substances contained in rhizomes, fruits, leaves or bark have been shown to have bactericidal effects [104–108].

The literature contains reports on the antibacterial effect of wild cherry components (Table 7). The beneficial effect of methanol extracts from seeds of *P. padus* on seventeen strains of pathogenic bacteria was demonstrated [89]. Earlier studies by the same authors confirmed the bactericidal effect of other methanol extracts obtained from the seeds of plants harvested in Scotland [109]. Methanol extracts obtained from seeds of *P. padus* were active against *Staphylococcus aureus*. In addition, the extracts also showed action against methicillin-resistant strains of *Staphylococcus aureus*, *Staphylococcus hominis* and *Proteus mirabilis*. Dichloromethane extracts, on the other hand, showed weak growth inhibition of *Enterococcus faecalis* and *Staphylococcus hominis* strains. The highest antibacterial activity was found for methanol extract from seeds of *P. padus* against *Staphylococcus aureus* ($1.0 \times 10^{-4} \text{ mg/mL}$) [89].

In another study, antimicrobial activity was assessed using methanol extracts from the leaves and branches of *P. padus*. Both leaf and branch extracts showed antimicrobial activity against most of the

gram-positive bacteria tested, but only branch extract showed any activity against Gram-negative bacteria. *P. padus* branch extract was most active against *Kocuria rhizophila* (MIC = 125 µg/mL) [88].

8.3. Antidiabetic Effect

Many herbal preparations are used in the prevention and support of diabetes treatment [110]. It has been shown that alpha-glucosidase inhibitors of natural origin such as aqarbose, 1-deoxynojirimycin and genistein, are beneficial in type 2 diabetes delaying the increase of blood glucose levels [111]. The literature points to such raw materials as: mulberry leaves [112,113], gurmar [114,115], Ceylon cinnamon [116,117], galega [118], common beans [119].

The beneficial antidiabetic effect was also confirmed for methanol extracts from the leaves and branches of *P. padus*, which were tested for their alpha-glucosidase inhibitory activity (Table 7). It was found that the extract from the branches showed higher activity, which was indicated by a higher content of polyphenols compared to the extract from leaves [88].

8.4. Cardiovascular Activity

One of the cardiovascular diseases is hypertension [120–122]. Currently, there is a search for plant preparations which action that will activate the main signaling pathways vasodilating endothelium [90]. In this respect, there are reports of beneficial effects of plant polyphenols in the literature. Positive effects in the prevention and treatment of hypertension have been found in the case of such plants as: *Nauclea latifolia* L. [123], green tea [124], grapefruit seeds [125], garlic [126] and globe amaranth (*Gomphrena globosa* L.) [127].

Studies on the influence of wild cherry on hypertension have confirmed that their efficacy is due to the presence of hyperoside and chlorogenic acid as smooth muscle relaxants in blood vessels (Table 7) [90]. Preparations made from the leaves and fruits of *P. serotina* may be a part of the diet supporting the treatment.

Other studies demonstrate that water and dichloromethane extracts from the fruit of *P. serotina* contain polar and non-polar vasodilating metabolites [31]. It was also noted that *P. serotina* fruits have a high content of phenolic compounds such as chlorogenic acid, gallic acid, coffee acid, catechin, epicatechin and quercetin and kaempferol glycosides, which are directly related to the high antioxidant activity and the resulting vasodilating effect [31]. These results were confirmed in another experiment where the effectiveness of wild cherry compounds in the H₂S hydrogen sulfide pathway, which is responsible for vasodilation, was confirmed [31]. Available data indicate that H₂S dilates vessels mainly by increasing the activity of ATP-sensitive potassium channels (KATP) in muscle cells. Triterpenes present in wild cherry fruits increased this activity. The study also confirmed the participation of wild cherry triterpenes in the NO/cGMP pathway. Moreover, it was shown that ursolic acid is able to activate both pathways simultaneously, which causes a synergic vasodilatory effect. Studies in silico confirmed the beneficial effects of these compounds [90].

8.5. Anti-Inflammatory and Anti-Nociceptive Properties

Inflammatory process is an important defense mechanism in the human body. It may be characterized by redness, swelling, pain, feeling of warmth and dysfunction of tissue and organs [128–131]. In response to inflammation, the body activates cells in the immune response, which increase the production of pro-inflammatory mediators including nitrogen and prostaglandin. Earlier studies demonstrated that excessive production of nitrogen and prostaglandin takes part in inflammatory diseases, including rheumatoid arthritis, asthma and cancer [132,133]. Their high levels are produced by inductive enzymes such as inductive nitric oxide synthase and cyclooxygenase-2 [134]. Thus, methods are being sought to inhibit the expression of these enzymes, which may prove to be an attractive therapeutic agent in the treatment of inflammatory diseases [135]. Studies demonstrated that *P. padus* contains anthocyanins, cyanogenic glycosides, flavonoids and chlorogenic acid, which are important in the treatment of inflammation (Table 7) [71]. It also has antioxidant and

antibacterial properties [89]. Methanol extracts from the stem of *P. padus* were analyzed, determining the anti-inflammatory and analgesic effects. In this study, a strong influence of the inhibitor on nitric oxide production induced by IFN-gamma/LPS was demonstrated. The extract suppressed not only the enzyme activity but also the expression of nitric oxide synthase. It also inhibited cyclooxygenase-2 expression depending on the dose. IFN-gamma/LPS stimulation induced NF-kB translocation to the nucleus, but was weakened by the presence of stem extract from *P. padus* [91]. The same study in vivo showed that the stem extract from *P. padus* may reduce paw swelling in six-week-old mice. It also shows a strong analgesic effect compared to tramadol and indomethacin. The study confirmed the strong anti-inflammatory properties of the extract not only by suppressing inflammatory mediators in vitro, but also by reducing inflammatory swelling in vivo. A strong anti-nociceptive effect through the central and peripheral mechanism acting as a partial opioid agonist was also proven. Based on these results, it can be suggested that *P. padus* has a strong anti-inflammatory and analgesic potential.

9. Cosmetic Properties of *P. padus* and *P. serotina*

Recently, the search for natural sources of active compounds, mainly of plant origin, and their beneficial properties has become a very clear trend in the cosmetics industry. Cosmetic companies are still looking for new natural ingredients that could enrich existing cosmetic compositions. Bark, leaves and fruits of *P. padus* are known for their anti-inflammatory, antimicrobial and antioxidant properties [91]. They contain polyphenols that indicate the potential of *Prunus* as a cosmetic ingredient. It is known that polyphenols have a strong antioxidant effect. Antioxidants delay the aging process of cosmetic products both during their use and storage. In practice, small amounts of antioxidants (0.001–0.01% by weight) can already deactivate free radicals and thus stop the chain oxidation of hydrocarbon lipid chains [136]. Phenolic contents in *P. padus* and *P. serotina* were very high compared to some known antioxidant plants such as thistle, mate, slippery elm bark or pine needles [137]. Polyphenols can also complement sun protection products and can be applied to treat inflammation caused by UV radiation, as well as to protect and prevent skin damage caused by oxidative stress or DNA damage.

P. padus fruits also contain a relatively high content of vitamin C, but it cannot be directly utilized to stabilize the oil phase of cosmetic products, as it is not soluble in fats. However, it is possible to conduct esterification with palmitic acid, and the obtained ascorbyl palmitate (ACP) is perfectly suited for use as an antioxidant both in cosmetic products and in the food industry.

Both *P. serotina* and *P. padus* fruits contained gallic acid. Antioxidants based on gallic acid are allowed in cosmetic products in weight concentrations from 0.005 to 0.01% [138]. Gallic acid (3,4,5-trihydroxybenzoic acid) esters are commonly used oil phase antioxidants for cosmetic preparations. Esters with low molecular weight alcohols and fatty acid esters are used to stabilize lipids, oils and cosmetic emulsions. It is also worth mentioning that in addition to antioxidant activity, gallic acid esters with long-chain alcohols also exhibit significant antifungal activity. A disadvantage of this group of antioxidants is that they cause unaesthetic yellow coloration of cosmetic preparations, especially in the presence of heavy metal cations [138].

Citric acid, present in *P. padus* fruits, also shows antioxidant activity; it binds heavy metal cations to non-dissociating complexes, thereby reducing the catalytic activity of metals in lipid auto-oxidation. Chlorogenic acid, which was also found in *P. padus* fruits, is listed among effective antioxidants as well. This compound binds iron ions, causing a loss of their ability to generate hydroxyl radicals, leading in turn to reduced lipid and protein peroxidation. It also prevents DNA damage induced by sunlight [139].

Essential oils from fruits, stems, leaves and bark of *P. padus* contain large amounts of benzoic acid and benzaldehyde. Benzoic acid has not only anti-bacterial, but also anti-fungal properties. This compound, already at a weight concentration of 0.1%, is an effective antimicrobial agent in an acidic environment, while at a weight concentration of about 1%, it can be added as a mild disinfectant, e.g., in ointments or mouth waters. The use of plant materials such as essential oils in cosmetic preparations

at relatively high concentrations provide many benefits for the skin. They exhibit antimicrobial effects [140] and can serve as effective cosmetic preservatives. At present, synthetic preservatives are applied to extend product shelf life. However, while common synthetic preservatives extend the life of products and help keep them free from microorganisms, many of them hold a negative opinion in the minds of consumers, and their use has become increasingly controversial in recent years. Some of them can even cause serious health issues, e.g., 4-hydroxybenzoic acid esters, better known as parabens, were the most widely used preservatives (included in approximately 80% of global cosmetics [141]), but have recently been reported to mimic estrogen, increase female breast cancer incidence and influence the development of malignant melanoma. Essential oils can also be used as scents to enrich cosmetic fragrance.

The bark contains tannins, cerebrosides and polyphenols [71]. Due to the content of the above-mentioned bioactive substances, it seems appropriate to state that *P. padus* may be a good candidate for the production of natural cosmetics with strong effects.

The bark extract of *P. padus* was evaluated in terms of the use as a natural ingredient in cosmetics manufacturing. Extract from the bark of *P. padus* was obtained by extraction with hot water. The use of such an extract was evaluated for safety (cell toxicity test) and an effectiveness test (antioxidants, anti-wrinkle and whitening effect). The total concentration of polyphenols was 714.7 ± 0.5 mg/g and flavonoids 72.1 ± 2.2 mg/g. In comparison with other natural antioxidants, the concentration of polyphenols in the *P. padus* bark extract was very high. The extract showed 71% free radical scavenging activity of DPPH (antioxidant), 36% elastase inhibition (anti-wrinkle action) and 38% tyrosinase inhibition (whitening action) at 350 µg/mL. The preparation in the form of lotion, containing 1% of *P. padus* bark extract, showed that the viscosity, pH, particle size and appearance of the lotion remained stable for 28 days [142]. The lotion particle size was stable (362–426 nm) during stability tests. This study showed that the *P. padus* bark extract has promising potential as a natural antioxidant cosmetic agent.

10. Summary

In recent years there has been a great interest in products of plant origin and their health-promoting properties. Plants of *P. padus* and *P. serotina* are common in many parts of the world and do not require special cultivation conditions. The fruit, bark, leaves and flowers of the wild cherry are a good source of minerals, vitamins and compounds with high antioxidant activity, especially polyphenols.

Current scientific results confirm the beneficial properties of both *P. serotina* and *P. padus* extracts. They are attributed with a beneficial effect in the prevention of cardiovascular diseases and hypertension. Their antioxidant, anti-inflammatory, anti-nociceptive, antidiabetic and antimicrobial effects were also confirmed in the studies. Due to their content of anthocyanins, which show strong antioxidant properties, they can also be used in the pharmaceutical industry as a component of creams, gels and other care and disinfection preparations.

In summary, preparations from *P. padus* and *P. serotina* can be a valuable raw material used in the food, pharmaceutical and cosmetic industries as a source of bioactive compounds with multi-directional action.

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Oświadczenia o współautorstwie

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**Rada Dyscypliny
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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Szulc, P., 2020, Phytopharmacological Possibilities of Bird Cherry *Prunus padus* L. and *Prunus serotina* L. Species and Their Bioactive Phytochemicals, *Nutrients*, 12(7), 1-21, mój indywidualny udział w jej powstaniu polegał na zebraniu materiałów źródłowych, analizie danych źródłowych, zestawieniu danych do manuskryptu z materiałów źródłowych, przygotowaniu zestawień tabelarycznych, opisie informacji zestawionych tabelarycznie, opisie wyników, przygotowaniu treści manuskryptu, zredagowaniu manuskryptu zgodnie z wymogami czasopisma, przygotowaniu zestawienia literatury cytowanej w manuskrypcie, prowadzeniu korespondencji z wydawnictwem, przesłaniu manuskryptu do wydawnictwa oraz na naniesieniu/uzupełnieniu manuskryptu po recenzjach.

Podpis

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Podpis



Research Article

Aleksandra Telichowska*, Joanna Kobus-Cisowska, Marta Ligaj, Kinga Stuper-Szablewska, Daria Szymanowska, Mariusz Tichoniuk, Piotr Szulc

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 ultra-performance liquid chromatography (UPLC) system showed a higher content of both phenolic acids and flavonols ($651.77^b \pm 18.12$ mg/100 g dw for acids and $3.85^b \pm 0.08$ mg/100 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

2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) cation radical ($37.39^b \pm 3.81$ 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 isorhamnetine glycosides were detected in less than 1 mg/kg fw [1]. On the other hand, *Prunus* is considered as an invasive species. It contains

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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 × 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 × 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 $L \times a \times b \times \text{CEN}$ 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 × 2.1 mm, particle size 1.7 µm) (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 µg/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, naringenin 17.50 min, rutin 19.00 min, and catechin 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 1 g (mg/1 g) 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 (2,2'-azino-bis-(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 three-

electrode 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 Savitzky–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 utilis* (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 ± 0.29 in the PPL sample and 34.59 ± 1.96 in the PSL sample. Parameter a^* , responsible for the color change in the range from green to red, was 9.95 ± 0.04 for PPL and 14.69 ± 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 ± 0.12) and higher for PSL (11.22 ± 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 ± 0.01	0.156 ^a ± 0.01
Freezing temperature (°C)	−0.289 ^a ± 0.01	−0.289 ^a ± 0.01
L^*	34.59 ^b ± 1.96	26.06 ^a ± 0.29
a^*	14.69 ^b ± 0.19	9.95 ^a ± 0.04
b^*	11.22 ^b ± 0.22	1.80 ^a ± 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 ± standard deviation. Values sharing the same letter in a line are not significantly different ($P \leq 0.05$).

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 ± 18.12 mg/100 g dw. The dominant acids were *p*-coumaric acid 157.6 ± 8.33 mg/100 g dw, ferulic acid 195.6 ± 5.64 mg/100 g dw, and sinapic acid 147.5 ± 2.21 mg/100 g dw. The lowest amounts among the tested acids were detected for vanillic acid 2.6 ± 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 ± 6.72 mg/100 g dw) and *p*-coumaric acid (103.6 ± 0.21 mg/100 g dw). The lowest levels were determined for vanillic acid (1.24 ± 0.06 mg/100 g dw) and syringic acid (5.62 ± 0.12 mg/100 g dw). PPL leaf extract contained a higher concentration of flavonols (3.85 ± 0.08 mg/100 g dw) than PSL extract. PPL leaves contained naringenin, rutin, quercetin, and dominant catechin (2.07 ± 0.02 mg/100 g dw). The extract from PSL

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

	PSL (mg/100 g dw leaves)	PPL (mg/100 g dw leaves)
Phenolic acids		
Gallic acid	19.56 ^a ± 0.64	22.3 ^b ± 0.64
2,5-Dihydroxybenzoic acid	14.52 ^a ± 0.44	16.52 ^b ± 0.24
4-Dihydroxybenzoic acid	23.6 ^a ± 0.12	29.45 ^b ± 0.13
Caffeic acid	11.45 ^a ± 0.61	13.65 ^b ± 0.09
Syringic acid	5.62 ^a ± 0.12	8.95 ^b ± 0.04
<i>p</i> -Coumaric acid	103.6 ^a ± 0.21	157.6 ^b ± 8.33
Ferulic acid	185.3 ^a ± 6.72	195.6 ^b ± 5.64
Chlorogenic acid	29.5 ^a ± 0.09	36.8 ^b ± 0.24
Sinapic acid	97.68 ^a ± 0.39	147.5 ^b ± 2.21
<i>t</i> -Cinnamic acid	13.4 ^a ± 0.08	20.8 ^b ± 0.05
Vanillic acid	1.24 ^a ± 0.06	2.6 ^b ± 0.11
Salicylic acid	ND	ND
Total phenolic acids	505.47 ^a ± 9.48	651.77 ^b ± 18.12
Flavonoids		
Naringenin	0.13 ^a ± 0.00	0.62 ^b ± 0.02
Vitexin	ND	ND
Rutin	ND	1.03 ± 0.02
Quercetin	0.17 ^b ± 0.01	0.13 ^a ± 0.02
Apigenin	ND	ND
Kaempferol	ND	ND
Luteolin	ND	ND
Catechin	1.01 ^a ± 0.01	2.07 ^b ± 0.02
Total flavonoids	1.31 ^a ± 0.02	3.85 ^b ± 0.08

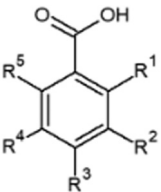
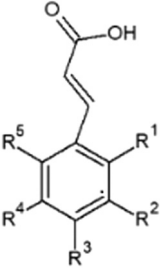
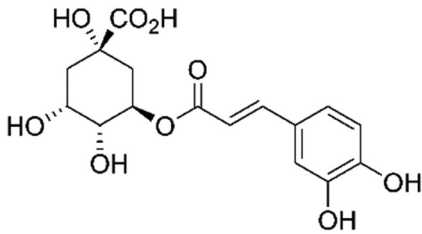
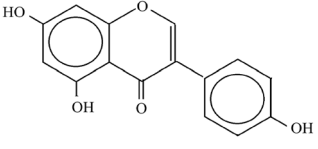
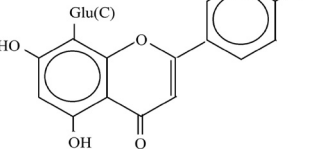
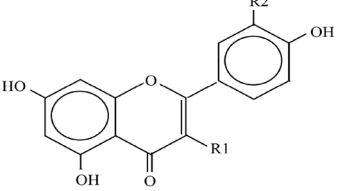
Abbreviation: as in Table 1, ND, not detected, results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($P \leq 0.05$).

