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Właściwości funkcjonalne pędów drzew iglastych i nowe możliwości ich wykorzystania w projektowaniu żywności funkcjonalnej

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Uniwersytet Przyrodniczy w Poznaniu

Poznań, 2023

SPIS TREŚCI

Streszczenie

Właściwości funkcjonalne pędów drzew iglastych i nowe możliwości ich wykorzystania w projektowaniu żywności funkcjonalnej

Na całym świecie obserwuje się wzrost zapotrzebowania na funkcjonalne produkty spożywcze. Obecnie pędy drzew iglastych są rzadko stosowane jako składnik żywności, pomimo ich szerokiej dostępności w wielu częściach świata. Badania prowadzone w ostatnich latach potwierdzają, że związki obecne w pędach drzew iglastych mogą być bogatym źródłem wielu związków bioaktywnych m.in. węglowodorów terpenoidowych, tanin i związków fenolowych.

Celem pracy była ocena pędów wybranych drzew iglastych pod względem zawartości związków bioaktywnych o potencjalnych właściwościach funkcjonalnych i możliwości zastosowania ich jako surowca w produkcji żywności. Materiałem badanym były pędy 6 wybranych drzew iglastych (świerk pospolity *Picea abies* L., modrzew europejski *Larix decidua* Mill, sosna zwyczajna *Pinus sylvestris* L., daglezja zielona *Pseudotsuga menziesii*, jodła pospolita *Abies alba* i jałowiec pospolity *Juniperus communis* L). Wykazano, że pędy badanych drzew iglastych i ich ekstrakty wodne oraz wodno-etanolowe są dobrym źródłem m.in. składników mineralnych oraz związków fenolowych tj.: kwas kawowy, kwas ferulowy, kwas chlorogenowy i kwas 4-hydroksybenzoesowy, oraz charakteryzują się właściwościami przeciwutleniającymi, hamującymi aktywność hialuronidazy w modelu *in vitro*, przeciwdrobnoustrojowymi i przeciwgrzybiczymi. W badaniach stwierdzono, że pędy sosny zwyczajnej umożliwiają wytworzenie piw tradycyjnych i niskoalkoholowych będących źródłem związków aktywnych o atrakcyjnych właściwościach sensorycznych. Zastąpienie części chmielu pędami sosny na etapie gotowania brzeczki nie wpływało negatywnie na przebieg procesu fermentacji a otrzymane piwa, cechowały się właściwościami przeciwutleniającymi w badaniach *in vitro* i dobrym profilem sensorycznym. Wyniki uzyskane w ramach pracy wskazują na właściwości funkcjonalne pędów drzew iglastych i ich potencjał jako składników w produkcji żywności funkcjonalnej w tym piwa rzemieślniczego.

Słowa kluczowe: pędy drzew iglastych, *Pinus sylvestris*, polifenole, przeciwutleniacze, żywność funkcjonalna, piwo, fermentacja

Summary

Functional properties of coniferous shoots and new possibilities for their use in functional food design

There is an increasing demand for functional food products all over the world. Currently, conifer shoots are rarely used as a food ingredient, despite their wide availability in many parts of the world. Research conducted in recent years confirms that compounds present in shoots of coniferous trees can be a rich source of many bioactive compounds, e.g. terpenoid hydrocarbons, tannins and phenolic compounds.

The aim of the study was to evaluate the shoots of selected coniferous trees in terms of the content of bioactive compounds with potential functional properties and the possibility of using them as a raw material in food production. The test material consisted of shoots of 6 selected coniferous trees (Spruce *Picea abies* L., European Larch *Larix decidua* Mill, Scots Pine *Pinus sylvestris* L., Douglas Fir *Pseudotsuga menziesii*, Silver Fir *Abies alba* and *Juniperus communis* L.). It was shown that the shoots of the studied coniferous trees and their water and water-ethanol extracts are a good source of minerals and phenolic compounds. Extracts from the tested shoots are rich in phenols, including: caffeic acid, ferulic acid, chlorogenic acid and 4-hydroxybenzoic acid, and are characterized by antimicrobial, antifungal, antioxidant properties and inhibit hyaluronidase activity in an in vitro model. In the further stages of the research, it was found that Scots pine shoots allow the production of traditional and low-alcohol beers rich in bioactive compounds with the desired sensory profiles. The addition of shoots at the stage of wort boiling did not adversely affect the course of the fermentation process and allowed to obtain beers that were characterized by antioxidant properties in in vitro tests, as well as an acceptable sensory profile. The results obtained as part of the doctoral dissertation indicate the bioactive properties of conifer shoots and the possibility of their use in functional food.

Keywords: conifer shoots, *Pinus sylvestris*, polyphenols, antioxidants, functional food, beer, fermentation

PUBLIKACJE WCHODZĄCE W SKŁAD ROZPRAWY DOKTORSKIEJ

a) Praca przeglądowa stanowiąca wprowadzenie do tematyki rozprawy doktorskiej

P I.:
Dziedziński, M., Kobus-Cisowska, J., Stachowiak, B. (2021). Pinus species as prospective reserves of bioactive compounds with potential use in functional food—Current state of knowledge. *Plants*, *10*(7), 1306. DOI: 10.3390/plants10071306
IF_{2023}: ***4.658***
$MEiN_{2023}$: ***70***

b) Prace oryginalne stanowiące cykl wyników badań rozprawy doktorskiej

P II.:
Dziedziński, M., Kobus-Cisowska, J., Szymanowska, D., Stuper-Szablewska, K., Baranowska, M. (2020). Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds. *Molecules*, *25*(15), 3527. DOI: 10.3390/molecules25153527
IF_{2023}: ***4.927***
$MEiN_{2023}$: ***100***

P III.:
Dziedziński, M., Kobus-Cisowska, J., Szymanowska-Powałowska, D., Stuper-Szablewska, K., Baranowska, M. (2020). Polyphenols composition, antioxidant, and antimicrobial properties of Pinus sylvestris L. shoots extracts depending on different drying methods. *Emirates Journal of Food and Agriculture*, 229-237. DOI: 10.9755/ejfa.2020.v32.i3.2080
IF_{2023}: ***1.1***
$MEiN_{2023}$: ***40***

P IV.:
Dziedzinski, M., Kobus-Cisowska, J., Stuper-Szablewska, K., Cielecka-Piontek, J., Wilk, R., Ludowicz, D. (2022). Antioxidant potential, mineral composition and inhibitory effects of conifer needle extract on hyaluronidase-prospects of application in functional food. *Journal of Elementology*, 27(4). DOI: 10.1016/j.ejbt.2023.01.001
IF_{2023}: ***0.923***
$MEiN_{2023}$: ***40***

P V.:
Dziedziński, M., Stachowiak, B., Kobus-Cisowska, J., Kozłowski, R., Stuper-Szablewska, K., Szambelan, K., Górna, B. (2023). Supplementation of beer with Pinus sylvestris L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles. *Electronic Journal of Biotechnology*. 63, 10-17. DOI: 10.1016/j.ejbt.2023.01.001
*IF_{2023}:**2.826***
*$MEiN_{2023}$: **70***

P VI.:
Dziedziński, M., Stachowiak, B., Kobus-Cisowska, J, Faria M., Ferreira I. (2023). Antioxidant, sensory and functional properties of low-alcoholic IPA beer with Pinus 2 sylvestris L. shoots addition fermented using unconventional yeast. *Open Chemistry*. DOI: 10.1515/chem-2022-0360
*IF_{2023}: **1.997***
*$MEiN_{2023}$: **70***

Sumaryczny Impact Factor dla publikacji wchodzących w skład rozprawy doktorskiej wyniósł 16,431

Suma punktów wg wykazu czasopism $MEiN_{2023}$ dla publikacji wchodzących w skład rozprawy doktorskiej wyniosła 390

1. WPROWADZENIE

1.1. Wstęp teoretyczny

Wstęp

Nowe badania wskazują, że spożywanie żywności funkcjonalnej o potencjale antyoksydacyjnym może być kluczowe w zachowaniu zdrowia. Nadmierny wzrost stężenia wolnych rodników na skutek czynników endogennych oraz egzogennych w organizmie może sprzyjać uszkodzeniom między innymi takich struktur biologicznych jak DNA, błony lipidowe i białka (El-Demerdash et al., 2018). Homeostaza oksydacyjno-redukcyjna komórek jest jednym z najważniejszych elementów regulujących funkcje organizmu na poziomie molekularnym, jej zaburzenie może sprzyjać dysfunkcjom, zwłaszcza pod względem aktywności enzymatycznej (Sies et al., 2017). Obecnie, poszukuje się naturalnych, ekologicznych i pozyskiwanych w zrównoważony sposób składników roślinnych, które mogą wspierać funkcje antyoksydacyjne organizmu.

Pędy drzew iglastych są rzadko stosowane jako składnik żywności mimo ich szerokiej dostępności w wielu częściach świata. Aktualnie na rynku dostępne takie produkty jak np. syropy z pędów sosny oraz piwa z dodatkiem składników z igliwia sosnowego oraz pędów, jednakże nadal są to produkty mało popularne (Penkina et al., 2017; Semeniuc et al., 2016). W sprzedaży dostępne są także syropy z pędów sosny z dodatkiem innych składników np. cytryny, malin, miodu i innych surowców naturalnych. Pędy sosny występują także jako składnik herbatek (np. Dary Natury, Plantago, Brown House & Tea) oraz mieszanek do przygotowania naparów z innymi ziołami lub owocami. W przeszłości pędy drzew iglastych często były stosowane w medycynie ludowej, m. in. w starożytnym Rzymie, w tradycyjnej medycynie chińskiej oraz islamskiej. Zarówno kora, pędy oraz żywice stosowane były w leczeniu chorób układu moczowego, układu pokarmowego, nerwowego, oddechowego oraz chorób skórnych (Akaberi et al., 2020; Bradley et al., 2014; Glaser & Zhao, 2012). Przeprowadzone w ostatnich latach badania potwierdzają, że związki zawarte w pędach drzew iglastych wykazują działanie terapeutyczne, a pędy są bogatymi źródłami polifenoli oraz cechują się właściwościami przeciwutleniającymi (Dziedziński et al., 2020; Salehi et al., 2019). Pędy drzew iglastych są źródłem pinenu, w formie izomerów alfa oraz beta. Alfa i beta-pinen mogą stanowić prekursor związków aromatycznych w produkcji żywności (Salehi et al., 2019). Związki zawarte w drzewach iglastych wykazują również działanie antyoksydacyjne (Emami et al., 2013).

(Burdock & Carabin, 2019). Literatura wskazuje na możliwe zastosowanie takich składników w napojach, produktach mlecznych, produktach mięsnych czy nawet pieczywie. Wskazano także na możliwość zwiększenia potencjału antyoksydacyjnego i przedłużenia trwałości produktów z dużym udziałem tłuszczu (López-Nicolás et al., 2014).

Stan wiedzy na temat właściwości i zastosowań komponentów drzew iglastych rosnących w Polsce jest niepełny. Nie przeprowadzono kompleksowych badań nad możliwościami zastosowania ich w żywności. Surowce te mogą być obiecującym składnikiem zarówno leków, ale także żywności funkcjonalnej lub suplementów diety, które należą do szybko rozwijającego się segmentu rynku żywnościowego. Dlatego w ramach pracy wykonano badania towaroznawcze oraz badania podstawowe dotyczące pędów modrzewia europejskiego, sosny zwyczajnej, daglezji zielonej, jodły pospolitej i jałowca pospolitego oraz badania aplikacyjne z wykorzystaniem wybranych pędów sosny zwyczajnej.

Charakterystyka botaniczna sosny zwyczajnej.

Pinus (*Pinaceae)* jest uznawany za najbardziej liczny rodzaj wśród drzew iglastych, do którego należy ponad 100 różnych gatunków (Tabela 1 i Tabela 2) (Dziedziński et al., 2021). Sosna (*Pinus* L.) jest największym i najbardziej heteromorficznym rodzajem roślin z rodziny sosnowatych (*Pinaceae* Lindl.), występującym prawie wyłącznie na półkuli północnej. Jest to rodzaj wszechstronny ekologicznie, rosnący od linii tundry w Eurazji po tropikalne sawanny przybrzeżne w Nikaragui i od strefy mgły solnej na wybrzeżu Pacyfiku po linię drzew alpejskich w Europie i zachodnich Stanach Zjednoczonych. Niektóre gatunki są również szeroko rozpowszechnione w regionach półpustynnych (Gernandt et al., 2005). Wysokość sosny jest gatunkowo zróżnicowana, *P. sylvestris* może osiągać 20-35 m, gdzie *P. nigra nawet 45 m* (Gernandt et al., 2005). Okres wegetacji sosny jest zależny od klimatycznych uwarunkowań oraz specyfiki gatunku. Przeważnie wegetacja sosny rozpoczyna się na wiosnę i trwa do jesieni (Gifford & Foster, 1989). Podłoże glebowe odgrywa kluczową rolę w rozwoju sosny. Optymalne warunki to żyzna gleba o dobrym drenażu. Optymalne pH gleby dla sosny wynosi od umiarkowanie kwaśnego do obojętnego (Gernandt et al., 2005).

Tabela 1. Hierarchia taksonomiczna rodzaju *Pinus L.* (Gifford & Foster, 1989)

Królestwo	*rośliny*
Podkrólestwo	*rośliny zielone*
Nadgromada	*rośliny telomowe*
Gromada	*rośliny naczyniowe*
Podgromada	*Rośliny nasienne*
Nadklasa	*nagonasienne*
Klasa	*iglaste*
Rząd	*sosnowce*
Rodzina	*sosnowate*
Rodzaj	*sosna*

Tabela 2. Klasyfikacja podrodzaju *Pinus* (Gernandt et al., 2005)

Sekcja *Pinus*		Sekcja *Trifoliae*		
Podsekcja *Pinus*	Podsekcja *Pinaster*	Podsekcja *Contortae*	Podsekcja *Australes*	Podsekcja *Ponderosae*
P. densata, densiflora, hwangshanensis, kesiya, luchuensis, massoniana, merkusii, mugo, nigra, resinosa, sylvestris, tabuliformis, taiwanensis, thunbergii, tropicalis, uncinata, yunnanensis	*P. brutia, canariensis, halepensis, heldreichii, pinaster, pinea, roxburghii.*	*P. banksiana, clausa, contorta, virginiana;*	*P. attenuata, caribaea, cubensis, echinata, elliottii, glabra, greggii, herrerae, jaliscana, lawsonii, leiophylla, lumholtzii, muricata, occidentalis, oocarpa, palustris, patula, praetermissa, pringlei, pungens, radiata, rigida, serotina, taeda, tecunumanii, teocote*	*P. cooperi, coulteri, donnell-smithii, devoniana, douglasiana, durangensis, engelmannii, hartwegii, jeffreyi, maximinoi, montezumae, nubicola, ponderosa, pseudostrobus, sabineana, torreyana, washoensis.*

Poszczególne części sosny cechują się różną zawartością składników odżywczych. Nasiona zawierają sumarycznie najwięcej badanych składników odżywczych, z wyjątkiem witaminy C, której zawartość wyższa jest w igliwiu. Nasiona mogą być dobrym źródłem magnezu, fosforu, a szczególnie cynku (Nergiz & Dönmez, 2004). Różne części roślin charakteryzują się

zróżnicowaną zawartością związków odżywczych (Edelman & Colt, 2016). Nasiona na ogół cechują się niższą zawartością witamin niż zielone części roślin, natomiast wyższą zawartością makroskładników, szczególnie tłuszczów (Sayeed et al., 2004). Pobieranie składników mineralnych i ich zawartość w roślinie uzależniona jest nie tylko od zawartości w glebie w formie dostępnej dla roślin, ale i od wzajemnego ilościowego stosunku poszczególnych składników mineralnych w środowisku i poziomu zalesienia (Jelonek et al., 2016; Szulc, Ambroży-Deręgowska, et al., 2020; Szulc, Barłóg, et al., 2020; Usowicz & Lipiec, 2017). Nie bez znaczenia są też inne czynniki takie jak pH gleby, pogoda i zmiany klimatyczne, roczne i dobowe wahania temperatury, nawodnienie, dostęp światła, (Borreani et al., 2018; Köhler et al., 2019; Kyriacou et al., 2018).

1.2. Związki fenolowe

Polifenole są wtórnymi metabolitami roślin, które umożliwiają wzrost i ich rozwój. Chronią również rośliny przed owadami i innymi czynnikami (Aggarwal et al., 2010; Ghahremani et al., 2020; Ren et al., 2021). Polifenole roślin biorą udział w kształtowaniu właściwości sensorycznych, tj.: koloru, goryczy i cierpkości (Debnath-Canning et al., 2020; Soares et al., 2020). Pod względem budowy, wspólną cechą polifenoli jest obecność pierścieni benzenu i grup hydroksylowych. Są jednak bardzo zróżnicowane i można je podzielić na kilka podgrup. Istnieją różne sposoby kategoryzacji tych związków w oparciu o ich źródło pochodzenia, funkcję biologiczną, lub strukturę chemiczną (Tsao, 2010). Często stosuje się klasyfikacje według liczby obecnych pierścieni fenolowych i składników strukturalnych, które łączą te pierścienie, różnicując cząsteczki na kwasy fenolowe, flawonoidy, stylbeny i lignany (Manach et al., 2004; Manasa et al., 2021). Proste fenole i flawonoidy odpowiadają większości naturalnych substancji fenolowych. Ponadto najbardziej rozpowszechnioną grupą tych związków są flawonoidy. Ich wspólna organizacja to C6 – C3 – C6, co odpowiada dwóm pierścieniom aromatycznym (pierścieniom A i B) połączonym z trzema atomami węgla w celu wytworzenia utlenionego heterocyklu (pierścień C). W wyniku rodzaju hydroksylacji i różnic w pierścieniu chromanowym (pierścień C), flawonoidy można dalej podzielić na odrębne podgrupy, w tym antocyjany, flawan-3-ole, flawony, flawanony i flawonole (Cutrim & Cortez, 2018; Debelo et al., 2020; Wang et al., 2020).

Zapotrzebowanie na kwasy fenolowe jest bardzo wysokie w przemyśle, ponieważ stosowane są jako prekursory innych istotnych bioaktywnych cząsteczek, które są niezbędne w celach leczniczych, kosmetycznych i spożywczych. Kwasy fenolowe są również dostępne w

handlu jako suplementy diety (Ferreira-Santos et al., 2020). Do ekstrakcji polifenoli można wykorzystać różne części sosny (igły, nasiona, kora i szyszka) oraz różne rozpuszczalniki. Chociaż wszystkie ekstrakty z sosny zawierają znaczące ilości polifenoli, zawartość ich w ekstrakcie zależy od rozpuszczalnika, metody ekstrakcji, użytej części rośliny czy gatunku sosny. Wynika to z naturalnej zmienności, takiej jak genotyp, różnice w uprawie i warunki zbioru, klimatu, rodzaj gleby itp. (Bindes et al., 2019; Ferreira-Santos et al., 2020). Ustalono, że polifenole zmniejszają zachorowalność i spowalniają postęp chorób sercowo-naczyniowych, neurodegeneracyjnych i nowotworowych. Mechanizm działania polifenoli silnie wiąże się z ich aktywnością przeciwutleniającą i redukcją reaktywnych form tlenu w organizmie człowieka (Dzah et al., 2020; Gorzynik-Debicka et al., 2018). Oprócz tego prozdrowotne właściwości polifenoli roślinnych obejmują działanie przeciwzapalne, przeciwalergiczne, przeciwmiażdżycowe, przeciwzakrzepowe i przeciwutagenne (Gabaston et al., 2017).
Obecnie na rynku dostępne są preparaty z sosny, które są skoncentrowanym źródłem polifenoli. Najpopularniejszym jest Pycnogenol® (Horphag Research Ltd., UK, Geneve, Switzerland), który jest ekstraktem z sosny *P. pinaster*. Jakość tego ekstraktu jest określona w Farmakopei Stanów Zjednoczonych (USP 28). Głównymi składnikami Pycnogenolu® są procyjanidyny, składające z podjednostek katechiny i epikatechiny o różnych długościach łańcucha. Inne składniki to monomery polifenolowe, kwasy fenolowe lub cynamonowe oraz ich glikozydy. Jak wynika z wielu badań, składniki piknogenolu są wysoce biodostępne (D'Andrea, 2010).

1.3. Właściwości funkcjonalne składników drzew iglastych w żywności

Wykazano, że kora, igły, pyłek jak i inne części wielu gatunków drzew iglastych są dobrymi surowcami do wytwarzania produktów i były wykorzystywane od wielu lat (Li et al., 2015). Pierwsze udokumentowane zastosowanie ekstraktów z kory sosny sięga 1535 roku, kiedy francuski odkrywca Jacques Cartier opisał wydarzenia, w których on i jego załoga uniknęli śmierci w wyniku szkorbutu, choroby spowodowanej niedoborem witaminy C, poprzez picie naparów z kory sosny. W 1951 r. francuski badacz Jacques Masquelier rozpoczął badania and surowcami roślinnymi w celu zidentyfikowania składników bioaktywnych, gdzie udało mu się wyekstrahować proantocyjanidyny z kory *P. pinaster*, w ilościach, które mogły być zastosowane w celach komercyjnych (D'Andrea, 2010).

Obecnie wiele standaryzowanych ekstraktów różnych gatunków sosny jest stosowanych jako składniki suplementów diety i preparatów wspomagających leczenie wielu jednostek

chorobowych m.in. przewlekłych stanów zapalnych, zaburzeń układu krążenia i astmy. Przeprowadzono wiele badań *in vitro*, a także z udziałem zwierząt oraz ludzi, które wskazują na profilaktyczny i terapeutyczny efekt ekstraktów z różnych gatunków sosny (Robertson et al., 2020). W opublikowanym przez Cochrane Collaboration przeglądzie, przeanalizowano 27 kontrolowanych randomizowanych badań klinicznych oceniających wpływ suplementów, zawierających ekstrakty z kory sosny, na choroby przewlekłe tj. astma, zespół nadpobudliwości psychoruchowej z deficytem uwagi, choroby układu krążenia cukrzyca, choroba zwyrodnieniowa stawów, osteopenia i in. Wykazano, że ograniczona liczba badań laboratoryjnych, zmienność wyników pomiarów wyników i różny sposób przeliczania wyników sprawia, że nie jest możliwe sformułowanie jednoznacznych wniosków dotyczących efektywności i bezpieczeństwa suplementów zawierających ekstrakt z kory sosny (Robertson et al., 2020). Aktualna literatura wskazuje jednak na możliwość zastosowania składników drzew iglastych w napojach, produktach mlecznych, produktach mięsnych czy nawet pieczywie. Dodatek ekstraktów *P. pinaster* zwiększał potencjał antyoksydacyjny soków i produktów mlecznych (Frontela-Saseta et al., 2011; López-Nicolás et al., 2014). Wykazano, że kefir wzbogacony syropem z pąków sosny charakteryzował się wysoką pożądalnością pod względem zarówno smaku jak i aromatu (Semeniuc et al., 2016). Ponadto potwierdzono, że dodatek ekstraktu z sosny do pieczywa i mielonego mięsa, wpływał zarówno na cechy sensoryczne jak również ograniczał rozwój niepożądanych drobnoustrojów oraz opóźniał zmiany oksydacyjne we frakcji tłuszczowej (Ahn et al., 2002, 2007). Wskazuje to, że ekstrakty z sosny mogą znaleźć w przyszłości szerokie zastosowanie jako dodatki konserwujące. Na chwilę obecną wiele ekstraktów z roślin iglastych znajduje się w bazie danych Everything Added to Food in the United States (EAFUS), które zostały zatwierdzone przez Food and Drug Administration (FDA) jako dodatki dozwolone do stosowania w produkcji żywności i są uznane za bezpieczne i posiadają status Generally Recognized as Safe (GRAS) (Ahn et al., 2007).