leaves contained the highest content of catechins (1.01 ± 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 ± 3.81 mg GAE/g 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 ± 0.06 mg TE/1 g dw, while DPPH anti-radical activity for PSL

Table 3: Chemical structure of compounds analyzed

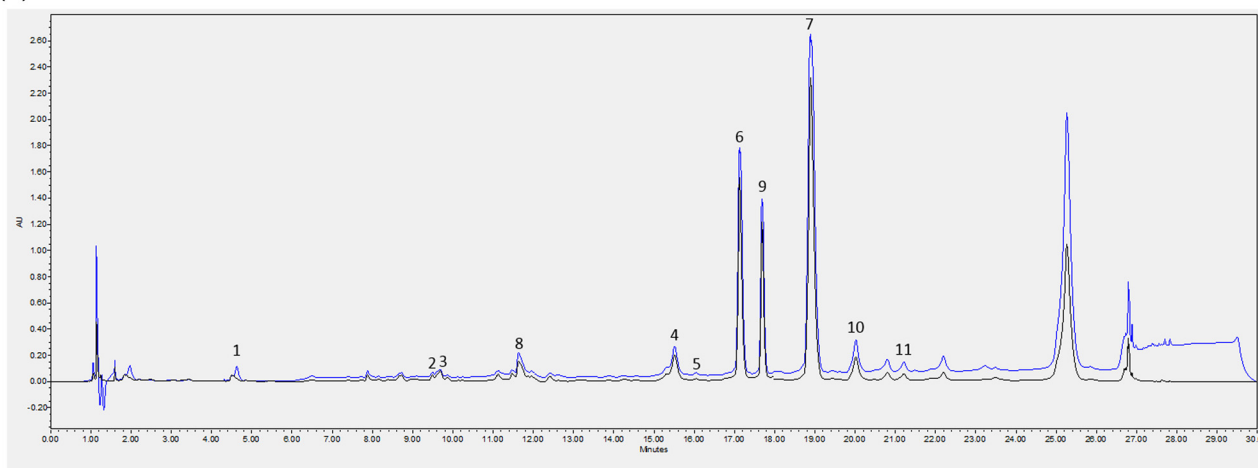
Phenolic acids		R1	R2	R3	R4	R5
	4-Hydroxybenzoic acid	-H	-H	-H	-OH	-H
	2,5-Dihydroxybenzoic acid	-H	-OH	-H	-H	-OH
	Gallic acid	-H	-OH	-OH	-OH	-H
	<i>t</i> -Cinnamic acid	-H	-H	-H	-H	-H
	Salicylic acid	-OH	-H	-H	-H	-H
	Syringic acid	-H	-OCH ₃	-OH	-OCH ₃	-H
	Vanillic acid	-H	-OCH ₃	-OH	-H	-H
	Ferulic acid	-H	-H	-OH	-OCH ₃	-H
	Caffeic acid	-H	-H	-OH	-OH	-H
	<i>p</i> -Coumaric acid	-H	-H	-OH	-OH	-H
	Sinapic acid	-H	-OCH ₃	-OH	-OCH ₃	-H
	Chlorogenic acid					
Flavonoids						
	Naringenin					
	Vitexin					
	Apigenin	R1	R2			
	Kaempferol	-H	-H			
	Quercetin	-OH	-H			
	Luteolin	-OH	-OH			
	Catechin	-H	-H			
	Rutin	<i>o</i> -gluk-ramn	-OH			

extract was slightly lower and amounted to 5.43 ± 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 ± 0.09 mg TE/g dw and was higher than for PSL extracts, where it was 8.55 ± 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

(a)



b)

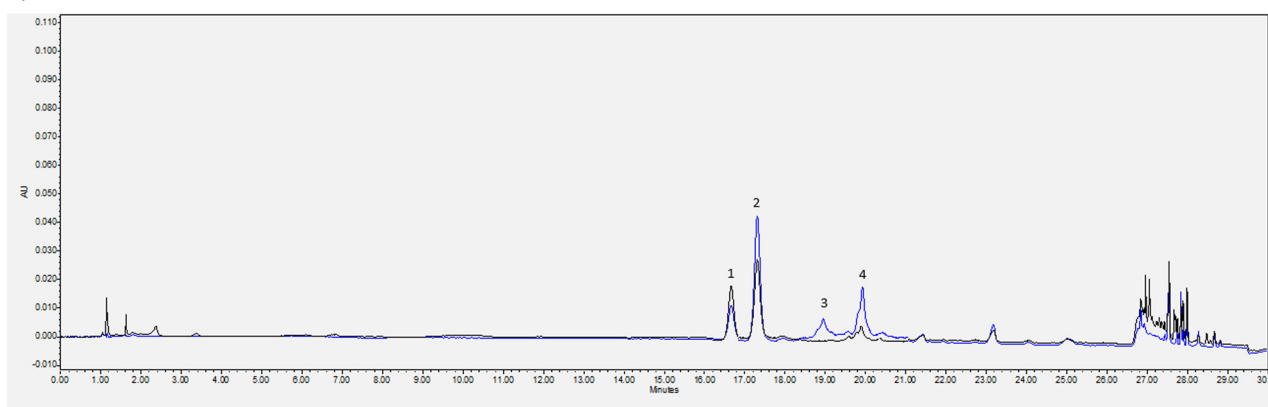


Figure 1: Comparison of chromatograms of phenolic acids for *P. serotina* and *P. padus* (a), comparison of chromatograms of flavonoids for *P. serotina* and *P. padus* (b). (a) Comparison of chromatograms of phenolic acids for *P. serotina* (black line) and *P. padus* (blue line) leaves: 1 – gallic acid, 2 – 2,5-dihydroxybenzoic acid, 3 – 4-dihydroxybenzoic 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.

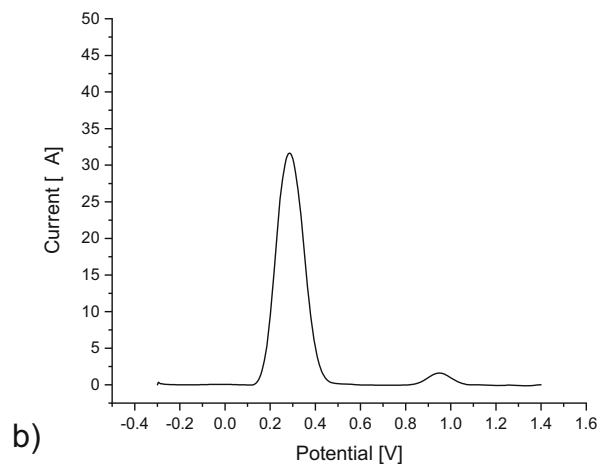
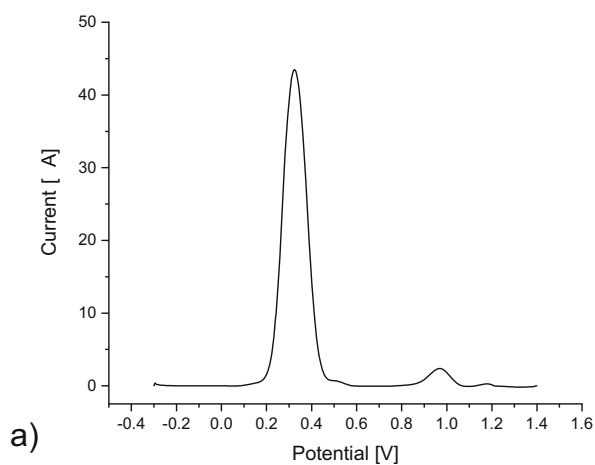


Figure 2: SWV signals for *P. padus* (a) and *P. serotina* (b) leaf extracts.

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)	21.54 ^a ± 3.34	37.39 ^b ± 3.81
DPPH (mg TE/1 g dw)	5.43 ^a ± 0.07	6.62 ^b ± 0.06
ABTS (mg TE/g dw)	8.55 ^a ± 0.08	9.65 ^b ± 0.09
FRAP (μM FeSO ₄ /1 g dw)	179.24 ^a ± 3.66	233.20 ^b ± 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 ± 0.117 V × μA, while EI reached the value of 142.417 ± 3.737 . These values for PSL leaf extract were 3.602 ± 0.439 V × μA 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 0.967 ± 0.010	0.285 ± 0.000 0.950 ± 0.000
Peak current I [μA]	27.102 ± 0.871 1.267 ± 0.087	29.065 ± 1.081 1.419 ± 0.225
Peak area $E_p \times I$ [V × μA]	3.469 ± 0.425 0.134 ± 0.014	4.280 ± 0.089 0.171 ± 0.027
Total peak current I [μA]	$28.379^a \pm 0.957$	$30.484^b \pm 1.306$
Total peak area [V × μA]	$3.602^a \pm 0.439$	$4.451^b \pm 0.117$
EI [V × μA/1 g dw]	$115.267^a \pm 14.043$	$142.417^b \pm 3.737$

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 gram-negative 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 ± 0.28 mg/100 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 ± 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 ± 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		
<i>Klebsiella pneumoniae</i> (ATCC 31488)	18 ± 30	19 ± 30
<i>Salmonella enteritidis</i> (ATCC 13076)	16 ± 30	14 ± 20
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	15 ± 20	17 ± 30
<i>Acinetobacter baumannii</i> (ATCC 19606)	8 ± 10	15 ± 30
Gram-positive bacteria		
<i>Enterococcus faecium</i> (ATCC 27270)	24 ± 40	22 ± 30
<i>Staphylococcus aureus</i> (ATCC 25923)	20 ± 30	15 ± 30
<i>Lactobacillus fermentum</i> (ATCC 14932)	18 ± 30	17 ± 30
<i>Clostridium butyricum</i> (ATCC 860)	19 ± 30	16 ± 30
<i>Listeria monocytogenes</i> (ATCC 19115)	23 ± 40	24 ± 40
<i>Bacillus coagulans</i> (GBI-30, 6086)	17 ± 30	19 ± 30
Fungi		
<i>Candida utilis</i> (ATCC 9950)	8 ± 10	7 ± 10
<i>Aspergillus</i> sp.	5 ± 10	3 ± 10
<i>Fusarium</i> 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 × 10⁻⁴ mg/mL). In addition, the extracts also showed the activity against methicillin-resistant 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 µg/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 plant-derived 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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Conflict of interest: Authors declare no conflict of interest.

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Oświadczenia o współautorstwie

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Podpis

Telichowska Aleksandra

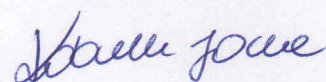
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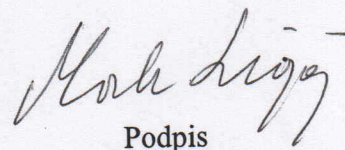
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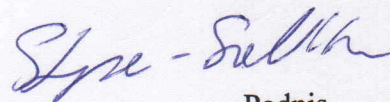
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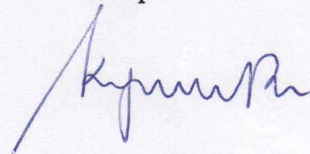
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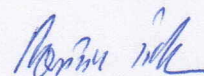
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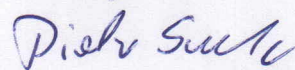
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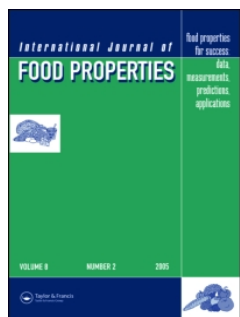
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Podpis





Exploring antimicrobial and antioxidant properties of phytochemicals from different anatomical parts of *Prunus padus* L

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Exploring antimicrobial and antioxidant properties of phytochemicals from different anatomical parts of *Prunus padus* L

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ABSTRACT

Prunus padus L. is a wild growing plant in various parts of the world with low soil requirements. This work evaluated the content of selected phytochemicals, antioxidant potential and determined the antimicrobial effect of individual anatomical parts of *P. padus* (i.e. leaves, bark, and fruits). It was shown that the examined anatomical parts of bird cherry (*Prunus padus* L.) contained polyphenols and low-molecular organic acids. The highest content of phenolic acids was found in *P. padus* leaves (1063 ± 26 mg/100 g dm). The analysis regarding flavonols confirmed that the highest content was found in the bark (1530 ± 61 mg/100 g dm). The content of low molecular weight organic acids, whose highest value was found in the fruits (1658 ± 27 mg/100 g dm) was also assessed. The highest antioxidant activity with DPPH radicals was confirmed for the bark (8.9 ± 0.03 mg TE/g dw). The FRAP test also showed the highest activity for the bark extract (380 ± 18 μ M FeSO₄/g dw). It was shown that signals located in the region of negative potentials in SWV (square wave voltammetry) indicated a high reducing ability of compounds present in *P. padus*, which means that they can be a very efficient protective barrier against oxidizing agents (i.e. the greatest intensity was for the leaves extract). The highest activity against Gram-negative bacteria was recorded in bird cherry leaves and the lowest in the bark. It showed that the leaves, fruits, and bird cherry bark can be a good source of antioxidant compounds and an attractive raw material with antimicrobial properties that can be widely applied, which is confirmed by the obtained results.

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Introduction

Antioxidants are substances that prevent the oxidation of other compounds or neutralize free radicals that contain an unpaired electron. Free radical causes oxidative stress responsible for damage to cellular macromolecules, including lipids, proteins, and DNA in organisms.^[1] Therefore, it is important to provide products in the diet that contain antioxidants that reduce the effects of oxidative stress.

Bird cherry is a popular plant belonging to the genus *Prunus* from the family Rosaceae. It is characterized by edible, dark fruits with a bitter aftertaste that is used as an ingredient in tinctures and syrups.^[2] Bird cherry bark, obtained from young twigs, together with leaf buds is also used as an herbal raw material. It was used in folk medicine as an astringent, diuretic, and anti-rheumatic agent. Black cherry juices and tinctures

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are known for their beneficial health properties. The fruit of the bird cherry is also used to color wines, most often apple ones. Heavily toasted fruit can also become a coffee substitute. In Siberia, dried fruit is made into flour, added to confectionery, cakes, dumplings, and even bread. In Sweden or Kalmykia (a region in Russia), black cherry fruits are eaten boiled in milk. You can also get a bitter oil similar to almond oil from the seeds. It can be used as an ingredient in meat spices. However, information on the application of bird cherry is limited.^[2]

Leaves, fruits, and bark of bird cherry (*P. padus*) contain phenolic compounds such as phenolic acids, e.g. gallic acid, ferulic acid, *p*-coumaric acid, or chlorogenic acid, as well as flavonoids like quercetin, catechin, or rutin. We can also find organic acids such as citric acid, shikimic acid, malic acid, or quinic acid in its composition. Data on antioxidant properties of individual plant parts are available in the literature,^[3–6] but they are not complete.

There is little research available on bird cherry and the compounds contained in its individual parts. No information was found regarding direct determination of their effect on the reduction of guanine oxidative damage caused by the OH[•] radical using an electrochemical DNA biosensor. Electrochemical biosensors show a significant advantage over other methods enabling the determination of 8-oxoguanine level, because they allow direct testing of the DNA sample, without the need for its hydrolysis, which is necessary in other highly sensitive methods. Hydrolysis can cause additional genetic damage in the tested genetic material samples, including oxidative damage, which may be the cause of false-positive test results.^[7]

Available literature also shows antimicrobial and antioxidant activity of selected cultivars and anatomical parts of bird cherry. Therefore, the purpose of the work was to determine the possible beneficial properties of *P. padus* fruits, bark, or leaves. Water extracts of individual parts of bird cherry have been tested for antioxidant and antimicrobial activity as well as for the content of selected phytochemicals. The aim of the study was to assess the possible pro-health effects of bird cherry and its beneficial properties for health, and thus the possibility of its use in diet therapy and functional food.

Material and methods

Material and extract preparation

The research material consisted of leaves, bark, and fruits of *P. padus* that had been selected in previous studies as a raw material with a higher antioxidant potential^[6] of leaves comparing with *P. serotina*.^[6] All anatomical parts of the *P. padus* were in the orchard farm in Ozierany Małe in Podlasie, Poland (53° 13' 14.865" N 23° 51' 9.327" E). The leaves, bark, and fruits were lyophilized (CHRIST 1-4 LSC freeze dryer – Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany). Lyophilization conditions were as follows: the condensation temperature in the freeze dryer was at –28°C, the temperature on the freeze dryer shelf at –20°C, and the product temperature at –4°C. After that research material was grinding (Grindomix GM 200 Retsch, Haan, Germany) for 150 seconds at 1800 x g at 22°C.

The water extraction was performed using distilled water, at 85 °C temperature. The procedure was as follows: 50 g of examined material was mixed with 1000 mL of distilled water was mixed and extracted in a water bath for 15 minutes. The extracts were filtered and centrifuged (800 x g, 15 minutes). The fractions were decanted and filtered (Whatman 1:11 µm). The prepared extracts were stored in dark tubes until examination at 4 °C. The experiment samples were named as follows: extract from *P. padus* leaves as PPL, extract from *P. padus* bark as PPB and extract from *P. padus* fruits as PPF.

Characteristic of color of *P. padus* extract

Color of leaves, bark, and fruit extracts was measured. Color measurement was run in the L* a* b* CEN unit system using a CM-5 spectrometer (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 6504 K. The observation angle of standard colorimetric observer was 10°. Measurements for each sample were repeated fivefold. The instrument calibration was performed with use of black pattern.