Niniejszy rozdział został opracowany na podstawie publikacji 1:

Dziedzinski, M., Kobus-Cisowska, J., Szymanowska, D., Stuper-Szablewska, K., Baranowska, M. (2020). Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds. *Molecules*, *25*(15), 3527. DOI: 10.3390/molecules25153527

2. CEL PRACY I HIPOTEZY BADAWCZE

2.1. Cel pracy

Problemem badawczym i celem nadrzędnym pracy była ocena właściwości funkcjonalnych pędów drzew iglastych i nowe możliwości ich wykorzystania w projektowaniu żywności funkcjonalnej.

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

1. Ocena towaroznawcza i zawartość składników aktywnych pędów wybranych drzew iglastych jako surowców o właściwościach przeciwutleniających, przeciwdrobnoustrojowych i właściwościach hamujących aktywność hialuronidazy.
2. Ocena wpływu suszenia na zawartość wybranych składników aktywnych i właściwości funkcjonalne pędów sosny *Pinus sylvestris* L.
3. Ocena przydatności pędów sosny *Pinus sylvestris* L. jako składnika recepturowego w technologii wytwarzania funkcjonalnego piwa pszenicznego typu Hefe-Weizen.
4. Ocena przydatności pędów sosny *Pinus sylvestris* L. jako składnika recepturowego w technologii wytwarzania funkcjonalnego piwa niskoalkoholowego typu IPA.

2.2. Hipotezy badawcze

Na podstawie zaplanowanych zadań sformułowano następujące hipotezy badawcze:

H.1. Pędy drzew iglastych są źródłem składników o potencjale przeciwutleniającym, przeciwdrobnoustrojowym oraz posiadają składniki hamujące aktywność hialuronidazy.

H.2. Zawartość związków aktywnych w pędach drzew iglastych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa ekstraktów zależą od gatunku drzewa, z którego pozyskano pędy, metody suszenia surowca i sposobu ekstrakcji.

H.3. Pędy sosny zwyczajnej (*Pinus sylvestris* L.) nie wpływają negatywnie na aktywność drożdży w procesie produkcji piwa typu Hefe-Waizen, a ich dodatek powoduje zwiększenie zawartości polifenoli i wzrost potencjału przeciwutleniającego jak również uzyskanie atrakcyjnego profilu sensorycznego piwa.

H.4. Pędy sosny zwyczajnej (*Pinus sylvestris* L.) nie wpływają negatywnie na aktywność drożdży w procesie produkcji piwa niskoalkoholowego, a ich dodatek powoduje zwiększenie zawartości polifenoli i wzrost potencjału przeciwutleniającego jak również uzyskanie atrakcyjnego profilu sensorycznego piwa.

3. PRZEDMIOT I METODY BADAŃ

3.1. Materiał badany

Materiałem do badań były pędy wybranych drzew iglastych: świerku pospolitego (*Picea abies* L.), modrzewia europejskiego (*Larix decidua* Mill), sosny zwyczajnej (*Pinus sylvestris* L.), daglezji zielonej (*Pseudotsuga menziesii*), jodły pospolitaej (*Abies alba*) i jałowca pospolitego (*Juniperus communis* L.), pozyskane w tym samym okresie wegetacyjnym z Arboretum Leśnego w Zielonce (17º06'33''E, 52º06'33''N) należącego do Leśnego Zakładu Doświadczalnego Uniwersytetu Przyrodniczego w Poznaniu. Materiał badany przechowywano w stanie zamrożonym (-28°C), a następnie przetwarzano zgodnie z metodyką badawczą w danym etapie.

3.2. Metody badań

W pracy zbadano:

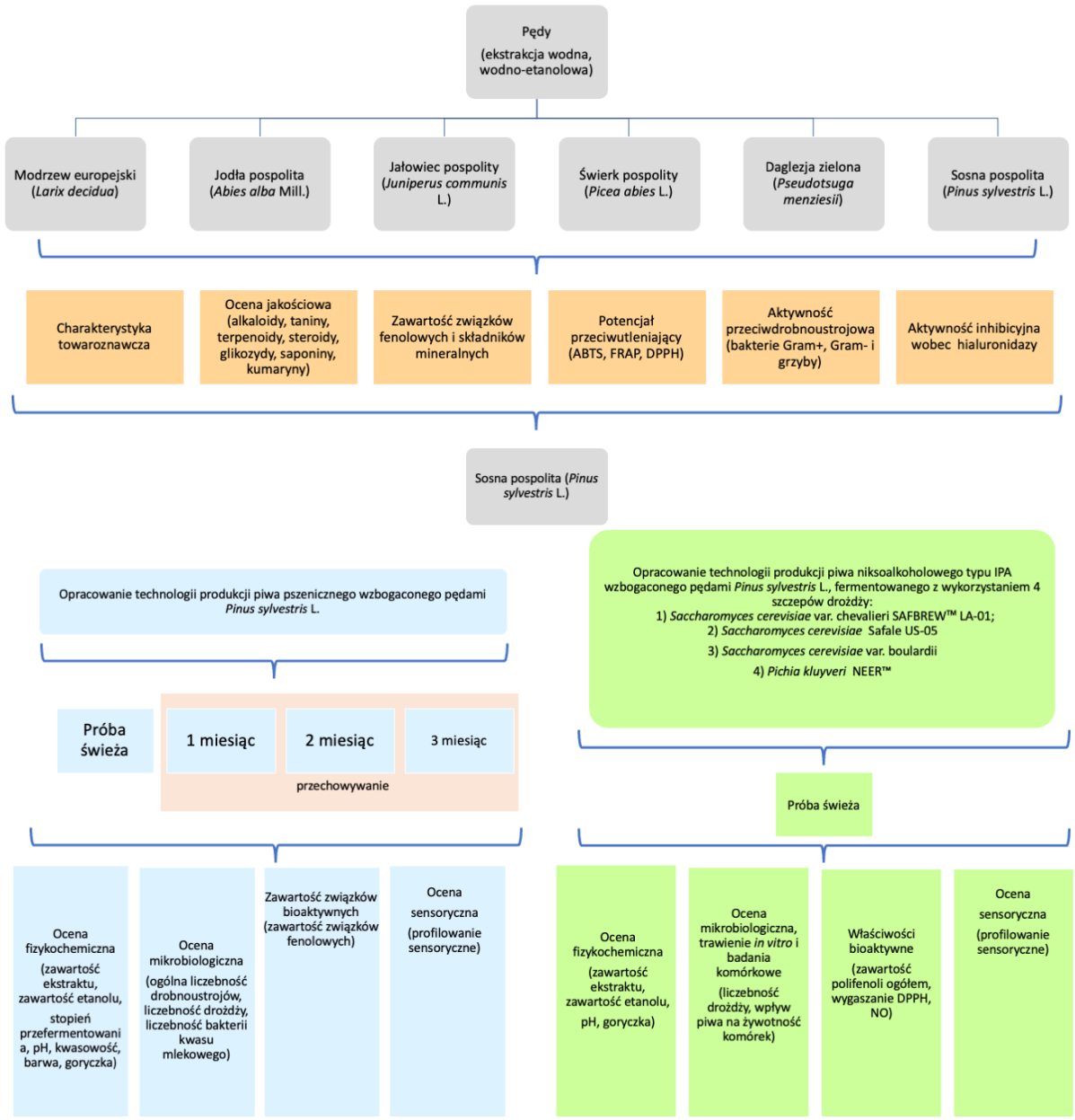
- Wydajność ekstrakcji (Pham et al., 2015)
- Barwę i osmolalność ekstraktów (Szczepaniak et al., 2021)
- Zawartość składników mineralnych (oznaczona techniką ICP-OES) (Lante et al., 2006)
- Zawartość witaminy C (metoda spektrofotometryczna) (Ajila et al., 2007)
- Zawartość polifenoli ogółem (metoda spektrofotometryczna) (Singleton et al., 1999)
- Zawartość flawonoidów ogółem (metoda spektrofotometryczna) (Meda et al., 2005)
- Zawartość indywidualnych związków polifenolowych (wysokosprawna chromatografia cieczowa, HPLC) (Stuper-Szablewska et al., 2017)
- Zawartość chlorofili i karotenoidów (metoda spektrofotometryczna)
- Obecność fitozwiązków tj. alkaloidy, saponiny, taniny, steroidy, terpenoidy, glikozydy, kumaryny (Auwal et al., 2014; Djaafar & Ridha, 2014; Morris, 2003)
- Aktywność przeciwutleniającą (metoda spektrofotometryczna: zdolność do zmiatania rodników DPPH, zdolność do wygaszania kationorodników ABTS, zdolność do

redukowania jonów żelaza (FRAP), aktywność chelatująca (O'Sullivan et al., 2013; Thaipong et al., 2006)

- Właściwości przeciwdrobnoustrojowe (metoda studzienkowo-dyfuzyjna) (Smith et al., 1985)
- Aktywność inhibicyjną wobec hialuronidazy (metoda spektrofotometryczna) (Chanaj-Kaczmarek et al., 2020)
- Właściwości fizyko-chemiczne piwa (metody instrumentalne) (Christensen et al., 2005)
- Liczebność wybranych drobnoustrojów w brzeczce i piwie (metoda płytkowa) (Clark, 1965)
- Wygaszanie wolnych rodników na liniach komórkowych (metoda spektrofotometryczna) (Pereira et al., 2017)
- Trawienie *in vitro* piwa suplementowanego pędami (Minekus et al., 2014)
- Analizę sensoryczną (profilowanie sensoryczne oraz ocena konsumencka) (*PN-EN ISO 8589:2010 Analiza Sensoryczna -- Ogólne Wytyczne Dotyczące Projektowania Pracowni Analizy Sensorycznej*, 2010)
- Analizę statystyczną (Statistica 13.1, TIBCO Software Inc.; RStudio 1.1.4, Posit Software)

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

3.3. Model badań



Rysunek 1. Model badań

4. OMÓWIENIE WYNIKÓW

4.1. Ocena przydatności i charakterystyka towaroznawcza pędów wybranych drzew iglastych jako surowców o właściwościach przeciwutleniających, przeciwdrobnoustrojowych i inhibicyjnych względem enzymów

Celem pierwszego etapu badań była ocena pędów wybranych drzew iglastych pod względem towaroznawczym i zawartości składników mineralnych, oraz ich ekstraktów pod względem

obecności i/lub zawartości wybranych metabolitów roślin, oceny aktywności przeciwutleniającej, przeciwdrobnoustrojowej i hamującej aktywność hialuronidazy.

Materiałem badanym były pędy drzew iglastych tj. świerk pospolity (*Picea abies* L.), modrzew europejski (*Larix decidua* Mill), sosna zwyczajna (*Pinus sylvestris* L.), daglezja zielona (*Pseudotsuga menziesii*), jodła pospolita (*Abies alba*) i jałowiec pospolity (*Juniperus communis* L.). Wytworzono ekstrakty wodne i etanolowo-wodne.

Najwyższą całkowitą zawartość składników mineralnych stwierdzono w próbce jodły pospolitej. Spośród analizowanych składników mineralnych, najwyższą zawartość stwierdzono potasu: w zakresie od 27 653,46 mg/kg w próbie jałowca pospolitego do 19 118,44 mg/kg w próbce sosny pospolitej. Ponadto stwierdzono wysoką zawartość wapnia i magnezu w badanych próbach. We wszystkich ekstraktach stwierdzono obecność garbników, terpenoidów, saponin i kumaryn.

Najwyższą aktywność redukującą mierzoną z odczynnika Folina-Ciocâlteu (FCR) (zwartość związków reagujących z odczynnikiem F-C) oraz całkowitą zawartość flawonoidów stwierdzono w ekstrakcie z pędów modrzewia europejskiego (14,83 mg GAE/g s.m.) i daglezji zielonej (14,53 mg GAE/g s.m.).

Najwyższą sumę badanych związków fenolowych stwierdzono w ekstrakcie ze świerku pospolitego (13,95 μg/g s.m.). Dominującym kwasem był kwas ferulowy, kwas chlorogenowy i kwas 4-hydroksybenzoesowy. Otrzymane ekstrakty z pędów roślin iglastych wykazały właściwości przeciwutleniające i przeciwdrobnoustrojowe *in vitro*. Ekstrakty ze świerku charakteryzowały się najwyższą aktywnością zmiatania rodnika DPPH (404,18 μM Trolox/g s.m.), a następnie ekstrakty z pędów jałowca (384,30 μM Trolox/g s.m.) Wyższą aktywność hamowania hialuronidazy (enzymu depolimeryzującego kwas hialuronowy) stwierdzono dla ekstraktów etanolowych niż wodnych badanych pędów, a spośród nich próbka ekstraktu modrzewia europejskiego charakteryzowała się najwyższym potencjałem (78,16%). Ekstrakty otrzymane z pędów świerku pospolitego i sosny zwyczajnej wykazywały najwyższą aktywność przeciwdrobnoustrojową wobec badanych bakterii Gram-ujemnych, Gram-dodatnich i grzybów.

PODSUMOWANIE

1. Pędy drzew iglastych takich jak świerk pospolity (*Picea abies* L.), modrzew europejski (*Larix decidua* Mill), sosna zwyczajna (*Pinus sylvestris* L.), daglezja zielona (*Pseudotsuga menziesii*), jodła pospolita (*Abies alba*) i jałowiec pospolity (*Juniperus*

communis L.). zawierają składniki mineralne, a spośród nich dominującymi są potas, magnez i wapń.

2. Ekstrakty wodne pozyskane z pędów drzew iglastych są źródłem związków aktywnych o działaniu przeciwutleniającym i przeciwdrobnoustrojowym.
3. Ekstrakty wodne z pędów jałowca pospolitego oraz świerku pospolitego wykazały najwyższą zdolność do wygaszania wolnych rodników DPPH.
4. Ekstrakty etanolowe z pędów wybranych drzew iglastych wykazują aktywność hamowania hialuronidazy.
5. Ekstrakty otrzymane z pędów świerku pospolitego i sosny zwyczajnej wykazywały najwyższą aktywność przeciwdrobnoustrojową wobec bakterii Gram-ujemnych, Gram-dodatnich i grzybów.

Na podstawie badań potwierdzono hipotezy:

H.1. Pędy drzew iglastych są źródłem składników o potencjale przeciwutleniającym, przeciwdrobnoustrojowym oraz posiadają składniki hamujące aktywność hialuronidazy.
H.2. Zawartość związków aktywnych w pędach drzew iglastych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa ekstraktów zależą od gatunku drzewa, z którego pozyskano pędy, metody suszenia surowca i sposobu ekstrakcji.

Powyższe wyniki zostały opisane na podstawie następujących publikacji wchodzącej w skład rozprawy doktorskiej:

Dziedzinski, M., Kobus-Cisowska, J., Szymanowska, D., Stuper-Szablewska, K., Baranowska, M. (2020). Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds. *Molecules*, *25*(15), 3527. DOI: 10.3390/molecules25153527

Dziedzinski, M., Kobus-Cisowska, J., Stuper-Szablewska, K., Cielecka-Piontek, J., Wilk, R., Ludowicz, D. (2022). Antioxidant potential, mineral composition and inhibitory effects of conifer needle extract on hyaluronidase-prospects of application in functional food. *Journal of Elementology*, 27(4). DOI: 10.1016/j.ejbt.2023.01.001

4.2. Ocena wpływu suszenia na zawartość wybranych składników bioaktywnych i właściwości funkcjonalne pędów sosny (*Pinus sylvestris* L.)

Celem etapu było zweryfikowano wpływu różnych metod suszenia tj. liofilizacja (PSL), suszenie próżniowe (PSP) i suszenie naturalne, na wolnym powietrzu (PSN) na zawartość wybranych składników bioaktywnych i właściwości funkcjonalne pędów *P. sylvestris* L. Wykazano, że zastosowane metody suszenia mają istotny wpływ na właściwości fizykochemiczne, zawartość związków bioaktywnych oraz aktywność przeciwutleniającą ekstraktów etanolowo-wodnych z pędów sosny zwyczajnej (*Pinus sylvestris* L.). Najwyższą całkowitą zawartość flawonoidów wykazano w próbce PSP, wynoszącej 5,51 mg kwercetyny/g s.m. Natomiast zdolność redukująca kształtowała się następująco: PSN>PSP>PSL w zakresie od 13,4 do 5,73 mg kwasu galusowego/g s.m. Najwyższą zawartością sumy polifenoli (HPLC) charakteryzował się ekstrakt z surowca liofilizowanego (91,51 mg/g), następnie suszonego próżniowo (8,26 mg/g), a najniższą zawartość związków fenolowych stwierdzono w ekstrakcie z pędów suszonych konwekcyjnie (7,62 mg/g). Badane ekstrakty wykazały właściwości przeciwutleniające, zarówno w teście badania aktywności do redukowania jonów żelaza (FRAP), jak również w teście zmiatania wolnych rodników (DPPH). Wszystkie badane ekstrakty wykazywały właściwości przeciwdrobnoustrojowe i grzybobójcze, a w szczególności aktywne były wobec bakterii Gram-ujemnych.

PODSUMOWANIE

1. Liofilizacja (PSL), suszenie próżniowe (PSP) i suszenie naturalne (PSN) mają istotny wpływ na właściwości fizykochemiczne, zawartość związków bioaktywnych i aktywność przeciwutleniającą ekstraktów wodno-etanolowych.
2. Najwyższą zawartość polifenoli stwierdzono w ekstrakcie z liofilizowanego surowca, a następnie w próżniowo suszonym, a najniższą zawartość związków fenolowych zaobserwowano w suszonych konwekcyjnie.
3. Badane ekstrakty wykazywały właściwości przeciwutleniające zarówno w teście FRAPjak i w pomiarze hamowania rodników DPPH.
4. Wszystkie badane ekstrakty wykazywały właściwości przeciwbakteryjne i przeciwgrzybicze, a szczególnie skuteczne były w przypadku bakterii Gram-ujemnych.

Na podstawie badań potwierdzono hipotezę badawczą:

H.2. Zawartość związków aktywnych w pędach drzew iglastych, potencjał przeciwutleniający i aktywność przeciwdrobnoustrojowa ekstraktów zależą od gatunku drzewa, z którego pozyskano pędy, metody suszenia surowca i sposobu ekstrakcji.

Powyższe wyniki zostały opisane na podstawie następującej publikacji wchodzącej w skład rozprawy doktorskiej:

Dziedziński, M., Kobus-Cisowska, J., Szymanowska-Powałowska, D., Stuper-Szablewska, K., Baranowska, M. (2020). Polyphenols composition, antioxidant, and antimicrobial properties of Pinus sylvestris L. shoots extracts depending on different drying methods. *Emirates Journal of Food and Agriculture*, 229-237. DOI: 10.9755/ejfa.2020.v32.i3.2080

4.3. Opracowanie innowacyjnej technologii wytwarzania piwa z udziałem pędów *Pinus sylvestris* L. (badania przechowalnicze, ocena sensoryczna oraz właściwości funkcjonalne napoju alkoholowego z pędami *Pinus sylvestris* L.)

Celem etapu była ocena możliwości zastosowania pędów sosny jako składnika piwa typu Hefe-Weizen. W procesie produkcyjnym zastąpiono część chmielu przewidzianego recepturą pędami sosny i oceniono ich wpływ na właściwości funkcjonalne i sensoryczne piwa. Na podstawie wcześniejszych badań i przeglądu literatury dokonano ewaluacji możliwości zastosowania pędów *Pinus sylvestris* L. w kształtowaniu cech antyoksydacyjnych i sensorycznych piwa pszenicznego. Analizie poddano podstawowe parametry fizykochemiczne, stężenie i profil polifenoli, stan mikrobiologiczny piwa oraz jego jakość sensoryczną (piwa doświadczalnego z pędami sosny oraz piwa kontrolnego).

Zawartość alkoholu w piwie doświadczalnym po przechowywaniu wynosiła 4,04% v/v, a goryczka kształtowała się na poziomie 15,83 IBU (jednostek goryczy). W badanej próbie stwierdzono wyższy poziom goryczki w porównaniu z piwem kontrolnym. Pozostałe analizowane parametry fermentacji (ekstrakt, stopień przefermentowania) oraz parametry fizykochemiczne (pH, kwasowość miareczkowa, barwa) były podobne dla obu rodzajów piwa. Stwierdzono, że dodatek pędów sosny na etapie warzenia wpłynął na profil związków biologicznie czynnych – zarówno kwasów polifenolowych jak i flawonoli. Zawartość obu grup

tych związków była o prawie 30% wyższa w próbce z pędami sosny w porównaniu z próbą kontrolną. Ocena sensoryczna potwierdziła wysoką atrakcyjność piwa z pędami sosny. Podczas trzymiesięcznego okresu przechowywania badane próbki były stabilne pod względem mikrobiologicznym.

Stwierdzono, że pędy sosny mogą być atrakcyjnym dodatkiem funkcjonalnym do smakowych piw rzemieślniczych. Ich dodatek nie wpływa negatywnie na atrakcyjność sensoryczną a jednocześnie wpływa na zwiększenie zawartości związków czynnych i potencjale antyoksydacyjnym.

PODSUMOWANIE

1. Pędy sosny nie wpływają negatywnie na proces fermentacji i właściwości fizykochemiczne piwa typu Hefe-Weizen.
2. Dodatek pędów sosny na etapie warzenia zwiększa zawartość związków biologicznie czynnych – zarówno kwasów polifenolowych jak i flawonoli w piwie typu Hefe-Weizen.
3. Piwo wzbogacane w pędy sosny na etapie warzenia cechuje się dobrą jakością organoleptyczną.

Na podstawie badań potwierdzono hipotezę badawczą:

H.3. Pędy sosny zwyczajnej (Pinus sylvestris L.) nie wpływają negatywnie na aktywność drożdży w procesie produkcji piwa typu Hefe-Waizen, a ich dodatek powoduje zwiększenie zawartości polifenoli i wzrost potencjału przeciwutleniającego jak również uzyskanie atrakcyjnego profilu sensorycznego piwa.

Powyższe wyniki zostały opisane na podstawie następujących publikacji wchodzącej w skład rozprawy doktorskiej:

Dziedziński, M., Stachowiak, B., Kobus-Cisowska, J., Kozłowski, R., Stuper-Szablewska, K., Szambelan, K., Górna, B. (2023). Supplementation of beer with *Pinus sylvestris* L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles. *Electronic Journal of Biotechnology,* 63, 10-17. DOI: 10.1016/j.ejbt.2023.01.001

4.4. Opracowanie innowacyjnej technologii piwa niskoalkoholowego z udziałem pędów *Pinus sylvestris* L. (ocena przechowalnicza, sensoryczna oraz ewaluacja właściwości funkcjonalnych piwa niskoalkoholowego z pędami *Pinus sylvestris* L.)

W ostatnim etapie badań oceniono możliwość wykorzystania pędów sosny (*Pinus sylvestris* L.) oraz standardowych i niekonwencjonalnych szczepów drożdży do produkcji piwa o obniżonej zawartości alkoholu.