Phenolic acids and flavonols

Phenolic compounds in samples were analyzed after alkaline and acidic hydrolysis. Samples were placed in sealed 17 ml culture test tubes, where first alkaline and then acid hydrolysis was run. In order to run alkaline hydrolysis, 1 ml distilled water and 4 ml 2 M aqueous sodium hydroxide was added to test tubes. Tightly sealed test tubes were heated in a water bath at 95°C for 30 min. After cooling (approx. 20 min), test tubes were neutralized with 2 ml 6 M aqueous hydrochloric acid solution (pH = 2). Next, samples were cooled in water with ice. Flavonoids were extracted from the inorganic phase using diethyl ether (2x2ml). Formed ether extracts were continuously transferred to 8 ml vials. Next, acid hydrolysis was run. For this purpose, the aqueous phase was supplemented with 3 ml 6 M aqueous hydrochloric acid solution. Tightly sealed test tubes were heated in a water bath at 95°C for 30 min. After being cooled in water with ice, the samples were extracted with diethyl ether (2 x 2 ml). Produced ether extracts were continuously transferred to 8 ml vials, after which they were evaporated to dryness in a stream of nitrogen. Prior to analysis, samples were dissolved in 1 ml methanol. 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×2.1 mm, particle size 1.7 µm) (Waters, Ireland). Elution was carried out in a gradient using the following mobile phase composition: A: acetonitrile with 0.1% formic acid, B: 1% aqueous formic acid mixture (pH = 2). Concentrations of phenolic compounds were determined using an internal standard at wavelengths $\lambda = 320$ nm and 280 nm and finally given in mg/100 g dm of *P. padus* leaves, bark, and fruits. Compounds were identified based on the comparison of retention time of the analyzed peak with standard retention time and by adding a specific amount of the standard to the analyzed samples and repeating the analysis. Detection level was 1 µg/g.

Low molecular weight organic acids (LMWOAs)

The analysis of LMWOAs was performed using a Waters Acquity H-class UPLC system. Separation was achieved on an Acquity UPLC BEH C₁₈ column (150 mm × 2.1 mm, 1.7 µm, Waters) thermostated at 35°C. The gradient elution was performed with water and acetonitrile (both containing 0.1% formic acid, pH = 2) at a flow rate of 0.4 mL/min. Detection was carried out in a Waters Photodiode Array Detector (Waters Corporation, Milford, MA, USA) at $\lambda = 280$ nm as the preferred wavelength for oxalic, maleic, citric, malic, quinic, and shikimic acids. Compounds were identified by comparing of retention time of the analyzed peaks with the retention time of standards or by adding a specific amount of the standard to the analyzed samples and a repeated analysis. The LMWOAs found were quantified by comparing of the area of their peaks recorded at 280 nm with calibration curves obtained from commercial standards of each compound obtained from Sigma (St. Louis, MO, USA). The results were expressed in milligrams per 100 g of dry weight of sample.

Electrochemical analysis of antioxidant activity

The content of redox compounds in bird cherry leaves, fruits, and bark extracts was determined using square wave voltammetry (SWV) and the procedure described previously Szczepaniak et al.^[8] Voltammetric measurements were performed using potentiostat PGSTAT12 with the GPES 4.9 control software (EcoChemie, The Netherlands). For the measurements was used a three-electrode 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). The CPE was made by itself according to a described procedure Ligaj et al.^[9] 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 carbon paste was renewed before use by removing its outer layer on a 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 conditioned in 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 Savitzky–Golay's filter.^[10] 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. On the basis of the our results for *P. padus* extracts^[8] were also determined 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. In the case of tasted samples, 1 ml of extracts was prepared from 0.063 g of weight, which after dilution in the buffer gave the final dry matter content of plant material in the tested sample of 0.03125 g.

Antioxidative potential analysis by spectrophotometric method

The total phenolic content (TPC) of the obtained extracts from individual anatomical parts of bird cherry was determined using the method described by Kulczyński et al. with minor modifications.^[11] The method consisted of: Aliquots of 100 μ L diluted in 900 μ L 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). Obtained 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 blank. The TPC content was then examined and expressed as mg of gallic acid (Sigma-Aldrich, Germany) equivalents (GAE) per 1 g (mg/1 g) of dry mass using the calibration curves of gallic acid.

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

The ABTS cation radical scavenging activity was measured according to the TEAC (Trolox Equivalent Antioxidant Capacity) test according to the methodology described by Kobus-Cisowska et al.^[12] Spectrophotometric measurement of the ability to scavenge $ABTS^{•+}$ formed from ABTS (2,20-azinobis-(3-ethylbenzothiazoline-6-sulfonic 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.

In the aqueous extracts obtained from the bird cherry, the antioxidant properties were determined using a ferric reducing/antioxidant power assay (FRAP method) according to 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 $FeCl_3 \cdot 6H_2O$ and 0.3 mol acetate buffer] was added to 1 μ L of each sample diluted in 999 μ L distilled H_2O . A calibration curve was constructed using $FeSO_4 \cdot 7H_2O$. Samples were incubated for 30 min and then the absorbance was measured at 593 nm (Metertech SP880, Taiwan). The obtained results were expressed as μ M $FeSO_4$ /1 g dw of extract.

Antimicrobial activity testing using the well-diffusion method

In order 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 μ L 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).

Indicator microorganisms like 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) and Gram-negative bacteria: *Klebsiella pneumoniae* (ATCC 31488), *Salmonella enteritidis* (ATCC 860), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 19606) and as well as fungi of the species *Candida utilis* (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.

Statistical analysis

All assays were conducted in triplicates and results expressed as mean $\pm$ SD. Statistical analysis was performed using Microsoft Excel 2013 software (USA) and Statistica 13 software (StatSoft, Poland). One way ANOVA testing was used to analyze statistical differences. The p value lower than 0.05 was assumed as the level of significance. Additionally, principal component analysis was used (PCA). 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.

Results

The obtained extracts were tested in terms of physicochemical properties and the results are presented in Table 1. The osmolality of the extracts that indicates the freezing point of the extract and its differences compared to the freezing of water, i.e. giving a measure of the osmotic pressure of the tested extract, showed that the highest values were obtained for bird cherry fruit extract (PPF) 0.21 ± 0.01 mOsm/kg H₂O, and the lowest for bark extract (PPB) amounting to 0.14 ± 0.01 mOsm/kg H₂O. The freezing point was the lowest for fruit extracts (PPF) and amounted to $-0.39^b \pm 0.01$ °C. The color of the extracts was also assessed, which, as demonstrated, differed in terms of parameters tested. However, the color of the leaf (PPL) and bark extracts (PPB) was similar. Parameter L*, responsible for determining the brightness of the extract, was $26^a \pm 0.3$ for leaves (PPL), $29^b \pm 1.7$ for bark (PPB) and $28^b \pm 0.3$ for fruits (PPF), respectively. Parameter a*, responsible for the color change in the range from green to red, was $11^b \pm 0.3$ for bark (PPB) and it was the highest value. Parameter b*, responsible for color change in the range from blue to yellow, showed lower values for the leaf extract (PPL) $1.8^a \pm 0.1$, while this parameter for bark and fruit extracts was similar and amounted to $3^b \pm 0.3$ (PPB) and $3^b \pm 0.7$ (PPF).

The highest content of phenolic acids was found in *P. padus* leaves (PPL), which was 1063 ± 26 , while the lowest content was found in the fruits (PPF) and amounted to 502 ± 25 (Table 2). Dominant acids in all parts of the plant studied were as follows: ferulic acid, *p*-coumaric acid, gallic acid, and chlorogenic acid. In leaves (PPL), *p*-coumaric acid was the most abundant (175 ± 9), while syringic acid the least (0.2 ± 0.01). Ferulic acid was dominant in bark (PPB) of *P. padus*, and vanillic acid (353 ± 11) was the least abundant (1 ± 0.01). Fruits (PPF), on the other hand, had the highest amount of *p*-coumaric acid (130 ± 5). The analysis regarding flavonols confirmed that the highest content was

Table 1. Characteristics of the tested leaves, bark, and fruits of *P. padus*, given in CIE L*a*b* units and osmolality.

Sample	PPL	PPB	PPF
Osmolality (mOsm/kg H ₂ O)	$0.16^a \pm 0.01$	$0.14^a \pm 0.01$	$0.21^b \pm 0.01$
Freezing temperature (°C)	$-0.289^a \pm 0.01$	$-0.263^a \pm 0.01$	$-0.390^b \pm 0.01$
L*	$26^a \pm 0.3$	$29^b \pm 2$	$28^b \pm 0.3$
a*	$10^a \pm 0.04$	$11^b \pm 0.3$	$11^{a,b} \pm 0.2$
b*	$2^a \pm 0.1$	$3^b \pm 0.3$	$3^b \pm 0.7$

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

Table 2. Content of phytochemicals compounds in *P. padus* leaves, bark, and fruits.

	PPL (mg/100 g dm of leaves)	PPB (mg/100 g dm of bark)	PPF (mg/100 g dm of fruits)
Phenolic acid			
Gallic acid	109 ^c ± 2.6	16 ^a ± 0.7	40 ^b ± 1
2,5-dihydroxybenzoic acid	1 ^a ± 0.04	106 ^c ± 2	16 ^b ± 0.4
4-dihydroxybenzoic acid	10 ^a ± 0.1	88 ^b ± 3	13 ^a ± 3
Caffeic acid	16 ^b ± 0.1	2 ^a ± 0.2	66 ^c ± 3
Syringic acid	0.2 ^a ± 0.01	46 ^c ± 2	12 ^b ± 0.1
p-coumaric acid	175 ^b ± 9	169 ^b ± 10	130 ^a ± 5
Ferulic acid	177 ^b ± 10	353 ^c ± 11	102 ^a ± 8
Chlorogenic acid	34 ^a ± 3	17 ^a ± 3	109 ^b ± 4
Sinapic acid	4 ^a ± 0.04	78 ^b ± 2	2 ^a ± 0.03
t-cinnamic acid	1 ^a ± 0.00	2 ^a ± 0.01	10 ^b ± 0.03
Vanillic Acid	1 ^a ± 0.03	1 ^a ± 0.01	0.30 ^a ± 0.00
Salicylic acid	4 ^a ± 3	99 ^b ± 2.54	2 ^a ± 0.01
Total phenolic acids	1063 ^b ± 26	978 ^b ± 35	502 ^a ± 25
Flavonoids			
Naringenina	0.10 ^a ± 0.01	17 ^b ± 0.05	1 ^a ± 0.02
Vitexin	1 ^a ± 0.01	7 ^b ± 0.1	1 ^a ± 0.01
Rutin	36 ^c ± 0.2	6 ^a ± 0.1	30 ^b ± 0.1
Quercetin	104 ^a ± 2	599 ^b ± 18	122 ^a ± 9
Apigenin	0.30 ^a ± 0.01	10 ^b ± 0.1	17 ^c ± 0.1
Kaempferol	2 ^a ± 0.01	2 ^a ± 0.01	6 ^b ± 0.02
Luteolin	0.10 ^a ± 0.02	11 ^b ± 0.03	ND ± 0.00
Catechin	110 ^a ± 9	879 ^c ± 43	566 ^b ± 24
Total flavonoids	252 ^a ± 11	1530 ^c ± 61	743 ^b ± 33
LMWOAs			
Oxalic acid	4 ^b ± 0.01	1 ^a ± 0.01	10 ^c ± 0.04
Maleic acid	1 ^a ± 0.01	7 ^b ± 0.04	16 ^c ± 0.04
Citric acid	106 ^a ± 6	115 ^a ± 3	517 ^b ± 7
Malic acid	89 ^a ± 4	356 ^b ± 11	690 ^c ± 4
Quinic acid	16 ^a ± 1	84 ^b ± 4	356 ^c ± 13
Shikimic acid	259 ^c ± 10	180 ^b ± 12	70 ^a ± 3
Total organic acids	474 ^a ± 21	742 ^b ± 30	1659 ^c ± 27

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

found in the bark (PPB) of bird cherry (1530 ± 61), and the lowest was in the leaves (PPL) (252 ± 11). Of the flavonols tested, catechin was the dominant in all anatomical parts of the plant. The content of low molecular weight organic acids, whose highest value was found in the fruits (PPF) (1658 ± 27) was also assessed. In contrast, the leaves (PPL) had the highest quantity of shikimic acid (259 ± 10) and citric acid (107 ± 6), and the lowest amount of maleic acid (1 ± 0.01), which in turn was found in a statistically significantly higher amount in the bark (PPB) (7 ± 0.5), which also contained the highest amount of malic acid (356 ± 11) and the least of oxalic acid (1 ± 0.01). Fruits (PPF), on the other hand, had a high content of malic acid and citric acid.

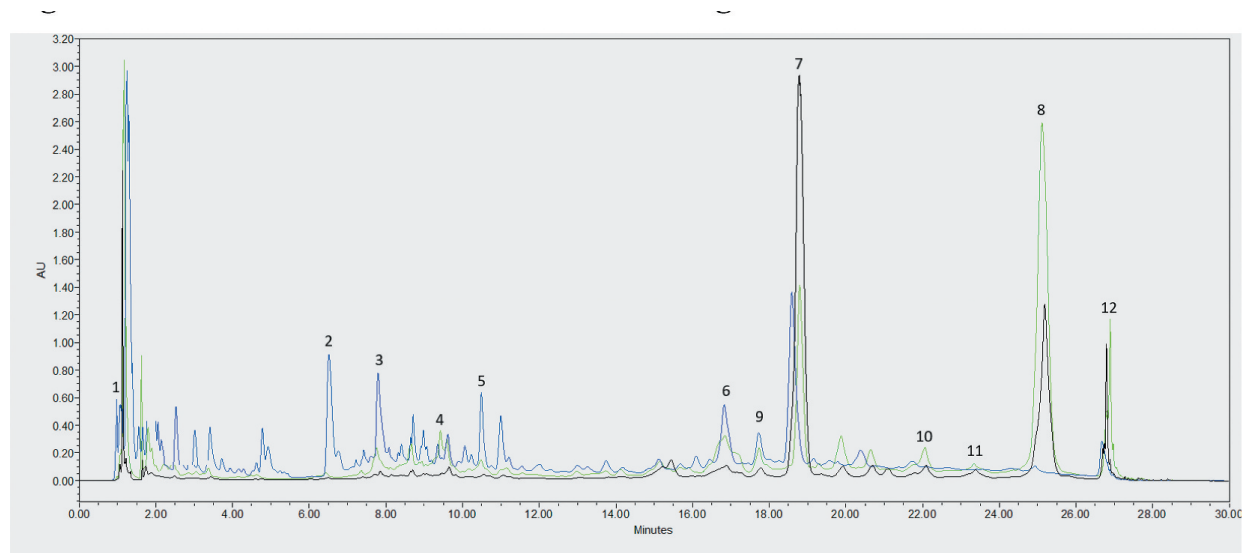
Antioxidant potential of the tested parts of bird cherry was evaluated by spectroscopic methods (Table 3). Differences between the properties of anatomical parts of *P. padus* were shown. All extracts contained compounds reacting with the Folin-Ciocalteu reagent; however, their highest amount was found for the bark (PPB), where their content amounted to $136^c \pm 9$ mg GAE/g dw. The FRAP test also showed the highest activity for the bark extract (PPB) ($379^b \pm 18$ μ M FeSO₄/1 g dw) compared to the other two samples. The results of the study have been extended by the DPPH radical test. In this case, the highest value was obtained again for the bark (PPB) ($9^c \pm 0.03$ mg TE/1 g dw). Only in the ABTS radical test, the bark activity (PPB) was the lowest (7^a and ± 0.02 mg TE/g dw), and the highest was confirmed for the leaves (PPL) ($10^c \pm 0.1$ mg TE/g dw).

The content of redox compounds in *P. padus* extracts was evaluated using square wave voltammetry (SWV). This very sensitive technique makes it possible to determine analytes at a very low concentration below 10^{-8} M.^[14] The voltamperograms in Figure 1 indicated the presence of one primary signal located in

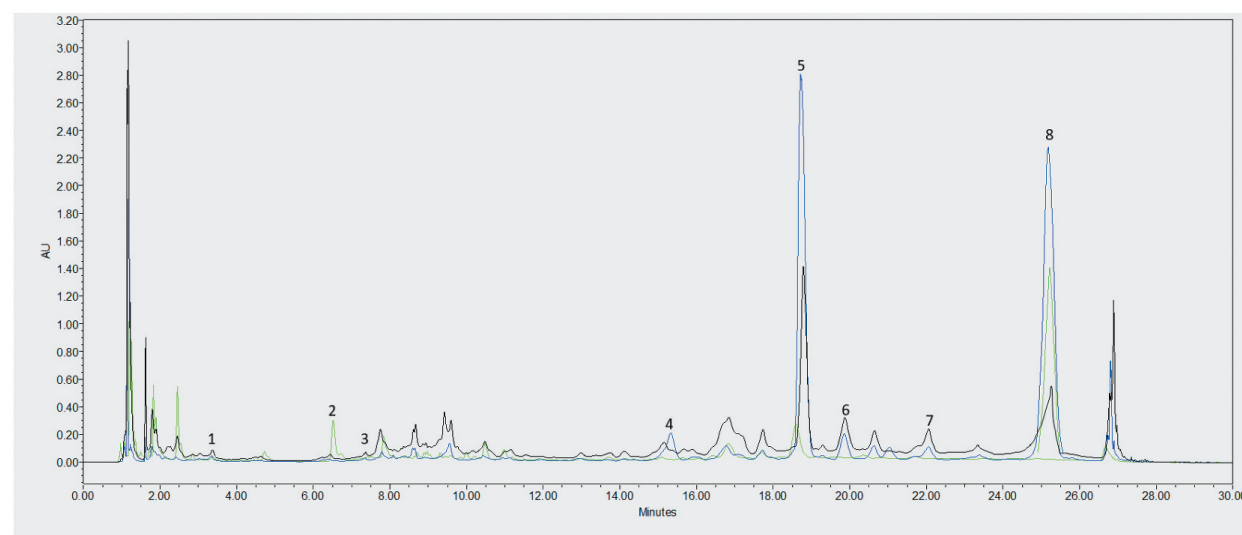
Table 3. Antioxidant activity of *P. padus* leaves, bark, and fruit extracts with FRAP, DPPH, ABTS radicals, and total polyphenol content.