W tym celu przygotowano brzeczki kontrolne bez dodatku pędów oraz brzeczki wzbogacane w pędy sosny w ilości 10 g/L. Brzeczki zaszczepiono drożdżami *Saccharomyces cerevisiae* var. chevalieri SAFBREW™ LA-01; *Saccharomyces cerevisiae* var. boulardii, *Pichia kluyveri* NEER™, *Saccharomyces cerevisiae* Safale US-05. Doświadczalne piwo zostało zbadane pod kątem liczebności drożdży, podstawowych właściwości fizykochemicznych piwa, oddziaływania na linie komórkowe i właściwości przeciwutleniających.

Wykazano, że pędy sosny oraz badane szczepy drożdży umożliwiają otrzymanie piw niskoalkoholowych o potencjalnym działaniu funkcjonalnym. Pędy sosny nie wpływają negatywnie na proces produkcji piwa i mogą zwiększać jego potencjał przeciwutleniający. Nie zauważono negatywnych zmian po miesiącu przechowywania. Wykazano, że piwa suplementowane pędami sosny charakteryzują się wyższą aktywnością do wygaszania wolnych rodników DPPH, natomiast wszystkie badane piwa niskoalkoholowe cechowały się aktywnością redukującą stan oksydacyjny indukowany na liniach komórkowych RAW264.7. W zależności od użytych drożdży pędy sosny wpływają w różny sposób na profil smakowy i zapachowy, mogąc maskować obce posmaki i zapachy, wynikające z zastosowania niekonwencjonalnych drożdży.

PODSUMOWANIE

1. Pędy sosny w stężeniu 10g/L brzeczki nie wpływają negatywnie na aktywność drożdży *Saccharomyces cerevisiae* Safale US-05; *Saccharomyces cerevisiae* var. chevalieri SAFBREW™ LA-01; *Saccharomyces cerevisiae* var. boulardii and *Pichia kluyveri* NEER™, a zastosowanie tych drożdży i pędów sosny pozwala na uzyskanie piwa niskoalkoholowego przechowywanego przez 1 miesiąc.

2. Szczep drożdży tj. *Saccharomyces cerevisiae* Safale US-05; *Saccharomyces cerevisiae* var. chevalieri SAFBREW™ LA-01; *Saccharomyces cerevisiae* var. boulardii i *Pichia kluyveri* NEER™ oraz pędy sosny *Pinus sylvestris* wpływają na smak i aromat piwa o obniżonej zawartości alkoholu.

Na podstawie badań potwierdzono hipotezę badawczą:

H.4. Pędy sosny zwyczajnej (*Pinus sylvestris* L.) nie wpływają negatywnie na aktywność drożdży w procesie produkcji piwa niskoalkoholowego, a ich dodatek powoduje zwiększenie zawartości polifenoli i wzrost potencjału przeciwutleniającego jak również uzyskanie atrakcyjnego profilu sensorycznego piwa.

Powyższe wyniki zostały opisane na podstawie następującej publikacji wchodzącej w skład rozprawy doktorskiej:

Dziedziński, M., Stachowiak, B., Kobus-Cisowska, J, Faria M., Ferreira I. (2023). Properties of Functional Low-Alcoholic Beer Fermented with *Pinus sylvestris* L. Shoots and Novel Yeast Strains. *Open Chemistry*. DOI:

5. PODSUMOWANIE I WNIOSKI

Wyniki przeprowadzonych badań pozwoliły na wysunięcie następujących stwierdzeń i wniosków:

- Pędy drzew iglastych tj. świerk pospolity (*Picea abies* L.), modrzew europejski (*Larix decidua* Mill), sosna zwyczajna (*Pinus sylvestris* L.), daglezja zielona (*Pseudotsuga menziesii*), jodła pospolita (*Abies alba*) i jałowiec pospolity (*Juniperus communis* L.). różnią się istotnie pod względem cech fizykochemicznych i towaroznawczych.
- Pędy wybranych drzew iglastych mogą być źródłem składników mineralnych tj. potas, magnez i wapń.
- Ekstrakty wodne oraz wodno-etanolowe z badanych pędów drzew iglastych cechują się wysoką zawartością związków fenolowych, oraz potencjałem przeciwutleniającym

mierzonym *in vitro* w testach FRAP, ABTS, DPPH. Zawartość ta jest zależna od metody ekstrakcji. Wykazano, że sposób utrwalania pędów sosny pospolitej (*P. sylvestris*) wpływa na właściwości przeciwutleniające i zawartość fenoli.

- W ekstraktach wykazano obecność garbników, terpenoidów, saponin i kumaryn.
- Badane ekstrakty wodne i wodno-etanolowe cechują się działaniem przeciwdrobnoustrojowym wobec bakterii Gram-ujemnych, Gram-dodatnich i grzybów.
- Ekstrakty etanolowe wykazały zdolność do inhibcji hialuronidazy *in vitro*. Najsilniejszym efektem inihbicyjnym cechował się ekstrakt z modrzewia europejskiego.
- Dodatek pędów sosny zwyczajnej w dodawane w stężeniu 10g/L brzeczki nie oddziałuje negatywnie na proces technologiczny warzenia konwencjonalnego piwa pszenicznego oraz piwa niskoalkoholowego typu IPA. Składniki pędów nie wpływają negatywniena aktywność drożdży tj.: *Saccharomyces cerevisiae* Safale US-05; *Saccharomyces cerevisiae* var. chevalieri SAFBREW™ LA-01; *Saccharomyces cerevisiae* var. boulardii and *Pichia kluyveri* NEER™.
- Piwo pszeniczne wytworzone z dodatkiem pędów sosny zwyczajnej w ilości 10g/L istotnie zwiększa ilość związków fenolowych w konwencjonalnym piwie pszenicznym.
- Piwo niskoalkoholowe suplementowane pędami sosny cechuje się wysoką zdolnością do wygaszania wolnych rodników DPPH *in vitro*.
- W zależności od zastosowanego szczepu drożdży, pędy sosny w różny sposób wpływają na postrzegany smak i aromat piwa alkoholowego i niskoalkoholowego.

6. ZASTOSOWANIE PRAKTYCZNE

Wyniki pracy wskazują na możliwości zastosowania składników pędów drzew iglastych, w tym przede wszystkim pędów sosny zwyczajnej *Pinus sylvestris* L., w technologii produkcji produktów spożywczych lub suplementów diety. Dodatek pędów sosny może wpłynąć na zwiększenie zawartości związków czynnych w środku spożywczym, które mogą działać przeciwdrobnoustrojowo, przeciwutleniająco i przeciwzapalnie. W pracy wykazano, że pędy sosny zwyczajnej mogą podwyższać zarówno potencjał przeciwutleniający jak i wpływać na

walory sensoryczne piwa typu Hefe-Weizen oraz niskoalkoholowego piwa typu IPA, co wskazuje na szerokie możliwości zastosowania pędów sosny w tego typu produktach.

7. ŹRÓDŁA FINANSOWANIA

Badania zrealizowane w ramach niniejszej pracy doktorskiej zostały sfinansowane ze środków:

- statutowych Katedry Technologii Gastronomicznej i Żywności Funkcjonalnej Uniwersytetu Przyrodniczego w Poznaniu (grant nr 506.751.03.00);
- programu Ministerstwa Nauki i Szkolnictwa Wyższego „Regionalna Inicjatywa Doskonałości" (nr 005/RID/2018/19); „Zintegrowanego Programu Uniwersytetu Przyrodniczego w Poznaniu na rzecz Innowacyjnej Wielkopolski" (nr POWR.03.05.00-00-ZR42/18)
- projektu rozwojowo-wdrożeniowego finansowanego przez ARiMR, program M16, Współpraca, „Opracowanie innowacji produktowej i procesowej związanej z technologią wytwarzania piwa ekologicznego zawierającego drobnoustroje probiotyczne, utrwalonego z zastosowaniem procesu refermentacji, prowadzonej z udziałem drobnoustrojów probiotycznych oraz wytwarzania dodatku spożywczego na bazie młóta będącego produktem ubocznym w procesie wytwarzania piwa, do przygotowania mieszanek wzbogacających pieczywo", Grupa Operacyjna: Biała Wrona (PROW 00053.DDD.6509.00062.2019.15, 2021-2023).

8. LITERATURA

1. Aggarwal, S., Thareja, S., Verma, A., Bhardwaj, T. R., & Kumar, M. (2010). An overview on 5α-reductase inhibitors. *Steroids*, *75*(2), 109–153. https://doi.org/10.1016/j.steroids.2009.10.005
2. Ahn, J., Grün, I. U., & Fernando, L. N. (2002). Antioxidant Properties of Natural Plant Extracts Containing Polyphenolic Compounds in Cooked Ground Beef. *Journal of Food Science*, *67*(4), 1364–1369. https://doi.org/10.1111/j.1365-2621.2002.tb10290.x
3. Ahn, J., Grün, I. U., & Mustapha, A. (2007). Effects of plant extracts on microbial growth, color change, and lipid oxidation in cooked beef. *Food Microbiology*, *24*(1), 7–14. https://doi.org/10.1016/j.fm.2006.04.006
4. Ajila, C. M., Bhat, S. G., & Prasada Rao, U. J. S. (2007). Valuable components of raw and ripe peels from two Indian mango varieties. *Food Chemistry*, *102*(4), 1006–1011.

https://doi.org/10.1016/j.foodchem.2006.06.036
5. Akaberi, M., Boghrati, Z., Amiri, M. S., Khayyat, M. H., & Emami, S. A. (2020). A review of conifers in Iran: Chemistry, biology and their importance in traditional and modern medicine. *Current Pharmaceutical Design*. https://doi.org/10.2174/1381612826666200128100023
6. Auwal, M. S., Saka, S., Mairiga, I. A., Sanda, K. A., Shuaibu, A., & Ibrahim, A. (2014). Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). *Veterinary Research Forum : An International Quarterly Journal*, *5*(2), 95–100.
7. Bindes, M. M. M., Cardoso, V. L., Reis, M. H. M., & Boffito, D. C. (2019). Maximisation of the polyphenols extraction yield from green tea leaves and sequential clarification. *Journal of Food Engineering*, *241*, 97–104. https://doi.org/10.1016/j.jfoodeng.2018.08.006
8. Borreani, G., Tabacco, E., Schmidt, R. J., Holmes, B. J., & Muck, R. E. (2018). Silage review: Factors affecting dry matter and quality losses in silages. *Journal of Dairy Science*, *101*(5), 3952–3979. https://doi.org/10.3168/jds.2017-13837
9. Bradley, W. G., Holm, K. N., & Tanaka, A. (2014). An orally active immune adjuvant prepared from cones of Pinus sylvestris, enhances the proliferative phase of a primary T cell response. *BMC Complementary and Alternative Medicine*, *14*(1), 163. https://doi.org/10.1186/1472-6882-14-163
10. Burdock, G. A., & Carabin, I. G. (2019). Chapter 10—Breaking down the barriers to functional foods, supplements, and claims1. In D. Bagchi (Ed.), *Nutraceutical and Functional Food Regulations in the United States and around the World (Third Edition)* (pp. 121–146). Academic Press. https://doi.org/10.1016/B978-0-12-816467-9.00010-1
11. Chanaj-Kaczmarek, J., Paczkowska, M., Osmałek, T., Kaproń, B., Plech, T., Szymanowska, D., Karaźniewicz-Łada, M., Kobus-Cisowska, J., & Cielecka-Piontek, J. (2020). Hydrogel Delivery System Containing Calendulae flos Lyophilized Extract with Chitosan as a Supporting Strategy for Wound Healing Applications. *Pharmaceutics*, *12*(7), E634. https://doi.org/10.3390/pharmaceutics12070634
12. Christensen, J., Ladefoged, A. M., & Nørgaard, L. (2005). Rapid Determination of Bitterness in Beer Using Fluorescence Spectroscopy and Chemometrics. *Journal of the Institute of Brewing*, *111*(1), 3–10. https://doi.org/10.1002/j.2050-0416.2005.tb00642.x
13. Clark, F. E. (1965). Agar-Plate Method for Total Microbial Count. In *Methods of Soil Analysis* (pp. 1460–1466). John Wiley & Sons, Ltd. https://doi.org/10.2134/agronmonogr9.2.c48
14. Cutrim, C. S., & Cortez, M. A. S. (2018). A review on polyphenols: Classification, beneficial effects and their application in dairy products. *International Journal of Dairy Technology*, *71*(3), 564–578. https://doi.org/10.1111/1471-0307.12515
15. D'Andrea, G. (2010). Pycnogenol: A blend of procyanidins with multifaceted therapeutic applications? *Fitoterapia*, *81*(7), 724–736. https://doi.org/10.1016/j.fitote.2010.06.011
16. Debelo, H., Li, M., & Ferruzzi, M. G. (2020). Processing influences on food polyphenol profiles and biological activity. *Current Opinion in Food Science*, *32*, 90–102. https://doi.org/10.1016/j.cofs.2020.03.001
17. Debnath-Canning, M., Unruh, S., Vyas, P., Daneshtalab, N., Igamberdiev, A. U., & Weber, J. T. (2020). Fruits and leaves from wild blueberry plants contain diverse polyphenols and decrease neuroinflammatory responses in microglia. *Journal of Functional Foods*, *68*, 103906. https://doi.org/10.1016/j.jff.2020.103906
18. Djaafar, Z., & Ridha, O. M. (2014). Phytochemical Study of Selected Medicinal Plant, Solanum Nigrum, the Algerian Desert. *International Letters of Chemistry, Physics and*

Astronomy, *20*, 25–30. https://doi.org/10.18052/www.scipress.com/ILCPA.20.25
19. Dzah, C. S., Duan, Y., Zhang, H., Authur, D. A., & Ma, H. (2020). Ultrasound-, subcritical water- and ultrasound assisted subcritical water-derived Tartary buckwheat polyphenols show superior antioxidant activity and cytotoxicity in human liver carcinoma cells. *Food Research International*, *137*, 109598. https://doi.org/10.1016/j.foodres.2020.109598
20. Dziedziński, M., Kobus-Cisowska, J., Powałowska, D. S.-, Szablewska, K. S.-, & Baranowska, M. (2020). Polyphenols composition, antioxidant and antimicrobial properties of Pinus sylvestris L. shoots extracts depending on different drying methods. *Emirates Journal of Food and Agriculture*, 229–237. https://doi.org/10.9755/ejfa.2020.v32.i3.2080
21. Dziedziński, M., Kobus-Cisowska, J., & Stachowiak, B. (2021). Pinus Species as Prospective Reserves of Bioactive Compounds with Potential Use in Functional Food—Current State of Knowledge. *Plants*, *10*(7), Article 7. https://doi.org/10.3390/plants10071306
22. Edelman, M., & Colt, M. (2016). Nutrient Value of Leaf vs. Seed. *Frontiers in Chemistry*, *4*. https://doi.org/10.3389/fchem.2016.00032
23. El-Demerdash, F. M., Tousson, E. M., Kurzepa, J., & Habib, S. L. (2018). Xenobiotics, Oxidative Stress, and Antioxidants. *Oxidative Medicine and Cellular Longevity*, *2018*, 9758951. https://doi.org/10.1155/2018/9758951
24. Emami, S. A., Shahani, A., & Hassanzadeh Khayyat, M. (2013). Antioxidant Activity of Leaves and Fruits of Cultivated Conifers in Iran. *Jundishapur Journal of Natural Pharmaceutical Products*, *8*(3), 113–117.
25. Ferreira-Santos, P., Zanuso, E., Genisheva, Z., Rocha, C. M. R., & Teixeira, J. A. (2020). Green and Sustainable Valorization of Bioactive Phenolic Compounds from Pinus By-Products. *Molecules*, *25*(12), Article 12. https://doi.org/10.3390/molecules25122931
26. Frontela-Saseta, C., López-Nicolás, R., González-Bermúdez, C. A., Peso-Echarri, P., Ros-Berruezo, G., Martínez-Graciá, C., Canali, R., & Virgili, F. (2011). Evaluation of Antioxidant Activity and Antiproliferative Effect of Fruit Juices Enriched with Pycnogenol® in Colon Carcinoma Cells. The Effect of In Vitro Gastrointestinal Digestion: PYCNOGENOL® IN FRUIT JUICES. *Phytotherapy Research*, *25*(12), 1870–1875. https://doi.org/10.1002/ptr.3625
27. Gabaston, J., Richard, T., Cluzet, S., Palos Pinto, A., Dufour, M.-C., Corio-Costet, M.-F., & Mérillon, J.-M. (2017). Pinus pinaster Knot: A Source of Polyphenols against Plasmopara viticola. *Journal of Agricultural and Food Chemistry*, *65*(40), 8884–8891. https://doi.org/10.1021/acs.jafc.7b04129
28. Gernandt, D. S., López, G. G., García, S. O., & Liston, A. (2005). Phylogeny and Classification of Pinus. *Taxon*, *54*(1), 29–42. https://doi.org/10.2307/25065300
29. Ghahremani, A., Ghasemi Pirbalouti, A., Mozafari, H., Habibi, D., & Sani, B. (2020). Phytochemical and morpho-physiological traits of mullein as a new medicinal crop under different planting pattern and soil moisture conditions. *Industrial Crops and Products*, *145*, 111976. https://doi.org/10.1016/j.indcrop.2019.111976
30. Gifford, E. M., & Foster, A. S. (1989). *Morphology and Evolution of Vascular Plants* (Third Edition). W. H. Freeman.
31. Glaser, A., & Zhao, G. (2012). Utilization of Scots Pine oil in chinese medicine. *International Journal of Traditional Chinese Medicine*, *34*(3), 229–231.
32. Gorzynik-Debicka, M., Przychodzen, P., Cappello, F., Kuban-Jankowska, A., Marino Gammazza, A., Knap, N., Wozniak, M., & Gorska-Ponikowska, M. (2018). Potential Health Benefits of Olive Oil and Plant Polyphenols. *International Journal of Molecular*

Sciences, *19*(3), Article 3. https://doi.org/10.3390/ijms19030686
33. Jelonek, T., Gzyl, J., Arasimowicz-Jelonek, M., Tomczak, A., & Remlein, A. (2016). The influence of the selected ratios of trees stability on the wall thickness of tracheids in the scots pine (Pinus sylvestris L.). *Acta Scientiarum Polonorum - Silvarum Colendarum Ratio et Industria Lignaria*, *15*(1), 13–21.
34. Köhler, I. H., Huber, S. C., Bernacchi, C. J., & Baxter, I. R. (2019). Increased temperatures may safeguard the nutritional quality of crops under future elevated CO2 concentrations. *The Plant Journal*, *97*(5), 872–886. https://doi.org/10.1111/tpj.14166
35. Kyriacou, M. C., Leskovar, D. I., Colla, G., & Rouphael, Y. (2018). Watermelon and melon fruit quality: The genotypic and agro-environmental factors implicated. *Scientia Horticulturae*, *234*, 393–408. https://doi.org/10.1016/j.scienta.2018.01.032
36. Lante, A., Lomolino, G., Cagnin, M., & Spettoli, P. (2006). Content and characterisation of minerals in milk and in Crescenza and Squacquerone Italian fresh cheeses by ICP-OES. *Food Control*, *17*(3), 229–233. https://doi.org/10.1016/j.foodcont.2004.10.010
37. Li, Y.-Y., Feng, J., Zhang, X.-L., & Cui, Y.-Y. (2015). Pine Bark Extracts: Nutraceutical, Pharmacological, and Toxicological Evaluation. *Journal of Pharmacology and Experimental Therapeutics*, *353*(1), 9–16. https://doi.org/10.1124/jpet.114.220277
38. López-Nicolás, R., González-Bermúdez, C. A., Ros-Berruezo, G., & Frontela-Saseta, C. (2014). Influence of in vitro gastrointestinal digestion of fruit juices enriched with pine bark extract on intestinal microflora. *Food Chemistry*, *157*, 14–19. https://doi.org/10.1016/j.foodchem.2014.01.126
39. Manach, C., Scalbert, A., Morand, C., Rémésy, C., & Jiménez, L. (2004). Polyphenols: Food sources and bioavailability. *The American Journal of Clinical Nutrition*, *79*(5), 727–747. https://doi.org/10.1093/ajcn/79.5.727
40. Manasa, V., Padmanabhan, A., & Anu Appaiah, K. A. (2021). Utilization of coffee pulp waste for rapid recovery of pectin and polyphenols for sustainable material recycle. *Waste Management (New York, N.Y.)*, *120*, 762–771. https://doi.org/10.1016/j.wasman.2020.10.045
41. Meda, A., Lamien, C. E., Romito, M., Millogo, J., & Nacoulma, O. G. (2005). Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chemistry*, *91*(3), 571–577. https://doi.org/10.1016/j.foodchem.2004.10.006
42. Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food—An international consensus. *Food & Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j
43. Morris, B. (2003). The components of the Wired Spanning Forest are recurrent. *Probability Theory and Related Fields*, *125*(2), 259–265. https://doi.org/10.1007/s00440-002-0236-0
44. Nergiz, C., & Dönmez, İ. (2004). Chemical composition and nutritive value of Pinus pinea L. seeds. *Food Chemistry*, *86*(3), 365–368. https://doi.org/10.1016/j.foodchem.2003.09.009
45. O'Sullivan, A. M., O'Callaghan, Y. C., O'Connor, T. P., & O'Brien, N. M. (2013). Comparison of the Antioxidant Activity of Commercial Honeys, Before and After In-Vitro Digestion. *Polish Journal of Food and Nutrition Sciences*, *63*(3), 167–171. https://doi.org/10.2478/v10222-012-0080-6
46. Penkina, N., Tatar, L., Kolesnyk, V., Karbivnycha, T., & Letuta, T. (2017). THE