Sample/activity	PPL	PPB	PPF
Folin-Ciocalteu (mg GAE/g dw)	37 ^a ± 4	136 ^c ± 9	43 ^b ± 3
DPPH(mg TE/g dw)	7 ^b ± 0.06	9 ^c ± 0.03	6 ^a ± 0.05
ABTS(mg TE/g dw)	10 ^c ± 0.1	7 ^a ± 0.02	9 ^b ± 0.04
FRAP (uM FeSO ₄ /g dw)	233 ^a ± 6	379 ^b ± 18	231 ^a ± 5

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



a)



b)

Figure 1. Phenolic acids and flavonoids chromatograms. Phenolic acid chromatograms: black line – PPL, blue line PPB and green line PPF. 1 – gallic acid, 2 – *p*-coumaric acid, 3 – 2,5-dihydroxybenzoic acid, 4 – 4-hydroxybenzoic acid, 5 – chlorogenic acid, 6 – caffeic acid, 7 – syringic acid, 8 – vanillic acid, 9 – sinapic acid, 10 – ferulic acid, 11 – salicylic acid, 12 – *t*-cinnamic acid. chromatogram for flavonoids: black line – PPL, blue line PPB and green line PPF. 1 – Vitexin, 2 – apigenin, 3 – kaempferol, 4 – luteolin, 5 – quercetin, 6 – naringenin, 7 – rutin, 8 – catechin

the +0.3 V region. It showed the greatest intensity for the leaf extract (PPL). The signal height was 26.065 μA and its area was 4.210 $\text{V} \times \mu\text{A}$. The additional signal located in the +0.95 V region had a definitely lower current. Slightly lower SWV signals were detected in the fruit extract (PPF), in this case, an additional signal was visible at +0.8 V. Bark extract (PPB) had clearly the lowest electroactivity. In addition to the basic signal, it also contained a blurred signal located in the region from +0.4 to +0.9 V with a maximum at +0.73 V, which indicated the presence of a mixture of various redox compounds in the tested extract. The detailed characteristics of electrochemical parameters determined for the tested extracts are presented in Table 4. In addition, on the basis of our previous research,^[8] the electrochemical index, expressing the general electroactivity of the tested extracts in relation to the dry matter of plant material was calculated.

All obtained extracts were also tested for the activity against fungi and bacteria from Gram-positive and Gram-negative groups (Table 5). The highest activity against Gram-negative bacteria was recorded in bird cherry leaves (PPL) and the lowest in the bark (PPB). The leaves (PPL) were the most active against *Klebsiella pneumoniae* (19 ± 30 mm) and the fruits (PPF) were the most active against *Pseudomonas aeruginosa* and showed value 19 ± 3 mm in this case. The lowest growth inhibition of Gram-negative bacteria was observed for the bark (PPB) against *Salmonella enteritidis* (1 ± 0 mm).

Table 4. Electrochemical parameters determined for extracts from *P. padus*.

Signal current – Square Wave Voltammetry (SWV)			
Sample	PPL	PPB	PPF
Peak potential E_p [V]	0.286	0.260	0.318
	0.950	0.732	0.799
			0.967
Peak current I [μA]	26.065	4.587	24.222
	1.419	0.209	1.395
			0.165
Peak area $E_p \times I$ [$\text{V} \times \mu\text{A}$]	4.210	0.747	3.287
	0.161	0.075	0.222
			0.023
Total peak current I [μA]	30.955	6.786	27.267
Total peak area [$\text{V} \times \mu\text{A}$]	4.371	1.510	3.735
EI [$\text{V} \times \mu\text{A}/1$ g dw]	139.857	48.312	119.531

Table 5. Microbiological activity of *P. padus* leaves, bark, and fruit extracts.

Indicator microorganisms	PPL	PPB	PPF
	Growth inhibition zone (mm)		
Gram-negative bacteria			
<i>Klebsiella pneumoniae</i> (ATCC 31488)	19 ± 3	2 ± 0	11 ± 2
<i>Salmonella enteritidis</i> (ATCC 13076)	14 ± 2	1 ± 0	13 ± 2
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	17 ± 3	7 ± 1	19 ± 3
<i>Acinetobacter baumannii</i> (ATCC 19606)	15 ± 3	2 ± 0	13 ± 2
Gram-positive bacteria			
<i>Enterococcus faecium</i> (ATCC 27270)	22 ± 3	2 ± 0	9 ± 1
<i>Staphylococcus aureus</i> (ATCC 25923)	15 ± 3	3 ± 1	11 ± 2
<i>Lactobacillus fermentum</i> (ATCC 14932)	17 ± 3	3 ± 1	13 ± 3
<i>Clostridium butyricum</i> (ATCC 860)	16 ± 3	3 ± 1	10 ± 1
<i>Listeria monocytogenes</i> (ATCC 19115)	24 ± 4	0 ± 0	7 ± 1
<i>Bacillus coagulans</i> (GBI-30, 6086)	19 ± 3	2 ± 0	10 ± 2
Fungi			
<i>Candida utilis</i> (ATCC 9950)	7 ± 1	0 ± 0	13 ± 2
<i>Aspergillus</i> sp.	3 ± 1	0 ± 0	12 ± 1
<i>Fusarium</i> sp.	4 ± 1	0 ± 0	10 ± 0

Similar to Gram-positive bacteria, the highest inhibition among Gram-positive bacteria was demonstrated for the leaves (PPL) and the weakest for the bark (PPB). Leaves (PPL) inhibited most effectively the growth of *Listeria monocytogenes* (24 ± 40 mm) and *Enterococcus faecium* (22 ± 30 mm). With respect to fungi, *Candida utilis* proved to be the most sensitive, for which the growth inhibition zone was 13 ± 2 when the fruit extract (PPF) was applied. The same part of *P. padus* (PPF) plant showed high activity against the remaining *Aspergillus* sp. (12 ± 1 mm) and *Fusarium* sp. (10 ± 0 mm) fungi. On the other hand, bird cherry bark (PPB) did not show any activity toward the tested microorganisms.

Principal Component Analysis (PCA) was used to observe possible relationships between the content of phytochemicals in bird cherry (*P. padus*) and antioxidant activity in its individual anatomical parts (Figures 2 and 3). The PCA projection (biplot) of the results of qualitative analysis in the system of the first two components (PC1 and PC2), responsible for over 60% composition variation, illustrated the diversity of samples in terms of the content of active components and the degree of influence of individual compounds on antioxidant activity. The close location of antioxidant activity vector, as measured by DPPH, FRAP, ferulic acid, naringenin, salicylic acid, sinapic acid, vitexin, or luteolin tests indicated a positive correlation of these indicators. In turn, the opposite position of the ABTS vectors to them indicated a negative correlation. Similarly, the opposite position of the SWV vector relative to apigenin, malic acid, catechin showed a negative correlation. Location of individual samples in different parts of the graph indicated the variability of anatomical parts of *P. padus* in terms of content of the analyzed compounds and antioxidant activity.

Discussion

Bird cherry is a not-so-popular plant in the context of its use in food technology or diet therapy, often referred to as forest weed. Available research results demonstrate that fruits such as bird cherry are a rich source of biologically active substances. In this work, the antioxidant potential of *P. padus* that results from the presence of polyphenolic compounds in individual parts of the plant has been demonstrated. Phenolic acids, whose highest amounts were found in *P. padus* leaves were, among others, responsible for this effect. Ferulic acid was dominant in all anatomical parts of the plant. This compound was also detected by other researchers^[4] in *P. padus* fruits (10.45 ± 3.65 mg/100 g FW); however, in our research also in fruits, it showed a much higher value (102 ± 8 mg/100 g dm). These authors pointed out that other compounds from this group, including chlorogenic acid, were also responsible for the antioxidant properties. Olszewska & Kwapisz also indicated a significant role of chlorogenic acid in the flowers of bird cherry, their result was 1.39–1.94% DW.^[3] In our work, the highest content of chlorogenic acid was in fruits and it was 109 ± 4 mg/100 g dm. Studies have shown that bird cherry contains a high quantity of flavonoids in its composition. The two dominant compounds in this group were quercetin and catechin, the highest values of which were found in the bark, where quercetin (599 ± 18 mg/100 g dm) and catechin (879 ± 43 mg/100 g dm). Other researchers have also marked these compounds in various anatomical parts of the bird cherry.^[3,4] Quercetin has also been found in flowers (1.37–1.56% DW).

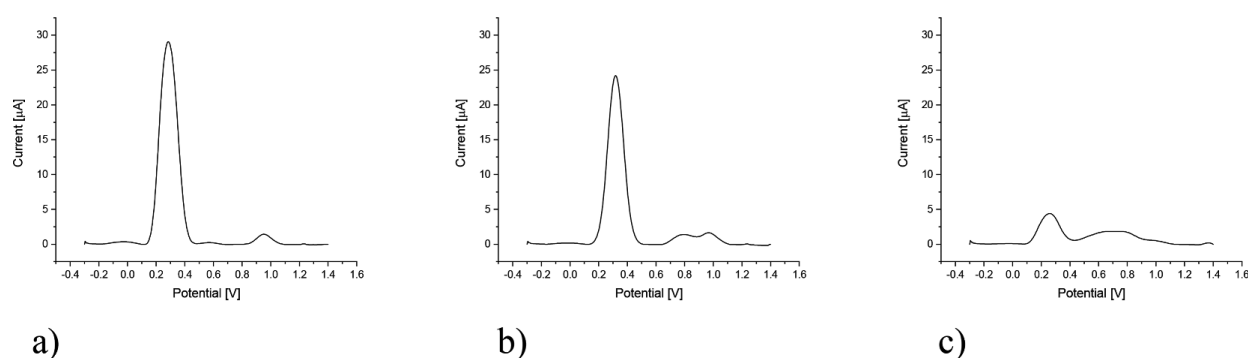
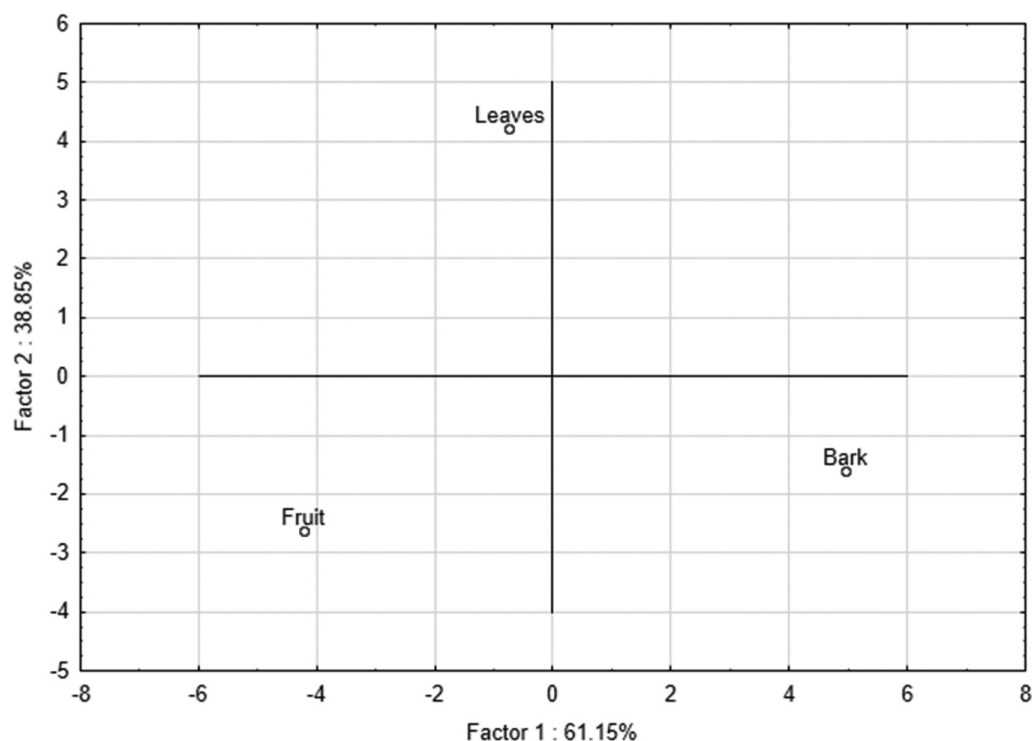
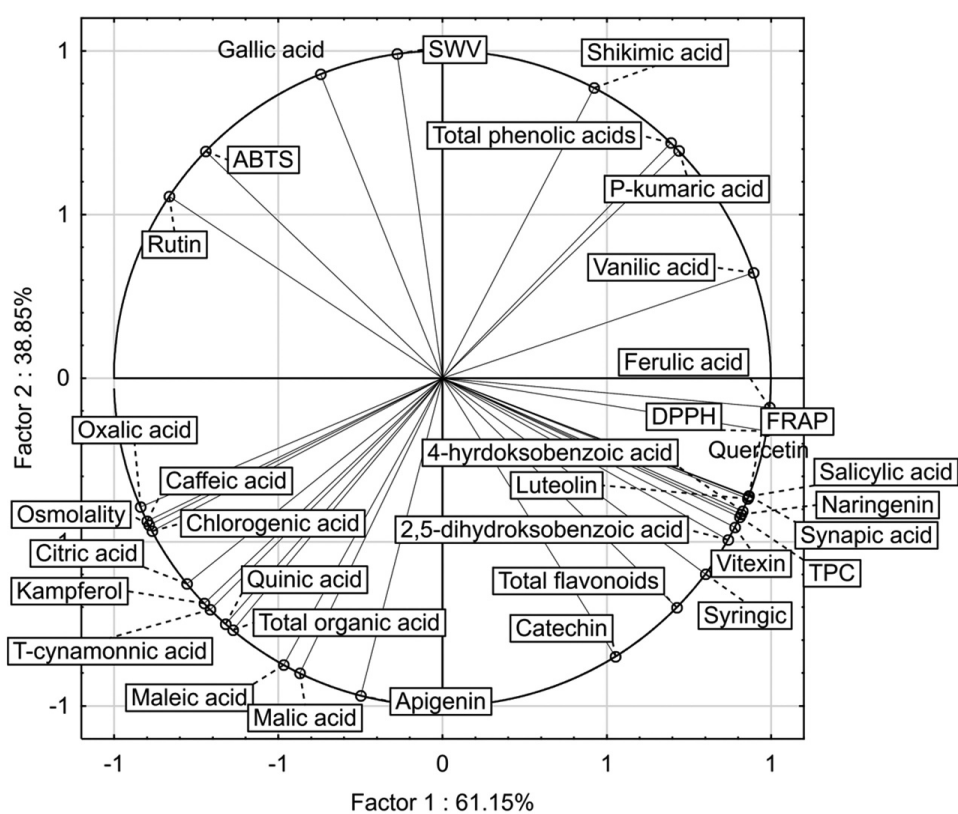


Figure 2. SWV signals of *P. padus* extracts from leaves (a), fruits (b), and bark (c).



a)



b)

Figure 3. PC analysis for quality indexes in the profile of the analyzed components in *P. padus* graph (load (a) and scatter plot (b)) related to the content of phenolic acids, flavonols, and LMWOAs as well as antioxidant activity measured by spectroscopic and electrochemical methods.

Plant products, primarily fruits, contain hydroxy acids, which are present in free form, unlike in vegetables. These acids protect organisms against microbes and unripe fruits contain their highest percentage. The study expanded research assessing the content of organic acids for individual parts of *P. padus*, and fruit extract contained their highest amount, which was consistent with the results of other Donno et al.^[4] and Mikulic-Petkovsek et al.^[15]

In addition to many reports on the antioxidant properties of polyphenols, much is also said about their bactericidal and bacteriostatic effects. Higher plants contain secondary metabolites active against bacteria and fungi. The study showed that anatomical parts of *P. padus* plant could have such properties where action against fungi and Gram-positive and Gram-negative bacteria was demonstrated (the highest antifungal effect was found toward *Candida utilis*). Gram-negative bacteria can cause many types of infections such as pneumonia, peritonitis, urinary tract infection, blood infection, wound infection, and meningitis.

The basic difference between Gram-positive and Gram-negative bacteria is that the former have a multilayered cell wall made of murein, and Gram-negative bacteria only have one layer. They are commonly treated with antibiotics, but they are not always effective. *P. padus* was also active against Gram-negative bacteria, such as *Enterococcus faecium*, for which the leaves showed the highest activity (22 ± 3 mm). *Enterococcus faecium* is responsible, among others, for urinary tract infections. The leaves were also active against *Staphylococcus aureus* causing, among others, skin infections, food poisoning, and arthritis. The effects of bird cherry on these two bacteria were also confirmed by Kumarasamy et al.,^[16] who studied the seed of *P. padus*. Dichloromethane extracts of seeds also showed activity against *Enterococcus faecium* (1.0 mg/ml) and against *Staphylococcus aureus* (1.0×10^{-4} mg/ml) in methanol extracts.

Antioxidants in the body protect lipid membranes by donating electrons to free radicals, thereby stopping the cascade of the reaction chain.^[1] They are the most active group in the process of detoxification and protection of genes, which due to damage by radicals, can cause many types of cancers. Many studies are available in the literature that indicate the high antioxidant potential of plant materials, and this applies to fruits as well as leaves, bark, and flowers. The current work demonstrated that bird cherry contained compounds with anti-radical activity. A positive correlation between antioxidant activity and flavonoid content was shown. The results in the work have confirmed that bird cherry can be a source of antioxidants in a multidirectional diet.