STUDY OF BEER QUALITY WITH THE REDUCED TOXIC EFFECT. *EUREKA: Life Sciences*, *0*(1), Article 1. https://doi.org/10.21303/2504-5695.2017.00303
47. Pereira, R. B., Pinto, D. C. G. A., Pereira, D. M., Gomes, N. G. M., Silva, A. M. S., Andrade, P. B., & Valentão, P. (2017). UHPLC-MS/MS profiling of Aplysia depilans and assessment of its potential therapeutic use: Interference on iNOS expression in LPS-stimulated RAW 264.7 macrophages and caspase-mediated pro-apoptotic effect on SH-SY5Y cells. *Journal of Functional Foods*, *37*, 164–175. https://doi.org/10.1016/j.jff.2017.07.053
48. Pham, H. N. T., Nguyen, V. T., Vuong, Q. V., Bowyer, M. C., & Scarlett, C. J. (2015). Effect of Extraction Solvents and Drying Methods on the Physicochemical and Antioxidant Properties of Helicteres hirsuta Lour. Leaves. *Technologies*, *3*(4), 285–301. https://doi.org/10.3390/technologies3040285
49. Pinho, S. C. M., Faria, M. A., Melo, A., Pinto, E., Almeida, A., Alves, R., Cabrita, A. R. J., Fonseca, A. J. M., & Ferreira, I. M. P. L. V. O. (2021). Effect of skimmed milk on intestinal tract: Prevention of increased reactive oxygen species and nitric oxide formation. *International Dairy Journal*, *118*, 105046. https://doi.org/10.1016/j.idairyj.2021.105046
50. *PN-EN ISO 8589:2010 Analiza sensoryczna—Ogólne wytyczne dotyczące projektowania pracowni analizy sensorycznej*. (2010). Polski Komitet Normalizacyjny. https://sklep.pkn.pl/pn-en-iso-8589-2010e.html
51. Ren, T., Zheng, P., Zhang, K., Liao, J., Xiong, F., Shen, Q., Ma, Y., Fang, W., & Zhu, X. (2021). Effects of GABA on the polyphenol accumulation and antioxidant activities in tea plants (Camellia sinensis L.) under heat-stress conditions. *Plant Physiology and Biochemistry: PPB*, *159*, 363–371. https://doi.org/10.1016/j.plaphy.2021.01.003
52. Robertson, N. U., Schoonees, A., Brand, A., & Visser, J. (2020). Pine bark (*Pinus* spp.) extract for treating chronic disorders. *Cochrane Database of Systematic Reviews*, *9*. https://doi.org/10.1002/14651858.CD008294.pub5
53. Salehi, B., Upadhyay, S., Erdogan Orhan, I., Kumar Jugran, A., L.D. Jayaweera, S., A. Dias, D., Sharopov, F., Taheri, Y., Martins, N., Baghalpour, N., C. Cho, W., & Sharifi-Rad, J. (2019). Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules*, *9*(11). https://doi.org/10.3390/biom9110738
54. Sayeed, M. A., Ali, M. A., Sohel, F. I., Khan, G. R. M. A. M., & Yeasmin, M. S. (2004). Physico-chemical characteristics of Mesua ferrea seed oil and nutritional composition of its seed and leaves. *Bulletin of the Chemical Society of Ethiopia*, *18*(2), Article 2. https://doi.org/10.4314/bcse.v18i2.61435
55. Semeniuc, C. A., Rotar, A., Stan, L., Pop, C. R., Socaci, S., Mireşan, V., & Muste, S. (2016). Characterization of pine bud syrup and its effect on physicochemical and sensory properties of kefir. *CyTA - Journal of Food*, *14*(2), 213–218. https://doi.org/10.1080/19476337.2015.1085905
56. Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative Stress. *Annual Review of Biochemistry*, *86*, 715–748. https://doi.org/10.1146/annurev-biochem-061516-045037
57. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology* (Vol. 299, pp. 152–178). Academic Press. https://doi.org/10.1016/S0076-6879(99)99017-1
58. Smith, L. B., Fox, T. L., & Busta, F. F. (1985). Comparison of a Dry Medium Culture Plate (Petrifilm SM Plates) Method to the Aerobic Plate Count Method for Enumeration of Mesophilic Aerobic Colony-Forming Units in Fresh Ground Beef. *Journal of Food Protection*, *48*(12), 1044–1045. https://doi.org/10.4315/0362-028X-48.12.1044
59. Soares, S., Brandão, E., Guerreiro, C., Soares, S., Mateus, N., & de Freitas, V. (2020).

Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. *Molecules*, *25*(11), Article 11. https://doi.org/10.3390/molecules25112590
60. Stuper-Szablewska, K., Kurasiak-Popowska, D., Nawracała, J., & Perkowski, J. (2017). Response of non-enzymatic antioxidative mechanisms to stress caused by infection with Fusarium fungi and chemical protection in different wheat genotypes. *Chemistry and Ecology*, *33*(10), 949–962. https://doi.org/10.1080/02757540.2017.1381689
61. Szczepaniak, O. M., Kobus-Cisowska, J., Nowosad, K., Stuper-Szablewska, K., Markowska, J., & Szulc, P. (2021). Relationship of colour with the phytocompounds present in Cornus mas cultivars. *International Journal of Food Properties*, *24*(1), 400–414. https://doi.org/10.1080/10942912.2021.1898420
62. Szulc, P., Ambroży-Deręgowska, K., Mejza, I., Kobus-Cisowska, J., & Ligaj, M. (2020). The Role of Agrotechnical Factors in Shaping the Protein Yield of Maize (Zea mays L.). *Sustainability*, *12*(17), Article 17. https://doi.org/10.3390/su12176833
63. Szulc, P., Barłóg, P., Ambroży-Deręgowska, K., Mejza, I., & Kobus-Cisowska, J. (2020). In-Soil Application of NP Mineral Fertilizer as a Method of Improving Nitrogen Yielding Efficiency. *Agronomy*, *10*(10), Article 10. https://doi.org/10.3390/agronomy10101488
64. Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., & Hawkins Byrne, D. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*(6), 669–675. https://doi.org/10.1016/j.jfca.2006.01.003
65. Tsao, R. (2010). Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients*, *2*(12), Article 12. https://doi.org/10.3390/nu2121231
66. Usowicz, B., & Lipiec, J. (2017). *Spatial variability of soil properties and cereal yield in a cultivated field on sandy soil*. https://depot.ceon.pl/handle/123456789/17303
67. Wang, H., Wang, C., Zou, Y., Hu, J., Li, Y., & Cheng, Y. (2020). Natural polyphenols in drug delivery systems: Current status and future challenges. *Giant*, *3*, 100022. https://doi.org/10.1016/j.giant.2020.100022

9. POZOSTAŁY DOROBEK NAUKOWY

I. Wykaz pozostałych artykułów naukowych posiadających współczynnik wpływu IF

1. Cielecka-Piontek, J., **Dziedziński, M.**, Szczepaniak, O., Kobus-Cisowska, J., Telichowska, A., Szymanowska, D. (2020). Survival of commercial probiotic strains and their effect on dark chocolate synbiotic snack with raspberry content during the storage and after simulated digestion. *Electronic Journal of Biotechnology*, 48, 62–71.
2. **Dziedziński, M**., Szczepaniak, O., Telichowska, A., Kobus-Cisowska, J. (2020). Antioxidant capacity and cholinesterase inhibiting properties of dietary infusions with *Humulus lupulus*. *Journal of Elementology*, 25(2).
3. Kobus-Cisowska, J., **Dziedziński, M**., Szczepaniak, O., Flaczyk, E., Telichowska, A., Krol, E., Staniek, H., Byczkiewicz, S. (2020). Effect of metal ions from Ginkgo biloba extracts on the oxidative stability of rapeseed oil and its triacylglycerols. *Journal of Elementology*, 25(3).
4. Kobus-Cisowska, J., **Dziedziński, M.**, Szczepaniak, O., Kusek, W., Kmiecik, D., Ligaj, M., Telichowska, A., Byczkiewicz, S., Szulc, P., Szwajgier, D. (2020). Phytocomponents and evaluation of acetylcholinesterase inhibition by *Ginkgo biloba* L. leaves extract depending on vegetation period. *CyTA - Journal of Food*, 18(1), 606–615.
5. Kobus-Cisowska, J., **Dziedziński, M.**, Szymanowska, D., Szczepaniak, O., Byczkiewicz, S., Telichowska, A., Szulc, P. (2020). The effects of *Morus alba* L. fortification on the quality, functional properties and sensory attributes of bread stored under refrigerated conditions. *Sustainability*, 12(16), 6691.
6. Kobus-Cisowska, J., Szczepaniak, O., Szymanowska-Powałowska, D., Piechocka, J., Szulc, P., **Dziedziński, M.** (2019). Antioxidant potential of various solvent extract from *Morus alba* fruits and its major polyphenols composition. *Ciência Rural*, 50.
7. Kobus-Cisowska, J., Szulc, P., Szczepaniak, O., **Dziedziński, M.**, Szymanowska, D., Szymandera-Buszka, K., Goryńska-Goldmann, E., Gazdecki, M., Telichowska,

A., Ligaj, M. (2020). Variability of *hordeum vulgare* L. cultivars in yield, antioxidant potential, and cholinesterase inhibitory activity. *Sustainability*, 12(5), 1938.

8. Szczepaniak, O., Ligaj, M., Kobus-Cisowska, J., Tichoniuk, M., **Dziedziński, M.**, Przeor, M., Szulc, P. (2020). The genoprotective role of naringin. *Biomolecules*, 10(5), 700.

II. Wykaz pozostałych artykułów naukowych

1. **Dziedziński, M.**, Goryńska-Goldmann, E., Kobus-Cisowska, J., Szczepaniak, O., Marciniak, G. (2019). Problem nadkonsumpcji suplementów diety przez Polaków. *Intercathedra*, *nr 40(3)*, 235–242.
2. **Dziedziński, M.**, Szczepaniak, O., Szymanowska, D., Szulc, P., Bielec, D., Kobus-Cisowska, J. (2019). Uprawa szparaga (*Asparagus officinalis* L.) jako surowca o właściwościach funkcjonalnych. Cz.II. Charakterystyka żywieniowa. *Technika Rolnicza Ogrodnicza Leśna*, 3, 5-8
3. Szczepaniak, O., Dziedzinski, M., Kobus-Cisowska, J., Szulc, P., Szymanowska, D., Sudyka, M., Goryńska-Goldmann, E. (2019). Chmiel (*Humulus lupulus* L.) jako surowiec o właściwościach prozdrowotnych: Aktualny stan wiedzy. *Technika Rolnicza Ogrodnicza Leśna*, *3*. 5-8
4. Szczepaniak, O., Dziedzinski, M., Szymanowska, D., Szulc, P., Bielec, D., Kobus-Cisowska, J. (2019). Uprawa szparaga (*Asparagus officinalis* L.) jako surowca o właściwościach funkcjonalnych. Cz.I. Charakterystyka towaroznawcza. *Technika Rolnicza Ogrodnicza Leśna*, 2, 2-6,
5. Telichowska, A., Dziedzinski, M., Szczepaniak, O., Kobus-Cisowska, J. (2019). Charakterystyka czeremchy (*Prunus*) pozyskanej z ekosystemów naturalnych. *Technika Rolnicza Ogrodnicza Leśna*, 5, 17-21
6. Kobus-Cisowska J., Szczepaniak O., Kmiecik D., **Dziedziński M**., Brzozowska A., Ligaj M., Fedko M., Szymanowska D., Szulc P., Goryńska-Goldmann E., (2019. Impact of *Salix viminalis* carbon as a bioactive component: developed a new proposal of application in functional bread. *Journal of Research and Applications* in *Agricultural Engineering*, 64(3), 25-30
7. Byczkiewicz, S., Kobus-Cisowska, J., Szulc, P., Telichowska, A., Szczepaniak, O.,

Dziedziński, M. (2019). Pigwowiec japoński (*Chaenomeles japonica* L.) jako surowiec o właściwościach prozdrowotnych – aktualny stan wiedzy. *Technika Rolnicza Ogrodnicza Leśna*, 5, 22-25.

8. Kobus-Cisowska, J., **Dziedziński, M.**, Szczepaniak, O., Jokiel, M., Szymandera-Buszka, K., Szymanowska, D., Ligaj, M. (2020). Evaluation of usefulness of selected food compounds as an active substances with modulating ability of bitter taste in chocolate. *Journal of Research and Applications in Agricultural Engineering*, 65(1), 9-13.
9. Zawadzka, A., **Dziedziński, M.**, Byczkiewicz, Sz., Baldys, W., Szulc, P., Kobus-Cisowska, J. (2020). Assessment of the content of polyphenol and the antioxidating potential in dried fruits populated on the market. *Journal of Research and Applications in Agricultural Engineering*, 65(1), 24-29.
10. Kozłowski, R., **Dziedziński, M.**, Stachowiak, B., Kobus-Cisowska, J. (2021). Non- and low-alcoholic beer - popularity and manufacturing techniques. *Acta Scientiarum Polonorum. Technologia Alimentaria,* 20(3), 347-357.
11. Kobus-Cisowska, J., Szablewski, T., Ziemniewska, P., **Dziedziński, M.** (2022). Skorupa kurzych jaj konsumpcyjnych: wartość odżywcza i właściwości funkcjonalne. *Zagadnienia Doradztwa Rolniczego*, 4(110), 36-48.
12. Zawadzka, A., Janczewska, A., **Dziedziński, M.**, Siwulski, M., Stuper-Szablewska, K., Szwajgier, D., Kobus-Cisowska, J. (2022). Effects of cultivation temperature on the content of selected active components in the anatomical parts of oyster mushroom (Plaurotus ostreatus L.). In Besztarda-Buszczak, M., Przeor, M. (Eds.), Food nowadays – local or global? Traditional or innovative? (pp. 53-64). Poznań: Uniwersytet Przyrodniczy w Poznaniu.
13. **Dziedziński, M.**, Kobus-Cisowska, J., Stachowiak, B. (2022). Ocena wpływu dodatku pędów sosny (Pinus sylvestris L.) na właściwości fizykochemiczne i zawartość wybranych witamin w piwie typu pszenicznego. In Besztarda-Buszczak, M., Przeor, M. (Eds.), Żywność dzisiaj Lokalna czy globalna, tradycyjna czy innowacyjna. Monografia prokonferencyjna (pp. 61-67). Poznań: Uniwersytet Przyrodniczy w Poznaniu.

III. Publikacje w czasopismach popularnonaukowych

1. **Dziedziński, M.**, Kobus-Cisowska, J. (2021). Nalewki – tradycyjny sposób Polaków na zdrowie, czy skuteczny. *Food Forum, 1*.
2. **Dziedziński, M.**, Kobus-Cisowska, J. (2021). Sosna jako źródło związków prozdrowotnych i leczniczych. *Naturoterapia w praktyce*, 24.
3. **Dziedziński, M.**, Kobus-Cisowska, J. (2021). Piwo w diecie świadomego konsumenta. Jakie korzyści i jakie zagrożenia wynikają z jego spożycia. *Food Forum,* 5(45), 96-99

IV. Doniesienia naukowe zaprezentowane na konferencjach naukowych

1. Szczepaniak, O., Mikołajczak-Ratajczak A., Malak M., Ligaj, M., **Dziedziński, M.**, Kobus-Cisowska J., Kikut-Ligaj, D. (2019). Antyoksydacyjne właściwości Yerba Mate w zależności od czasu i temperatury parzenia naparu. II Ogólnopolska Konferencja „Biotechnologia Niejedno ma imię", Uniwersytet Przyrodniczy w Poznaniu, Poznań
2. Telichowska, A., Baranowska M., **Dziedziński M**, Szczepaniak, O., Kobus-Cisowska J. (2019). Charakterystyka liści czeremchy amerykańskiej oraz czeremchy zwyczajnej jako potencjalnego źródła związków aktywnych w preparatach farmaceutycznych. II Ogólnopolska Konferencja „Biotechnologia Niejedno ma imię", Uniwersytet Przyrodniczy w Poznaniu, Poznań
3. **Dziedziński, M.**, Kobus-Cisowska J., Szczepaniak, O., Baranowska M., Meres, B. (2019). Ocena igieł drzew iglastych jako bioaktywnego składnika produktów funkcjonalnych. II Ogólnopolska Konferencja „Biotechnologia Niejedno ma imię". Uniwersytet Przyrodniczy w Poznaniu, Poznań
4. **Dziedziński, M.**, Kobus-Cisowska, J., Stachowiak, B., Szymanowska, D., Baranowska, M., Kozłowski R. (2020). Charakterystyka właściwości funkcjonalnych pędów sosny zwyczajnej (*Pinus sylvestris* L.) jako składnika piwa. III Ogólnopolska Konferencja „Biotechnologia Niejedno ma imię" [online], Uniwersytet Przyrodniczy w Poznaniu, Poznań
5. **Dziedziński, M.**, Telichowska, A., Kobus-Cisowska, J. (2021). Pędy sosny (*Pinus sylvestris* L.) jako źródło związków biologicznie aktywnych w dietoterapii. IV

Ogólnopolska Konferencja Naukowa: Rośliny w naukach medycznych i przyrodniczych [online]. Fundacja na rzecz promocji nauki i rozwoju TYGIEL

6. **Dziedziński, M.**, Kobus-Cisowska, J. (2019). Ocena możliwości wykorzystania szyszek i liści chmielu jako składnika herbatek. W XXIV Sesja Naukowa Sekcji Młodej Kadry Naukowej PTTŻ "Żywność – wczoraj, dziś i na zdrowe jutro" (s. 80). Olsztyn, 23-24.05.2019.
7. Przeor, M., **Dziedziński, M.**, Szczepaniak, O., Kobus-Cisowska, J., Ligaj, M., Szymanowska, D., Szulc, P., Kmiecik, D. (2019). Ocena możliwości wykorzystania nasion konopii siewnej Cannabis Sativa jako składnika batonów owocowo-zbożowych decydującego o potencjale przeciwutleniającym. W XXIV Sesja Naukowa Sekcji Młodej Kadry Naukowej PTTŻ "Żywność – wczoraj, dziś i na zdrowe jutro" (s. 81). Olsztyn, 23-24.05.2019.
8. Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P., Szczepaniak, O., **Dziedziński, M.** (2019). Ocena możliwości wykorzystania wybranych szczepów probiotycznych do zaprojektowania nowych przekąsek czekoladowych. W Nauka, technologia i innowacje w żywności i żywieniu: XLIV Sesja Komitetu Nauk o Żywności i Żywieniu PAN (s. 60). Łódź, 3-4.06.2019.
9. Kobus-Cisowska, J., Szymanowska, D., Maciejewska, P., Szczepaniak, O., **Dziedziński, M.** (2019). Wpływ leżakowania orzechów kokosowych na aktywność enzymatyczną i właściwości fizyko-chemiczne wody kokosowej. W Nauka, technologia i innowacje w żywności i żywieniu: XLIV Konferencja Komitetu Nauk o Żywności i Żywieniu PAN. Łódź, 3-4.07.2019.
10. Maciejewska, P., Szczepaniak, O., **Dziedziński, M.**, Kobus-Cisowska, J., Szymanowska, D. (2019). Multifunkcjonalne suplementy diety z kwasem alfa-ketoglutarowym. W Nauka, technologia i innowacje w żywności i żywieniu: XLIV Konferencja Komitetu Nauk o Żywności i Żywieniu PAN. Łódź, 3-4.07.2019.
11. **Dziedziński, M.**, Szczepaniak, O., Kobus-Cisowska, J. (2019). Wpływ warunków suszenia na zawartość polifenoli w igłach jodły pospolitej. W PIMR, Konferencja krajowa: "Techniki, technologie, badania w przemyśle rolno-spożywczym", 5.12.2019.
12. Kobus-Cisowska, J., Szczepaniak, O., Szymanowska, D., **Dziedziński, M.**, Ligaj, M., Szymandera Buszka, K., Szulc, P., Goldman, E. G. (2019). Evaluation of usefulness of selected food compounds as an active substances with modulating ability of bitter taste in chocolate. In International Conference on Agriculture, Food Security and

Safety (AgroFood 2019) "Global Food Security; Reality and Challenges" (s. 15). Colombo, Sri Lanka, 7-8.11.2019.

13. **Dziedziński, M.**, Kobus-Cisowska, J. (2019). Problem nadkonsumpcji suplementów diety przez Polaków. W XXIV Sesja Naukowa Sekcji Młodej Kadry Naukowej PTTŻ; VII International Session of Young Scientific Staff "Żywność - wczoraj, dziś i na zdrowe jutro" (s. 48). Olsztyn, 23-24.05.2019.
14. Kobus-Cisowska, J., Szczepaniak, O., **Dziedziński, M.**, Telichowska, A., Szymanowska, D., Szulc, P. (2019). Suszenie dyni zwyczajnej (Cucurbita pepo L.) metodą MIRVAC i nowe możliwości projektowania żywności funkcjonalnej z jej udziałem. W PIMR Konferencja krajowa ROL-EKO 2019 (s. 2). Poznań, 10.10.2019.
15. Kobus-Cisowska, J., Szczepaniak, O., **Dziedziński, M.**, Byczkiewicz, S., Telichowska, A., Szulc, P. (2019). Pasteryzacja, a aktywność enzymatyczna surowców roślinnych w technologii produkcji napojów funkcjonalnych. W Konferencja krajowa: "Techniki, technologie, badania w przemyśle rolno-spożywczym" (s. 5). Poznań, 5.12.2019.
16. Kobus-Cisowska, J., Szulc, P., **Dziedziński, M.**, Szczepaniak, O., Byczkiewicz, S., Telichowska, A. (2020). Wartość odżywcza dyni i zawartość poszczególnych fitozwiązków w zależności od odmiany. W XXIII Międzynarodowa Konferencja Naukowo-Praktyczna. Konferencja naukowa "Nowoczesne technologie produkcji rolniczej" (s. 84-86). Grodno, 24.03.2020.
17. Kobus-Cisowska, J., Szczepaniak, O., **Dziedziński, M.**, Szulc, P., Telichowska, A., Byczkiewicz, S. (2020). Wpływ odmiany jęczmienia Hordeum vulgare L. na wartość odżywczą trawy jęczmiennej. W XXIII Międzynarodowa Konferencja Naukowo-Praktyczna. Konferencja naukowa "Nowoczesne technologie produkcji rolniczej" (s. 83-84). Grodno, 24.03.2020.
18. Janczewska, A., Zawadzka, A., Kobus-Cisowska, J., **Dziedziński, M.**, Siwulski, M., Stuper-Szablewska, K. (2021). Wpływ warunków uprawy na zawartość wybranych składników aktywnych w częściach anatomicznych boczniaka ostrygowatego (Pleurotus ostreatus L.). W IV Ogólnopolska Konferencja "Biotechnologia niejedno ma imię" (s. 17). Poznań, 27.11.2021.
19. Szczepaniak, O., Brózdowski, J., **Dziedziński, M.**, Przeor, M., Kobus-Cisowska, J. (2021). Phytochemical composition and antioxidant activity of children-dedicated low-volume beverages enriched in Cornus mas L., Berberis vulgaris L. and Prunus

spinosa L. fruits. W Plant productivity and food safety, Soil science, Microbiology, Agricultural Genetics and Food quality, 15-17.09.2021.

20. Kobus-Cisowska, J., Waszkowiak, K., Wojdyła, I., **Dziedziński, M.**, Hęś, M., Szymandera-Buszka, K., Stuper-Szablewska, K. (2022). Zawartość związków bioaktywnych i potencjał antyoksydacyjny nowych liofilizowanych dań dla dzieci powyżej 6 miesiąca życia. W Polskie Towarzystwo Technologów Żywności Oddział Wielkopolski, III Wielkopolska Konferencja Nauka Gospodarce "Partnerstwo nauki i przemysłu źródłem rozwoju" (s. 63). Poznań, 9-10.06.2022.
21. Zawadzka, A., Janczewska, A., **Dziedziński, M.**, Siwulski, M., Kubara, M., Kobus-Cisowska, J. (2022). Wpływ dodatku kiełków słonecznika (Helianthus annuus L.) na właściwości funkcjonalne kiełbasek wegańskich. W Polskie Towarzystwo Technologów Żywności Oddział Wielkopolski, III Wielkopolska Konferencja Nauka Gospodarce "Partnerstwo nauki i przemysłu źródłem rozwoju" (s. 64). Poznań, 9-10.06.2022.
22. **Dziedziński, M.**, Stachowiak, B., Kobus-Cisowska, J. (2022). Zastosowanie pędów sosny zwyczajnej w produkcji piwa funkcjonalnego o obniżonej zawartości alkoholu. W Polskie Towarzystwo Technologów
23. Żywności Oddział Wielkopolski, III Wielkopolska Konferencja Nauka Gospodarce "Partnerstwo nauki i przemysłu źródłem rozwoju" (s. 81). Poznań, 9-10.06.2022.
24. **Dziedziński, M.**, Stachowiak, B., Kobus-Cisowska, J. (2022). Zastosowanie niekonwencjonalnych szczepów drożdży i pędów sosny zwyczajnej w produkcji piwa funkcjonalnego o obniżonej zawartości alkoholu. W XXVI Sesja Naukowa Sekcji Młodej