Conclusion

The total antioxidant effect is difficult to predict, because the total antioxidant capacity is determined not only by the properties of individual compounds but above all by their mutual interaction. Therefore, it has been shown in the study that each of the anatomical parts of *P. padus* is rich in compounds from the group of polyphenols, which may also determine their diverse health-promoting properties. Based on the results, it has been found that it is often difficult to compare the antioxidant capacity using different methods. Studies have confirmed the variety of antioxidant mechanisms present in *P. padus*. Bird cherry bark was characterized by high antioxidant potential measured by Folin-Ciocalteu, ABTS and DPPH tests, resulting from the properties of active compounds present in them, and above all a rich content of phenolic acids and flavonoids. However, electrochemical methods demonstrated higher activity for leaves. In the antimicrobial test, the fruits were the most active against mold and fungi. Thus, it has been shown that *P. padus* bark, leaves, and fruits can be an inexpensive and pro-ecological source of antioxidants with multidirectional action.

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Conflict of interest

The authors declare that they have no conflict of interest.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Stuper-Szablewska, K., Ligaj, M., Tichoniuk, M., Szymanowska, D., Szulc, P. Exploring antimicrobial and antioxidant properties of phytocomponents from different anatomical parts of *Prunus padus* L, 2020, *International Journal of Food Properties*, 23 (1), 2097-2109, mój indywidualny udział w jej powstaniu polegał na zebraniu materiałów źródłowych, opracowaniu modelu doświadczenia, wykonaniu analiz chemicznych, przygotowaniu zestawień tabelarycznych/wykresów, interpretacji i opisie wyników, przygotowaniu treści manuskryptu, zredagowaniu manuskryptu zgodnie z wymogami czasopisma, przygotowaniu zestawienia literatury cytowanej w manuskrypcie, przesłaniu manuskryptu do wydawnictwa, prowadzeniu korespondencji z wydawnictwem oraz na naniesieniu zmian i uzupełnieniu manuskryptu zgodnie z uwagami recenzentów.

Podpis

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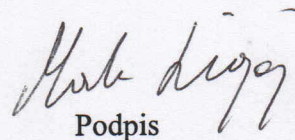
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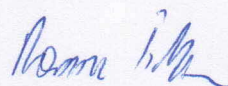
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Podpis



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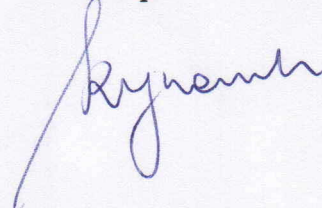
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Podpis



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Podpis

Piotr Szulc

Article

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

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Abstract: *Prunus padus* L., is not very popular plant, it is commonly found due to low soil requirements and easy to settle in various places. As for now, concerning food technology, there is no wide application for *P. padus*. Therefore, the aim of this study is to evaluate the possibility of using bird cherry bark as an ingredient in herbal functional teas. In the first step, the conditions for extraction of the bark were electrochemically optimized. It was proven that the highest content of polyphenols could be found in the sample consisting of chamomile, linden flower, and calendula (7939.8 ± 106.6 mg/100 g dm). In the beta-carotene bleaching test, the highest activity could be spotted for calendula tea ($16.7 \pm 1.1^{\text{c}}$ %) and chamomile tea ($15.0 \pm 2.0^{\text{c}}$ %) and concerning the test for linden flower tea without added bark ($134.4 \pm 15.1^{\text{b}}$ µg ascorbic acid /mL). The property of the tested teas to inhibit cholinesterases was proven. What is more, *P. padus* bark infusion showed the highest activity of $15.8 \pm 1.1^{\text{d}}$ µg neostigmine/mL, for acetylcholinesterases (AChE) inhibition and $21.2 \pm 1.0^{\text{c}}$ µg neostigmine/mL for butyrylcholinesterases (BChE). The same tea also showed the highest activity to reduce ions of iron (Fe(III)): $25.3 \pm 0.9^{\text{c}}$ µg Trolox /mL and glutathione reductase and glutathione peroxidase inhibition, $87.0 \pm 1.1^{\text{e}}$ % and $64.9 \pm 2.0^{\text{d}}$ % respectively. The use of *P. padus* bark may be vital in the preventive care concerning modern-age diseases and allow for the production of a new range of products with distinctive sensory characteristics and functional properties and, at the same time, in combating the spread of *P. padus* in the farm and forest ecosystem.

Keywords: *Prunus padus*; bird cherry; antioxidants; SWV; cholinesterase; COX-2; SOD; catalase; glutathione reductase; inhibition; bioactive food

1. Introduction

1.1. Soil Conditions and Physiology of Bird Cherry as Invasive Plant

Prunus padus L. (bird cherry) belongs to the Rosaceae family, *Padus* subgenus of *Prunus* genus L. Taking into consideration all plants from *Prunus* species, bird cherry grows farthest to the north in Europe, spreading to the coasts of the Arctic Ocean. What is

more, in the Alps it grows at a higher altitude than any other deciduous tree [1]. This species is highly invasive, tolerant of low and high temperatures, as well as tolerant of many soil types. It owes its ecological invasion to the high efficiency of its vegetative and generative propagation, thanks to characteristics such as the high capacity and speed of seed germination, its overproduction, its longevity, its spread, the rapid growth and development of individuals, its low requirements in relation to habitat factors, and its high tolerance to climatic conditions. In many European countries, the bird cherry is called a “forest weed” due to its dense undergrowth, which prevents the regeneration and growth of many tree species. Dense bird cherry bushes cause displacement of native species and completely prevent their natural regeneration, leading to the complete degradation of forest phytocenoses. Therefore, the negative impact of *P. padus* on the natural environment involves the displacement of native species. The intensity of this displacement depends on the original condition of the existing forest community. Despite determining many methods of removal of bird cherry, the most effective one has not yet been discovered.

Therefore, the expansion of this species is a threat to biodiversity conservation, and the development of effective strategies to combat invasive species is one of the most challenging tasks facing nature conservation, both concerning the preservation of natural vegetation and native plants. The literature on the topic contains statements that show that depriving bird cherry of their bark can limit and weaken their growth [2].

1.2. Nutritional and Functional Potential Related to the Phytochemicals Present in *Prunus padus* L.

Bird cherry contains numerous compounds that might be healthy. Its bark has a pleasant, distinctive pungent scent, similar to that of black currant. As a herbaceous plant, it is harvested in summer. It contains, among others: tocopherols, vitamins, tannins, terpenes, and polyphenols [3].

P. padus bark is known for its anti-inflammatory, antimicrobial, and antioxidant properties [4]. Bird cherry bark has the property of iron ions chelation and reduction [5]. It is used to brew infusions with medical properties [6]. The compounds present in bird cherry may prove vital for herbal medicine or in the production of extracts, as they have health-promoting properties, e.g., lowering the risk of cancer, cardiovascular disease, and diabetes. In addition, they reduce inflammation [7]. This is due to the polyphenols which improve the oxidant/antioxidant balance in the organism. Polyphenols can support the effects of health-promoting substances [8].

Modern medicine uses synthetic acetylcholinesterases (AChE) and butyrylcholinesterases (BChE) inhibitors for the symptomatic treatment of dementia, but these drugs often cause side effects that could be minimized by supporting therapy using phytochemicals [9]. The literature confirms the possible effect of polyphenols as preventive and supportive agents for the treatment of neurodegenerative diseases. Polyphenols may also be useful in inhibiting other enzymes that are crucial in eliminating the effects of oxidative stress. This is due to the multidirectional action of polyphenols as antioxidants [10].

In recent years, the nutritional use of *P. padus* is increasing. Due to the presence of active compounds, its health-promoting effects may be expected in terms of chelating and reducing metal ions, and it may also be a source of substances helpful in inhibiting cholinesterase activity and inhibiting glutathione reductase and glutathione peroxidase. The aim of this study was to evaluate the possibility of using it as an ingredient in functional teas with a broad spectrum of health-promoting properties, while also inhibiting the spread of the species.

2. Materials and Methods

2.1. Preparation of Tea Blends and Infusions with *P. padus* Bark

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) and was prepared as described above [5]. The ingredients for the preparation of infusions (*Prunus padus* L. bark), linden

flower (*Tilia europaea* L.), chamomile flower (*Matricaria Chamomilla* L.) and calendula (*Calendula officinalis* L.) were ground in a Retsch (Haan, Germany) Grindomix GM 200 for 15 s at 500 rpm at 21 °C to obtain a particle size of 0.5–0.9 mm. The teas were prepared by mixing the dry ingredients and weighing 2-gram portions, which were packed in disposable tissue paper teabags. Eleven samples were prepared, four of which contained only one component: P (100% *P. padus*), Ch (100% *Matricaria Chamomilla* L.), C (100% *Calendula officinalis* L.) and T (100% *Tilia europaea* L.), the first sample was a base labeled B (33.33% Ch + 33.33% C + 33.33% T), and six samples contained *P. padus* (P) bark at different concentrations: BP5 (5% P + 95%B), BP10 (10% P + 90%B), BP15 (15% P + 85%B), BP20 (20% P + 80%B), BP25 (25% P + 75%B), BP30 (30% P + 70%B). The infusion was obtained by extracting the bag, containing dry product, with 200 mL of water at 80 °C in 15 min.

2.2. An Electrochemical Method for Optimizing Bird Cherry Bark Extraction Conditions

The content of redox compounds in bark extracts was determined using square wave voltammetry (SWV) and the procedure described previously [11]. Measurements were performed using a potentiostat PGSTAT12 with the GPES 4.9 software (EcoChemie, The Netherlands) and a three-electrode system consisting of a reference (Ag/AgCl, 3 M KCl) (Mineral, Poland), auxiliary (platinum) (Mineral, Poland), and working (carbon paste electrode: CPE). The CPE was made by itself according to a described procedure [12]. 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 reestablished before use by removing its outer layer on a filter paper, application of fresh paste, and polishing it to a smooth finish on a frosted glass slide. Before carrying out measurement, the surface of CPE was conditioned in 0.05 M phosphate buffer with 0.01 M KCl (pH 7.0) at +1.7 V for 60 s. Next, the electrodes were immersed for 120 s in the extract dissolved in phosphate buffer (1:1, *v/v*) and the three measurements of SWV in the range from −0.3 V to +1.4 V were taken. SWV parameters were: step potential of 5 mV, frequency of 50 Hz, and amplitude of 40 mV. Baselines with moving average were subtracted from SWV voltammograms, and the peak potential, peak height (current), peak area for each signal, and the total peak areas were determined. Additionally, the electrochemical index (EI) was calculated, describing the electrochemical activity of extracts, expressed as the total area of all redox signals, in relation to 1 g dry matter of plant material [11]. One ml of the tested extract contained 0.03125 g dry plant material.

2.3. Phenolic Acids, Flavonols, and Low Molecular Weight Organic Acid (LMWOA)

Phenolic compounds in the samples were analyzed after alkaline and acid hydrolysis. The analysis was performed using a Waters Acquity Class H UPLC system equipped with a Waters Acquity Photodiode Array (PDA) detector (Waters, USA). Chromatographic separation was performed on an Acquity UPLC®BEH C18 column (100 mm × 2.1 mm, particle size 1.7 µm) (Waters, Ireland). Gradient elution was conducted using the following mobile phase composition: A: acetonitrile with 0.1% formic acid, B: 1% aqueous formic acid mixture (pH = 2). The concentrations of phenolic compounds were determined using an internal standard at wavelengths $\lambda = 320$ nm and 280 nm and expressed in mg/100 g dry weight. The detection level was 1 µg/g.

Low molecular weight organic acid (LMWOA) analysis was performed using a Waters Acquity Class H UPLC system. The separation was performed on an Acquity UPLC BEH C18 column (150 mm × 2.1 mm, 1.7 µm, Waters) thermostatically heated to 35 °C. Gradient elution was performed with water and acetonitrile (both containing 0.1% formic acid, pH = 2) at a flow rate of 0.4 mL/min. Detection was performed using a Waters Photodiode Array (PDA) Detector (Waters Corporation, Milford, MA, USA) at $\lambda = 280$ nm. Results were expressed as milligrams per 100 g dry weight of tea.

2.4. Linoleic Acid Oxidation, Beta-Carotene Bleaching Test and FRAP Method

Linoleic acid oxidation. The study used the method published by Kozarski et al. (2012) with some modifications [13]. Linoleic acid (800 mg) was dissolved in 20 mL of absolute MeOH and 200 mL of 0.2 M sodium phosphate buffer (pH 6.5) and Tween 20 (6.5 mM conc. of Tween 20 was obtained). The emulsion was sonicated for 10 min. 1.8 mL linoleic acid emulsion was mixed with 0.2 mL of the sample and incubated at 37 °C. Samples (0.1 mL) were taken out after 6 h of incubation and mixed with 1.2 mL of absolute MeOH. The absorbance against the blank sample was measured at 234 nm, without the studied solution. Ten ascorbic acid solutions (concentration in the range 124.5–1824.3 µg ascorbic acid/mL) were used for the preparation of the calibration curve in the same way as the tested samples. In addition, the content was measured at 234 nm (a mixture containing the studied sample and buffer only [13]). Each sample was analyzed at least four times.

Beta-carotene bleaching test. The study used the method published by Öztürk et al. (2011) with some modifications [14]. Seven mg of β-carotene in 5 mL of chloroform was mixed with 350 µL of linoleic acid and 2.8 g of Tween 80. Vacuum evaporation of chloroform was performed (40 °C) and 100 mL of DDI (doubly distilled water) saturated with oxygen was added followed by vigorous shaking. The sample (200 µL) was mixed with β-carotene/linoleic acid emulsion (200 µL). Zero time absorbance and the change in absorbance were measured at 463 nm after 4 h at 50 °C. A positive sample was prepared using ascorbic acid. A total of 0.94 mg/mL of stock solution, used instead of samples, was prepared followed by a serial dilution (9.4–94 µg/mL), as described above. In addition, the content of samples was measured (a mixture containing the studied sample and DDI water only). The percentage of activity of the samples was calculated according to the blank samples containing emulsion and DDI water only [14]. Each sample was analyzed at least four times.

The FRAP method of Hanafy et al. (2017) with slight modifications was used [15]. The amount of 20 µL of the tested solution was mixed with 1900 µL of FRAP (ferric ion reducing antioxidant power) solution, shaken for 30 min at room temperature and the absorbance was read at 593 nm, after shaking for 30 s. The FRAP solution was prepared by mixing 2.5 mL 5 mM TPTZ solution (prepared in 40 mM HCl solution), 2.5 mL 5 mM FeCl₃ solution, and 25 mL acetate buffer (0.3M pH 3.6), and warming in water at 37 °C for 20 min. A blank (reagent) sample was prepared with buffer instead of the sample and the content of the sample was measured (a mixture containing the studied sample and buffer only). All samples were measured at 593 nm. The calibration curve was prepared using Trolox solution: 0.51 mg of Trolox was dissolved in 1 mL of DDI water. An amount of 20 µL of 20 standard solutions (containing 0.0243–0.524 mg Trolox/mL) was studied, as described above [15]. Each sample was analyzed at least four times.

2.5. Cholinesterase (AChE and BChE), Catalase and SOD Inhibition

Inhibition of AChE and BChE tests were performed as described in a work by Baranowska-Wójcik et al. (2020) [16]. Neostigmine standard solutions (neostigmine bromide, Sigma Aldrich N2001, 2.57–22.85 µg/mL in ethanol: DDI (doubly distilled water) 1:1) were prepared and analyzed using the same method as tested samples. The calibration curve was developed and the inhibition of enzymes was expressed in the equivalent concentration of neostigmine (µg/mL). Each sample was analyzed at least four times.

Inhibition of catalase test. The method of Watanabe et al. (2007), with slight modifications, was used [17]. The reaction mixture was composed of 50 samples, 945 µL EDTA (ethylenediaminetetraacetic acid) solution (6.2 mg EDTA in 1 M Tri-HCl, pH 7.0, previously filtered through 0.45 µm membrane), and 12 µL of 30% H₂O₂ solution (final conc. 0.035 M/L). The reaction was started by adding 2.5 µL catalase solution (Sigma C3515, previously diluted 100-fold using 1M Tris-HCl, pH 7.0). Absorbances were measured at 240 nm at the start and after 2 min. In controls, the tested sample was replaced by Tris buffer. The calculations were performed as follows: Inhibition [%] = 100 – 100 × (Abs. of

the sample 0 min – Abs. of the sample 2 min)/(Abs. of the control 0 min – Abs. of the control 2 min) [17]. Each sample was analyzed at least four times.

This study adopted methods for inhibition of SOD from Parschat et al. (2001) implementing their own modifications [18]. The test mixture was composed of 50 µL of the sample, 10 µL SOD (0.24 U), 160 µL of nitro blue tetrazolium (NBT, 0.0025 M), 140 µL phosphate buffer (0.2 M, pH 7.5), 30 µL xanthine (150 mM in 1 M NaOH), and 10 µL xanthine oxidase (0.065 U, Sigma Aldrich X4875). The change in the absorbance in the control sample (without test sample) at 550 nm was 0.02/min. The change in the absorbance was measured after 0 min and 20 min. The calculations were performed as follows: Inhibition [%] = $100 - 100 \times (\text{Abs. of the sample 0 min.} - \text{Abs. of the sample 2 min}) / (\text{Abs. of the control 0 min} - \text{Abs. of the control 2 min})$ [18]. Each sample was analyzed at least four times.

2.6. Inhibition of Glutathione Reductase, Inhibition of Glutathione Peroxidase

Inhibition of glutathione reductase. The method of Moreira et al. (2014) was used [19]. The studied sample (150 µL) was mixed with 660 µL of 0.1 mM sodium phosphate buffer. Then, 25 µL of EDTA solution and 30 µL of GSSG (glutathione disulfide) solution were added, samples were incubated for 5 mins at 25 °C, and 40 µL of NADPH (nicotinamide adenine dinucleotide phosphate) solution was added (all reagents were dissolved in 0.1 mM sodium phosphate buffer, pH 7.6) (concentrations of reagents in the final mixture (805 µL) were as follows: 0.5 mM EDTA, 10 mM GSSG, and 10 mM NADPH). The initial absorbance (340 nm) was recorded, and the reaction was initiated by adding 2 U enzyme (2 µL, Sigma Aldrich G3664). The absorbance (340 nm) was recorded after 2.5 min of incubation at 25 °C. A blank sample was prepared with buffer instead of the sample and the content of the sample was measured (a mixture containing the studied sample and buffer only). One unit of enzyme activity was defined as nmol of NADPH consumed/min – mL of the sample, in comparison with nmol of NADPH consumed/min in the blank (reagent) sample [19]. Each sample was analyzed at least four times.

Inhibition of glutathione peroxidase. In the study, the method of Singh et al. (2000) was used with some modifications [20]. The sample (150 µL) was a mix of 20 µL EDTA solution, 3 µL of glutathione reductase (0.2 U Sigma G3664), 10 µL of GSH solution, 3 µL of glutathione peroxidase (0.04 U Sigma G6137), 55 µL of H₂O₂, and 663 µL of 50 mM sodium phosphate, pH 7.0). To start the reaction, 10 µL of NADPH solution (N5130) was added and, after 2 min of incubation at 25 °C, the decrease in the absorbance (340 nm) was read. All solutions were prepared in a 50 mM buffer solution and the concentration of the reagents in the final mixture was as follows: 1 mM EDTA, 0.2 U glutathione reductase, 2 mM GSH, 0.04 U glutathione peroxidase, 1.5 mM H₂O₂, and 0.8 mM NADPH. A blank sample was prepared with buffer instead of the sample and the content of the sample was measured (a mixture containing the studied sample and buffer only). One unit of enzyme activity has been defined as nmol of NADPH consumed/min – mL of the sample, in comparison with nmol of NADPH consumed/min in the blank (reagent) sample [20]. Each sample was analyzed at least four times.

2.7. Sensory Analysis by Sensory Profiling

For a detailed sensory description of these samples the method of quantitative descriptive analysis, i.e., sensory profiling, was applied, which was performed by a 20-person team specially trained for this purpose. Individual quality descriptors selected in preliminary studies were evaluated: color (clear, straw, brown, green), taste (sweet, sour, bitter, fruity, astringent, grassy, foreign), scent (fruity, earthy, sweet, sour, unfamiliar, intense) and overall desirability of the teas. The intensity of each quality descriptor was determined using a 10-cm unstructured linear scale with appropriate boundary markings. The results obtained were described as numerical values expressed in conventional units.

2.8. Statistical Analysis

All tests were conducted three times and the results are expressed as mean $\pm$ SD. The routine statistical tests (average values and standard deviation) were performed. Statistical differences were calculated with significant differences identified at $p < 0.05$ (Statistica Software ver. 13.1 StatSoft, Cracow, Poland). Additionally, the analysis of the principal components was performed (PCA). 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.

3. Results

3.1. Electrochemical Analysis

Optimization of the process conditions is aimed at determining the best solutions concerning the criterion of the quality of the final product. The stage that determines the content of phenolic compounds, as well as the antioxidant activity of infusions, is the extraction process. What is more, a vital element also includes the choice of time and temperature. Electrochemical activity (antioxidant potential) of the bark of *P. padus* was tested using different extraction temperatures (80 °C and 100 °C) and extraction duration (15, 30, or 45 min). The electrochemical (SWV: square wave voltammetry) measurement procedure was identical to the one adopted in the previous studies [5,21].

The total peak current, total peak area and electrochemical activity of the extracts from the bark of *P. padus*, prepared using different temperature and extraction time, was tested.

Below (Table 1), there is a summary of average results (number of measurements $n = 3$).

Table 1. Electrochemical parameters determined for *Prunus padus* L. (*P. padus*) bark extracts.

Sample <i>P. padus</i> Bark Extract	Signal Current—Square Wave Voltammetry (SWV)					EI [V $\times$ μ A/1 g d.m.]
	Peak Potential E_p [V]	Peak Current I [μ A]	Peak Area $E_p \times I$ [V $\times$ μ A]	Total Peak Current I [μ A]	Total Peak Area [V $\times$ μ A]	
80 °C/15 min	0.247	5.491	0.925	7.403	1.411	45.154
	0.694	1.912	0.486			
80 °C/30 min	0.262	7.918	1.529	12.734	3.413	109.220
	0.594	4.816	1.884			
80 °C/45 min	0.247	9.689	1.874	15.315	3.933	125.851
	0.562	5.626	2.059			
100 °C/15 min	0.254	3.592	0.719	7.438	1.993	63.781
	0.623	3.581	1.110			
100 °C/30 min	0.271	4.143	0.858	9.190	2.736	87.541
	0.564	5.047	1.878			
100 °C/45 min	0.262	5.518	1.160	10.872	3.114	99.632
	0.559	5.354	1.954			

Concerning the electrochemical test, one could see that the highest antioxidant activity was characteristic for an infusion prepared at 45 min at 80 °C (total peak area $3.933 \text{ V} \times \mu\text{A}$) (Figure 1).

Extending the extraction process time from 15 to 45 min increased the tested electrochemical activity by 170%, however, due to the very long brewing time, the most practical brewing time, from the consumer's point of view, of 15 min and a temperature of 80 °C were chosen for further analysis. The infusions obtained in this way were also characterized by high electrochemical activity.

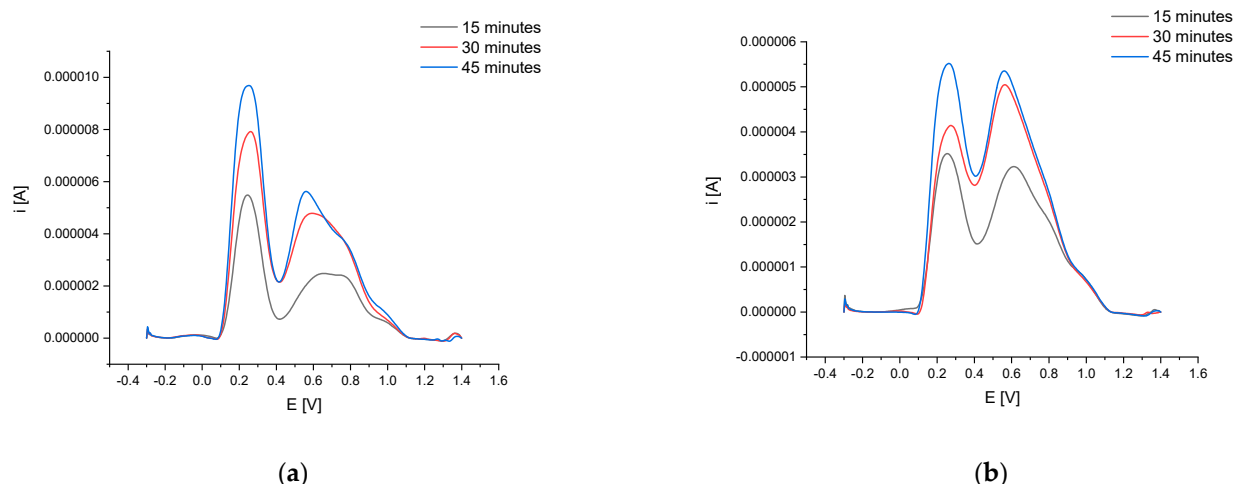


Figure 1. Square wave voltammetry (SWV) voltammograms for *P. padus* bark extracts (80 °C (a) and 100 °C (b)).

3.2. Content of Phytochemicals Compounds in Infusions with *P. padus* Bark Added

Of the samples tested, sample B (Table 2) was characterized by the highest polyphenol content, in which the total content of polyphenolic compounds was 7939.8 ± 106.6 mg/100 g dw. It had the highest content of isorhamnetins (1951.3 ± 3.6 mg/100 g dw), as well as catechins (1251.6 ± 3.2 mg/100 g dw) and apigenins (900 ± 1.5 mg/100 g dw). Nonetheless, oxalic acid (1.2 ± 0.1 mg/100 g dw) and maleic acid (7.1 ± 0.1 mg/100 g dw) were compounds with the lowest content in the sample. Among the infusions with added *P. padus* bark, the total polyphenols content ranged from 7509.2 ± 189.6 mg/100 g in sample BP5 to 6546.4 ± 66.5 mg/100 g in sample BP30. One could see that the content of isorhamnetin (between 1841.6 ± 20.7 and 1362.2 ± 3.2 mg/100 g dm) and catechins (between 1206.4 ± 4.8 and 1140.9 ± 2.5 mg/100 g dm) was the highest. On the contrary, the infusions with bird cherry bark added were characterized by low content of oxalic acid (between 0.9 ± 0.1 and 1.1 ± 0.1 mg/100 g dm) and vanillic acid (between 4.8 ± 0.3 and 6.2 ± 0.1 mg/100 g dm). Concerning low molecular weight organic acids (LMWOA), their lowest value was determined in sample Ch, while the highest value was obtained for the infusion prepared from bark alone (B) and was 1300% higher than in the tea with chamomile. All teas with *P. padus* bark contained a very high amount of acids.

Table 2. Content of phytochemicals compounds in dietary infusions with the bark of *P. padus*.

SAMPLE mg/100 g dw	P	Ch	C	T	B	BP5	BP10	BP15	BP20	BP25	BP30
PHENOLIC ACIDS											
Gallic acid	16 ± 0.8	1.4 ± 0.2	ND	0.3 ± 0.1	18.3 ± 0.7	18 ± 0.7	18 ± 0.5	18 ± 0.3	18 ± 0.1	18 ± 0.1	17 ± 0.2
2,5-dihydroxybenzoic acid	102.8 ± 5.1	15 ± 2	ND	72 ± 1.3	188 ± 0.7	178 ± 2.3	172 ± 5	174 ± 0.6	168 ± 2.4	161 ± 1	155.6 ± 3
4-hydroxybenzoic acid	89.1 ± 1	108.7 ± 2.6	ND	3.8 ± 0.4	196.8 ± 4.7	188 ± 2.6	183.2 ± 3	178 ± 2	175.3 ± 1.7	168 ± 0.7	159 ± 0.1
Caffeic acid	3.4 ± 0.4	49 ± 1	102.1 ± 3.3	2 ± 0.2	151.8 ± 1.5	138.2 ± 4.8	132.9 ± 4.5	124 ± 4	119.9 ± 0.3	113.1 ± 1.4	100.9 ± 1.3
Syringic acid	43.2 ± 0.9	3.1 ± 0.2	ND	0.3 ± 0.2	44.2 ± 1.4	39.5 ± 0.9	43.5 ± 0.6	43.8 ± 0.5	42.5 ± 1.1	43.3 ± 1.7	41 ± 0.8
P-coumaric acid	172.1 ± 2	1.6 ± 0.1	ND	0.7 ± 0.1	169.5 ± 1.5	164.6 ± 5.5	159.4 ± 1.2	166.1 ± 3.4	166.5 ± 4.3	168.7 ± 1.4	171.3 ± 1.1
Ferulic acid	349.8 ± 7.5	87.5 ± 0.9	10.5 ± 0.4	13.9 ± 0.3	455.4 ± 1.6	438 ± 2.7	439.8 ± 0.6	432.9 ± 2.6	434 ± 1.6	427.7 ± 1.9	420.1 ± 1.5
Chlorogenic acid	16.5 ± 0.7	54.8 ± 2.2	38 ± 0.4	ND	100.6 ± 2	87.4 ± 2.3	87.8 ± 2.4	87.2 ± 1.1	81.9 ± 2.1	76.6 ± 0.9	73.7 ± 1.3
Sinapic acid	71 ± 2.5	1.5 ± 0.2	ND	0.6 ± 0.1	70.6 ± 0.7	68.2 ± 2.1	69.3 ± 0.7	69.5 ± 0.7	70.1 ± 0.6	70.2 ± 0.3	71.4 ± 1.1
T-cinnamic acid	1.7 ± 0.2	0.7 ± 0.2	6.3 ± 0.6	0.9 ± 0.1	9 ± 0.2	7.7 ± 0.3	7.7 ± 0.4	7.4 ± 0.2	7.2 ± 0.3	6.9 ± 0.2	6.5 ± 0.3
Vanillic acid	0.7 ± 0.2	ND	ND	7.1 ± 0.2	7 ± 0.2	6 ± 0.5	6.2 ± 0.1	6 ± 0.1	5.6 ± 0.2	5.1 ± 0.2	4.8 ± 0.3
Salicylic acid	96 ± 1.3	2.4 ± 0.2	0.3 ± 0.1	ND	90.7 ± 1.8	88.4 ± 0.8	87.9 ± 2.9	90.3 ± 0.8	88.7 ± 1.2	87.9 ± 1.8	92.6 ± 0.9
Total phenolic acids	962.2 ± 22.3	325 ± 9.6	157.2 ± 9.3	101.1 ± 2.8	1501.5 ± 33.6	1421.7 ± 50.6	1407.5 ± 42	1396.9 ± 33	1377.2 ± 31.6	1346.4 ± 23.7	1314.2 ± 25
FLAVONOIDS											
Naringenina	18 ± 2.4	169.1 ± 3.4	16.3 ± 0.6	0.1 ± 0.1	198.9 ± 1.9	168.6 ± 1.8	176.4 ± 1	169.9 ± 1.4	161.2 ± 4.4	149.2 ± 0.9	140.9 ± 0.7
Vitexin	6.4 ± 0.3	0.6 ± 0.1	24.3 ± 1.6	ND	29.3 ± 0.4	25.2 ± 1.1	24.4 ± 0.8	24.9 ± 0.3	24.1 ± 0.4	22.5 ± 1.1	20.7 ± 0.8
Rutin	5.4 ± 0.3	25.2 ± 0.6	9.9 ± 0.3	24.5 ± 1.2	60.4 ± 0.7	53.8 ± 1.5	51.6 ± 1.3	51.4 ± 1	48.6 ± 0.6	45.5 ± 1.3	40 ± 1.3
Quercetin	593 ± 7.9	70.8 ± 3.3	24 ± 1.4	0.1 ± 0.1	665.3 ± 2.9	657.9 ± 2.5	657.5 ± 2.5	648.3 ± 6.4	647.4 ± 2.6	643.2 ± 3.1	641.8 ± 1.6
Apigenin	10.4 ± 0.3	886.6 ± 26	10.1 ± 0.6	0.2 ± 0.1	900 ± 1.5	850.8 ± 1.3	809 ± 2.9	764 ± 3.2	719.5 ± 1.1	670 ± 1.5	630.3 ± 1.3
Kaempferol	3.1 ± 0.2	11 ± 0.8	0.5 ± 0.2	10.9 ± 0.6	22.4 ± 0.9	20.1 ± 0.6	19.5 ± 0.7	19 ± 0.4	17.2 ± 1.6	17.3 ± 0.4	16.3 ± 0.1
Luteolin	9.5 ± 0.3	1.9 ± 0.2	3.6 ± 0.2	0.2 ± 0.1	14.2 ± 0.6	12.8 ± 0.3	13.2 ± 0.3	13.4 ± 0.5	13.1 ± 0.3	12.9 ± 0.2	12.2 ± 0.1
Catechin	887.4 ± 4.6	149.3 ± 4.9	241.3 ± 3.3	5.5 ± 0.3	1251.6 ± 3.2	1208.8 ± 8.4	1206.4 ± 4.8	1191.1 ± 2.1	1172 ± 2.7	1157.6 ± 2.4	1140.9 ± 2.5
Isohamethn	ND	ND	207.4 ± 3.5	ND	1951.3 ± 3.6	1841.6 ± 20.7	1747.7 ± 3.1	1652.2 ± 5.3	1555.73 ± 3.9	1454.4 ± 5.4	1362.2 ± 3.2
Lycorine	ND	ND	289 ± 3.6	ND	275.9 ± 5.4	253.9 ± 4.08	241.9 ± 2	232.3 ± 1.8	217.1 ± 3.1	203.4 ± 2.2	191.9 ± 1.6
Myricetin	ND	ND	ND	31.5 ± 1.1	29.6 ± 0.9	27.3 ± 0.7	24.4 ± 0.7	24.3 ± 0.8	23 ± 0.6	21.1 ± 0.9	19.6 ± 0.6
Total flavonoids	1533.1 ± 32.4	1314.6 ± 78.4	826.4 ± 30.1	73.1 ± 6.5	5398.8 ± 45.4	5120.9 ± 85.5	4972 ± 41.2	4790.8 ± 45.7	3426.9 ± 21.2	4397.2 ± 38.2	4216.6 ± 27.5
LMWOAs											
Oxalic acid	1.3 ± 0.2	ND	ND	ND	1.2 ± 0.1	1 ± 0.1	0.9 ± 0.2	1 ± 0.2	0.9 ± 0.1	1.1 ± 0.1	0.9 ± 0.1
Maleic acid	7.3 ± 0.4	ND	ND	0.2 ± 0.1	7.1 ± 0.1	7 ± 0.2	6.7 ± 0.3	7 ± 0.2	7.2 ± 0.1	7.1 ± 0.1	7.1 ± 0.1
Citric acid	118.4 ± 2.1	11.3 ± 0.1	0.7 ± 0.2	6.5 ± 0.3	129.4 ± 1	124.3 ± 3.5	121.9 ± 1.9	127.2 ± 1.7	128.1 ± 1.4	125.5 ± 1.6	125.8 ± 0.8
Malic acid	319 ± 5.9	ND	ND	27 ± 1.4	337.8 ± 2.6	324.7 ± 7.4	330.7 ± 1.8	330.7 ± 3	327.1 ± 6.5	329.7 ± 0.9	331.6 ± 0.8
Quinic acid	80.4 ± 1	6.3 ± 0.5	13.1 ± 0.6	165.8 ± 1.3	246.9 ± 6.9	223.1 ± 9.4	227.7 ± 2.4	220.9 ± 0.9	211.5 ± 1.1	202.8 ± 3.1	192.1 ± 3.2
Shikimic acid	166.5 ± 0.8	57.7 ± 1.9	104.4 ± 2.6	203.3 ± 2.8	317.1 ± 3.2	286.5 ± 6.2	299.4 ± 3.7	294.5 ± 3.7	280.7 ± 7.9	272.8 ± 3.5	268.3 ± 2
Total LMWOAs	692.8 ± 10.5	75.3 ± 6	118.3 ± 6.5	402.7 ± 11.5	1039.5 ± 27.6	966.6 ± 53.4	987.3 ± 20.5	981 ± 9.7	955.4 ± 34	938.9 ± 18.5	925.7 ± 14
Total Polyphenols	3188.1 ± 65.2	1714.9 ± 94	1102 ± 9.2	576.8 ± 41.5	7939.8 ± 106.6	7509.2 ± 189.6	7366.8 ± 103.9	7168.7 ± 88.7	5759.6 ± 86.8	6682.5 ± 80.4	6546.4 ± 66.5

ND—not detected, DW—dry weight. Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($p \leq 0.05$).

3.3. Linoleic Acid Oxidation, Beta-Carotene Bleaching Test and FRAP Method

It was decided to broaden the scope of the study by incorporating the linoleic acid oxidation test, in which the highest antioxidant activity against this acid was determined in tea without added bird cherry bark (B) (134.4 ± 15.1^b μg ascorbic acid /mL), and the lowest in tea with its 20% content (BP20) (107.3 ± 7.2^a μg ascorbic acid /mL). Concerning the beta-carotene bleaching test, a common method used to assess antioxidant activity, the highest results were obtained for samples: C ($16.7 \pm 1.1^c\%$) and Ch ($15.0 \pm 2.0^c\%$), and the lowest was shown by the BP20 sample ($9.2 \pm 0.4^a\%$). The infusions with the addition of bark were characterized in terms of their ability to reduce Fe (III) to Fe (II). This ability represents antioxidant activity as expressed by its mechanism of action as the ability to donate an electron. This reaction is associated with an increase in the negative charge and, as a result, a decrease in the degree of oxidation. In the assay for the ability to reduce Fe (III) iron ions, one could see that this property was most visible for infusions containing only bird cherry bark (P), respectively: 25.3 ± 0.9^c μg Trolox /mL, and for an infusion with 20% addition of the tested material (BP20) 22.1 ± 0.3^b (Table 3).

Table 3. Linoleic acid oxidation and beta-carotene bleaching test.

Sample	Linoleic Acid Oxidation Activity (μg Ascorbic Acid/mL)	Beta-Carotene Bleaching Test Inhibition (%)	FRAP Activity (μg Trolox/mL)
Blank samples	134.4 ± 15.1^b	12.4 ± 0.9^{bc}	23.1 ± 3.7^{bc}
C	122.2 ± 21.5^{ab}	16.7 ± 1.1^c	19.3 ± 0.7^{ab}
Ch	114.0 ± 20.0^{ab}	15.0 ± 2.0^c	16.5 ± 1.5^a
P	103.2 ± 11.3^a	10.2 ± 0.8^{ab}	25.3 ± 0.9^c
T	117.9 ± 14.6^{ab}	12.6 ± 1.7^{bc}	20.0 ± 2.0^{ab}
BP5	112.4 ± 10.0^{ab}	11.4 ± 0.5^b	18.3 ± 1.7^{ab}
BP10	115.0 ± 18.4^{ab}	10.2 ± 2.1^{ab}	20.7 ± 0.3^b
BP15	104.2 ± 11.9^a	12.5 ± 0.0^b	21.5 ± 1.0^b
BP20	107.3 ± 7.2^a	9.2 ± 0.4^a	22.1 ± 0.3^b
BP25	100.4 ± 16.1^a	11.0 ± 3.2^{ab}	20.9 ± 2.4^{ab}
BP30	108.1 ± 15.1^{ab}	12.7 ± 0.2^b	19.4 ± 2.2^a

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

3.4. Inhibition of AChE, BChE, Catalase and SOD

The ability to inhibit acetylcholinesterases (AChE) and butyrylcholinesterases (BChE) was demonstrated for all tested extracts. The highest ability to inhibit cholinesterase was determined for an infusion of bird cherry bark alone (P) 15.8 ± 1.1^d μg neostigmine/mL for AChE and 21.2 ± 1.0^c μg neostigmine/mL for BChE (Table 4). Concerning bark teas, the highest activity was determined for the infusion with 20% addition of the tested material (BP20) 12.1 ± 0.1^c μg neostigmine/mL for AChE and 18.5 ± 1.5^{bc} μg neostigmine/mL for BChE. Cholinesterases are enzymes that cleave choline esters. There are two enzymes belonging to the cholinesterase group, AChE, which is present in the nervous system and interrupts neurotransmission, and BChE found in the brain, serum, and nervous system.

Table 4. Inhibition of acetylcholinesterases (AChE), butyrylcholinesterases (BChE), catalase and (SOD) superoxide dismutase in teas with *P. padus* bark added.

Sample	Inhibition of AChE (μg Neostigmine/mL)	Inhibition of BChE (μg Neostigmine/mL)	Inhibition of Catalase (%)	Inhibition of SOD (%)
Blank samples	9.6 ± 1.8^b	13.9 ± 1.0^a	34.5 ± 3.6^a	23.9 ± 1.3^b
C	8.9 ± 1.1^b	14.4 ± 1.0^{ab}	41.7 ± 4.4^{ab}	26.4 ± 0.9^{bc}
Ch	5.9 ± 1.7^a	16.2 ± 2.0^{ab}	37.2 ± 3.8^a	19.8 ± 1.4^a
P	15.8 ± 1.1^d	21.2 ± 1.0^c	62.9 ± 2.8^e	37.5 ± 2.3^e
T	7.2 ± 0.9^a	14.0 ± 0.3^a	43.9 ± 1.5^b	24.5 ± 1.3^b
BP5	10.6 ± 1.1^{bc}	14.7 ± 2.5^{ab}	45.7 ± 2.5^{bc}	26.9 ± 1.4^{bc}
BP10	12.0 ± 1.0^b	16.8 ± 1.4^b	50.1 ± 3.1^c	29.3 ± 1.9^c
BP15	11.0 ± 1.8^{bc}	17.3 ± 1.0^b	54.8 ± 2.0^{cd}	31.7 ± 2.2^{cd}
BP20	12.1 ± 0.1^c	18.5 ± 1.5^{bc}	57.2 ± 2.2^d	32.0 ± 1.5^{cd}
BP25	11.2 ± 0.6^{bc}	16.4 ± 1.8^b	55.7 ± 2.9^{cd}	31.5 ± 1.0^c
BP30	12.0 ± 0.3^c	16.5 ± 1.6^b	58.9 ± 1.1^{de}	33.6 ± 0.5^d

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

Catalase, on the other hand, is a protein enzyme, a type of haemoprotein that plays a vital role in cell protection and as an antioxidant. Superoxide dismutase enzymes catalyze the dismutation of superoxide radicals to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2), providing cellular defense against reactive oxygen species and consequently constitute an important defense mechanism against superoxide radicals' toxicity. As indicated by the statistical analysis of the results, the mixture containing the addition of bird cherry bark in the range of 10–30% of the share was characterized by a statistically higher activity than the raw material base or the mixture with the addition of 5% bark. Catalase and SOD inhibition for the BP20, BP25, and BP30 trials, although nominally different, did not differ statistically.

The results obtained for the inhibition of catalase and SOD enzymes were similar. Again, the highest inhibitory activity was determined for the infusion of bird cherry bark alone (P), 62.9 ± 2.8^e % for catalase and 37.5 ± 2.3^e % for SOD, and the lowest for the infusion consisting of the base alone (B), 34.5 ± 3.6^a % and 23.9 ± 1.3^b %, respectively. Concerning the bark teas, the highest activity was discovered for the infusion with 20% addition of the tested material, the same as for AChE and BChE.

3.5. Inhibition of Glutathione Reductase, Inhibition of Glutathione Peroxidase

Glutathione reductase is a homodimeric flavoprotein and key antioxidant enzyme in regard to maintaining an intracellular reducing environment, which is crucial for the cell in combating oxidative stress. Glutathione peroxidase-1 is an intracellular antioxidant enzyme that decreases hydrogen peroxide to water enzymatically in order to limit its damaging effects.

Testing the ability to inhibit glutathione reductase and glutathione peroxidase, one could spot that in both cases the highest values were obtained for the infusion in which the only ingredient was bird cherry bark— 87.0 ± 1.1^e % for glutathione reductase and 64.9 ± 2.0^d % for peroxidase (Table 5). Among teas with added bark, the highest activity was determined for infusions with 25% addition of the tested material (BP25) (77.9 ± 1.6^d % and 53.3 ± 3.2^c %) as well as 20% addition of bark (BP20) (74.2 ± 2.9^{cd} and 53.7 ± 2.7^c %) (Table 5).

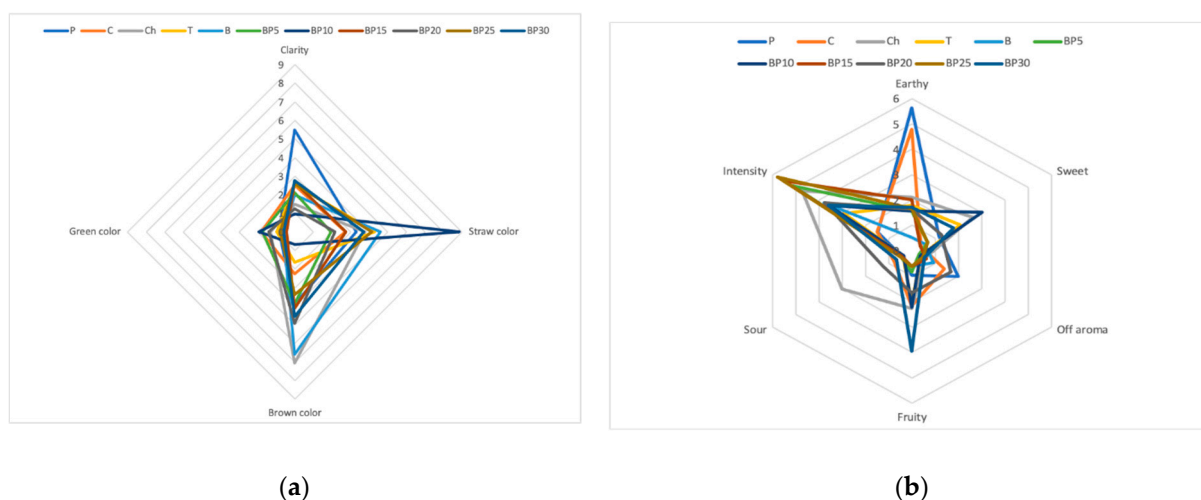
Table 5. Inhibition of glutathione reductase, inhibition of glutathione peroxidase.

Sample	Glutathione Reductase Inhibition (%)	Glutathione Peroxidase Inhibition (%)
Blank samples	65.8 ± 3.1 ^b	40.9 ± 1.3 ^a
C	57.2 ± 2.2 ^a	44.3 ± 0.9 ^b
Ch	55.6 ± 4.1 ^a	39.8 ± 1.7 ^a
P	87.0 ± 1.1 ^e	64.9 ± 2.0 ^d
T	63.8 ± 4.5 ^b	44.4 ± 1.3 ^b
BP5	66.8 ± 2.5 ^b	46.1 ± 3.5 ^b
BP10	70.3 ± 3.1 ^{bc}	48.6 ± 4.1 ^{bc}
BP15	76.1 ± 0.9 ^d	52.2 ± 1.6 ^c
BP20	74.2 ± 2.9 ^{cd}	53.7 ± 2.7 ^c
BP25	77.9 ± 1.6 ^d	53.3 ± 3.2 ^c
BP30	72.0 ± 1.8 ^c	49.3 ± 4.1 ^{bc}

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

3.6. Sensory Analysis of Teas with the Addition of the Bark of *P. padus*

The dominant color for most samples of the tested teas containing the bark of bird cherry was brown (Figure 2). Among all samples, green was the least noticeable color and it ranged, in the terms of value, from 0.62 to 1.92. The samples were of heterogeneous clarity, with sample P having the highest mean score of 5.5 and sample BP10 with the lowest score (0.96). The teas containing bark of bird cherry were characterized by a variety of flavors, the distinguishing feature of a sour aftertaste was dominant and it was followed by grassy flavor, which was the second dominant flavor. It was demonstrated that sweet flavor was rated on a 10-point scale ranging from 0.9 for sample C to 5.7 in the case of sample BP30. It was also discovered that, among all studied types of teas, the fruity flavor was found to be poorly recognizable, with intensity scores ranging from 0.7 to 3.44. Almost all samples of the tea that contained the bark of bird cherry were scored very similarly in the terms of color distinguishing feature and it was closer to brown. The Ch sample was of a more intense brown color and was clearer.

**Figure 2.** Cont.

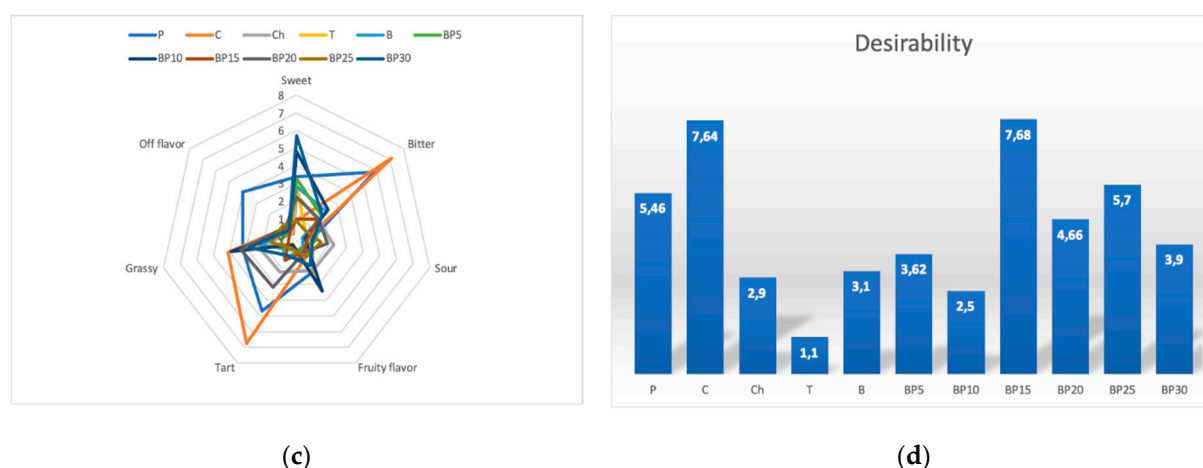


Figure 2. Sensory profiles of teas with added bark of *P. padus*: (a) color, (b) taste, (c) flavor, (d) overall desirability.

4. Discussion and Conclusions

In agriculture as well as in forestry, bird cherry is an issue because it disrupts the ecosystem. Due to the abundant occurrence of bird cherry in natural ecosystems and its low requirement when it comes to the growth conditions, and thus its ability to spread easily and quickly, it seems unquestionable that finding an effective method of using its properties in a wide industrial or food spectrum should become one of the most important tasks for scientists and technologists nowadays. The use, and the elimination of bird cherry at the same time, can limit its growth and its bark can be a source of many bioactive compounds. Due to the high content of active compounds and the antioxidant and inhibitory activities of bird cherry, as determined in this study, a new method can be indicated for the elimination of the tree from the environment, with simultaneous efficient use of its bark for nutritional purposes.

Products such as herbal infusions rich in polyphenol can support the prevention of chronic diseases [5,10,22,23]. Bird cherry, due to the fact that it contains a lot of active compounds, can be an attractive raw material to use in teas, however, the high content of those substances can also make the taste of herbal infusions unattractive and specific. Therefore, to meet the expectations of consumers and to improve the sensory values, it can be used as an ingredient in mixtures that are used to prepare herbal teas. Such practices are common in food technology, for example, to enrich products with bioactive components. Similar research was conducted by Dziejzinski et al. (2020), and it was related to infusions with the addition of hops, bringing an intense aroma, and in that case, it was also proved that the developed recipe, which included hops as an ingredient, could be an interesting alternative and a proposal in supporting the treatment and preventing the development of neurodegenerative diseases [24].

New raw materials, such as bird cherry bark, which have not been used in industry so far, may be a source of active compounds increasing the health-promoting value of food. Currently, there are visible trends for the search for this type of raw materials, as well as trends related to the management of waste materials or by-products of the food industry, such products are, for example, bran, spent grain, or pulp after juice production. Therefore, sustainable development that takes into account the acquisition of raw materials that do not require agrotechnical treatments (bark of invasive plants) as well as the use of by-products and additional processes increasing the stability of compounds in the processing process, e.g., through the process of microencapsulation, allows you to maintain balance in the environment and increase the nutritional value and functional properties of products [25].

In this study, it was confirmed that the addition of the bark of bird cherry can influence the functional properties, which is the result of the specific composition of the compounds present in the bark. According to earlier studies, a significant amount of polyphenols was identified in certain anatomical parts of *P. padus* [5]. This may be the future of the

management of bird cherry and the use of its bark for the purposes of food production. The bark of bird cherry contains many bioactive compounds that are particularly rich in catechins and their derivatives which contribute to the beneficial effects of herbal or tea infusions, they effectively eliminate reactive oxygen species in vitro and may also act indirectly as antioxidants thanks to their effects on transcription factors and enzyme activity [26]. In 2015, scientists from Korea also studied the total content of polyphenol in methanolic extracts from the bark of *P. padus* [27]. The bark was also a research material for another group of scientists who determined the high content of phenolic in water extracts and it was suggested by them that this could be a way to eliminate it from the environment [28]. Those benefits may also be the result of the high antioxidant potential, e.g., the reduction activity [27].

The classic antioxidant activity of polyphenols, based on their reducing properties, includes the direct scavenging of reactive oxygen and nitrogen species (ROS and RFA) and the reduction of the amount of ROS and/or RFA formed. This action is indirect through the regeneration of other antioxidants such as α -tocopherol or β -carotene and by chelating or reducing transition metals. Polyphenols have antioxidant properties due to their chemical structure which determines the ability to easily donate hydrogen atoms and/or electrons. Moreover, the phenoxy radicals of the polyphenols may participate in subsequent stages in reactions characteristic for radicals, such as dimerization or reactions with other radicals, they may also undergo further oxidation to the oquinone form. Moreover, in addition to their classic antioxidant activity, polyphenols can act as antioxidants, influencing the intracellular redox balance, thanks to other mechanisms such as inhibition of “pro-oxidative” enzymes, such as eg lipoxygenase [5,27].

Such actions may indicate the personalized use of the bark of bird cherry in the prevention of neurodegenerative diseases. Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two enzymes that are sensitive to different chemicals and have the ability to bind to key parts of enzymes. They are sensitive to a broad spectrum of molecules. The research on natural inhibitors which resulted in creating, e.g., physostigmine and galantamine, began years ago, and more recently, synthetic versions of these products were also introduced [29]. Our study presented a highly visible property of inhibiting cholinesterases in bird cherry bark. The search for this type of material was commenced by other researchers. Yang et al. (2015) analyzed *Peganum harmala* L. and stated that the 2-aldehyde tetrahydroharmine contained in it can inhibit both AChE and BChE [30]. Polyphenols may also have other properties concerning catalase inhibition. Catalase is produced by aerobic organisms. It takes part in cell protection by degrading reactive oxygen species. Thus, catalase may prove helpful in chronic inflammation [31], whereas SOD is one of the main antioxidant enzymes that protect against the harmful effects of reactive oxygen species on the cell [32]. Our results for inhibition of catalase and SOD enzyme indicate high inhibitory activity of bird cherry bark, proving that it can be a relevant ingredient for improving the functional properties of food products.

The research also evaluated the property of infusions to inhibit glutathione peroxidase, which is an intracellular antioxidant enzyme, and glutathione reductase, which catalyzes the reduction of glutathione disulfide to the sulfhydryl form, a molecule crucial for combating oxidative stress and maintaining the intracellular reducing environment [33,34].

The quality of food consists of its nutritional value, health safety, and, above all, sensory characteristics which should be accepted by consumers. This can be achieved by using different types of combinations, mixing together ingredients with a specific taste, aroma, and color to obtain a product with the best health-promoting property for the consumer and a pleasant and desirable taste. When carrying out this study, a product both tasty and having many health-promoting properties resulting from the antioxidant and inhibitory activity against key enzymes in neurodegenerative diseases was obtained.

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Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Szulc, P., Ligaj, M., Stuper-Szablewska, K., Szwajgier, D., Bujak, H. 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., 2021, *Sustainability*, 13(7), 3913, mój indywidualny udział w jej powstaniu polegał na zebraniu materiałów źródłowych, opracowaniu modelu doświadczenia, wykonaniu analiz chemicznych, przygotowaniu zestawień tabelarycznych/wykresów, interpretacji i opisie wyników, przygotowaniu treści manuskryptu, zredagowaniu manuskryptu zgodnie z wymogami czasopisma, przygotowaniu zestawienia literatury cytowanej w manuskrypcie, przesłaniu manuskryptu do wydawnictwa, prowadzeniu korespondencji z wydawnictwem oraz na naniesieniu zmian i uzupełnieniu manuskryptu zgodnie z uwagami recenzentów.

Podpis

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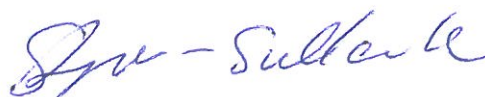
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Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Szulc, P., Ligaj, M., Stuper-Szablewska, K., Szwajgier, D., Bujak, H. Comparative Analysis of Infusions with the addition *P. padus* bark: Assessment of the antioxidant potential and their inhibitory effect on enzymes associated with oxidative stress., 2021, *Sustainability*, 13(7), 3913, mój indywidualny udział w jej powstaniu polegał na nadzorowaniu badań laboratoryjnych (chromatograficznych), nadzorowaniu prac analitycznych oraz na weryfikacji wnioskowania z wykonanych analiz chemicznych.



Podpis

Oświadczenia o współautorstwie

Warszawa, 11.04.2022 r.

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**Rada Dyscypliny
Technologia Żywności i Żywienia
Szkoły Głównej Gospodarstwa
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Podpis



Oświadczenia o współautorstwie

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Podpis



Research Article

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

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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

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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.

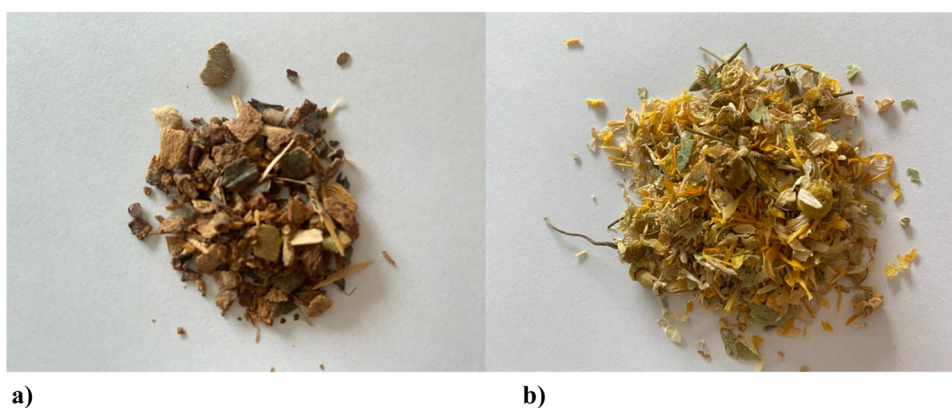


Figure 1: (a) bird cherry bark (b) base mix (*Matricaria chamomilla* L. + *Tilia cordata* Mill. + *Calendula officinalis* L.).

(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% HNO₃) 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% Na₂CO₃ water solution (750 µL). After 2 h, the absorbance was read at 760 nm against the reagent sample (containing water, F–C reagent, and Na₂CO₃) [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% Na₂CO₃ 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 H₂O₂ solution (10 µL) and CoF₂ × 4H₂O 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

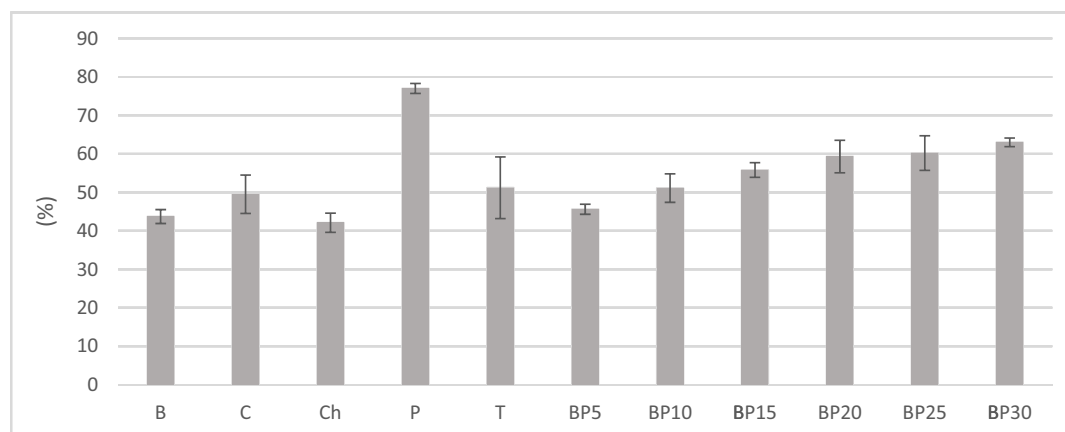


Figure 2: COX 2 inhibition in infusions with the bark of bird cherry.

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.

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Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Szulc, P., Wilk, R., Szwajgier, D., Szymanowska, D., 2021, *Prunus padus* L. bark as a functional promoting component in functional herbal infusions – cyclooxygenase-2 inhibitory, antioxidant, and antimicrobial effects., *Open Chemistry*, 19(1), 1052-106, mój indywidualny udział w jej powstaniu polegał na zebraniu materiałów źródłowych, opracowaniu modelu doświadczenia, wykonaniu analiz chemicznych, przygotowaniu zestawień tabelarycznych, przygotowaniu grafik, interpretacji i opisie wyników, przygotowaniu treści manuskryptu, zredagowaniu manuskryptu zgodnie z wymogami czasopisma, przygotowaniu zestawienia literatury cytowanej w manuskrypcie, przesłaniu manuskryptu do wydawnictwa, prowadzeniu korespondencji z wydawnictwem oraz na naniesieniu zmian i uzupełnieniu manuskryptu zgodnie z uwagami recenzentów.

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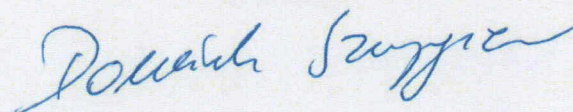
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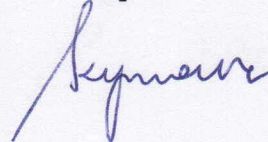
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Podpis



RESEARCH ARTICLE

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

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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 IC₅₀ 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 plicae 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 (Truschein 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,

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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 Male in the region of Podlasie, 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 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 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^{•+} sweep factor was calculated from the standard curve for $y = 95.12x + 31.54$ ($R^2 = 0.938$) 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 $y = 185.21x + 19.33$ ($R^2 = 0.941$) 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 (Fe^{2+})

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 (Fe^{2+}) 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 content 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 alpha-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 = $((A_c - A_s)/A_c) \times 100$, 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 $\pm$ SD. The IC_{50} values were calculated using OriginPro 9 software with nonlinear regression. IC values₅₀ (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 [%] = $100 - 100 \times (\text{Sample Abs. 0 min} - \text{Sample Abs. 2 min}) / (\text{Control Abs. 0 min} - \text{Control Abs. 2 min})$.

Statistical analysis

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

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 - $1.23^c \pm 0.01$ mM Trolox/g d.m.

and FA - $1.22^c \pm 0.02$ 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 ($1.22^c \pm 0.04$ mM Trolox/g d.m.) and water extract from bark - KW $0.94^b \pm 0.02$ mM 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 $2.43^d \pm 0.13$ mM Trolox/g d.m.), two other extracts also showed high capacity in the test (FA - $2.32^c \pm 0.04$ mM Trolox/g d.m. and KW - $2.01^c \pm 0.01$ 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, $0.89^a \pm 0.02$ mM Trolox/g d.m. and $1.76^b \pm 0.09$ 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 $\lambda = 765$ 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 ±0.02	1.22 ^c ±0.04	0.76 ^a ±0.11	0.89 ^a ±0.02	1.22 ^c ±0.02	1.23 ^c ±0.01
DPPH (mM Trolox/ g d.m.)	2.01 ^c ±0.01	1.23 ^a ±0.02	1.32 ^a ±0.02	1.76 ^b ±0.09	2.32 ^c ±0.04	2.43 ^d ±0.13

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 ($p \leq 0.05$)

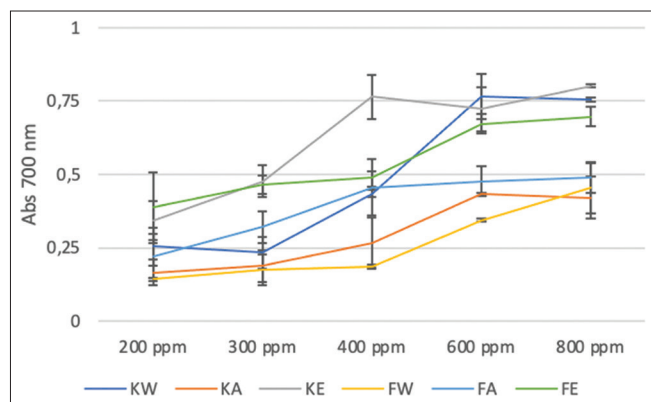


Fig 2. The reducing activity of iron ions of bird cherry bark and fruit extracts.

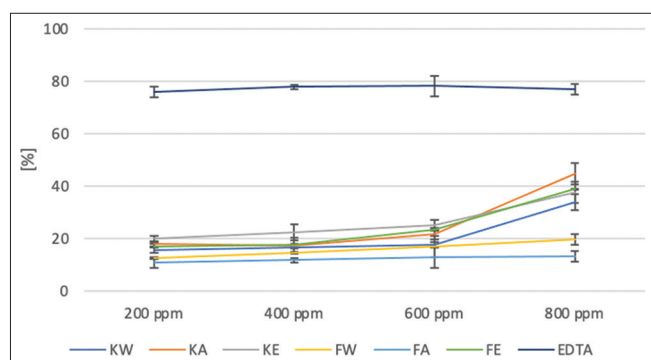


Fig 3. Chelating activity of extracts of bird cherry bark and fruit.

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 - $71.0^a \pm 1.7\%$), and the highest ethanol extract from bird cherry bark (KE - $77.6^a \pm 1.7\%$). In the case of the ability to inhibit glutathione peroxidase, the lowest value was determined for the acetone-water extract from fruit (FE - $58.3^a \pm 0.7\%$) and acetone-water extract from bark (KA - $58.3^a \pm 1.1\%$), and the water extract was the most active. from bark (KW - $63.8^a \pm 2.0\%$). In the case of catalase inhibition studies, the lowest activity was demonstrated by the acetone-water extract from fruit (FA - $41.1^a \pm 0.8\%$), and the highest by water extract from bird cherry fruit (FW - $60.2^c \pm 0.9\%$) (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 ($2.43^d \pm 0.13$ 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 (μ g/mL)	<i>Prunus padus</i> L. fruit (%)	<i>Prunus padus</i> L. bark (%)	Acarbose
165	16.77 $\pm$ 0.09	20.43 $\pm$ 0.11	-
200	23.59 $\pm$ 0.21	36.72 $\pm$ 0.13	-
235	32.02 $\pm$ 0.33	53.25 $\pm$ 0.12	-
265	46.98 $\pm$ 0.76	76.42 $\pm$ 0.32	-
300	78.68 $\pm$ 0.63	89.91 $\pm$ 0.66	-
IC ₅₀ mg/mL	27.11 $\pm$ 0.19	44.98 $\pm$ 0.08	1.22 $\pm$ 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 $\pm$ 1.1	72.9 $\pm$ 0.9	77.6 $\pm$ 1.7	71.0 $\pm$ 1.7	79.4 $\pm$ 0.6	72.2 $\pm$ 1.3
Glutathione Peroxidase	63.8 $\pm$ 2.0	58.3 $\pm$ 1.1	59.2 $\pm$ 2.6	62.3 $\pm$ 2.4	59.9 $\pm$ 1.2	58.3 $\pm$ 0.7
Catalase	47.4 $\pm$ 2.0	53.2 $\pm$ 1.2	52.2 $\pm$ 0.6	60.2 $\pm$ 0.9	41.1 $\pm$ 0.8	43.4 $\pm$ 0.9

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

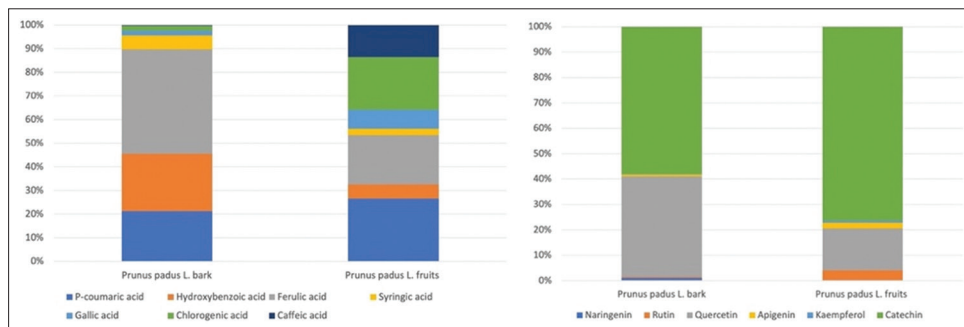


Fig 4. Polyphenols composition in *Prunus padus* L. bark and fruit aqueous extract.

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^{-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 $11,053.3 \pm 491.28 \text{ mg GAE/kg}$ of fruit weight (FW), and the antioxidant activity (FRAP) was $31.54 \pm 0.26 \text{ mM trolox/kg}$ (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 ($43.9 \pm 0.7\%$) (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.

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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.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy: Telichowska, A., Kobus-Cisowska, J., Cielecka-Piontek, J., Sip, Sz., Stuper-Szablewska, K., Szulc, P., 2022, *Prunus padus* L. as a source of functional compounds antioxidant activity and antidiabetic effect., *Emirates Journal of Food and Agriculture*, 34 (2), mój indywidualny udział w jej powstaniu polegał na zebraniu materiałów źródłowych, opracowaniu modelu doświadczenia, wykonaniu analiz chemicznych, przygotowaniu zestawień tabelarycznych/wykresów, przygotowaniu grafik, interpretacji i opisie wyników, przygotowaniu treści manuskryptu, zredagowaniu manuskryptu zgodnie z wymogami czasopisma, przygotowaniu zestawienia literatury cytowanej w manuskrypcie, przesłaniu manuskryptu do wydawnictwa, prowadzeniu korespondencji z wydawnictwem oraz na naniesieniu zmian i uzupełnieniu manuskryptu zgodnie z uwagami recenzentów.

Podpis

Oświadczenia o współautorstwie

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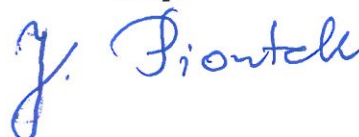
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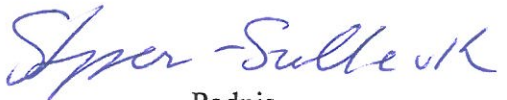
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Podpis

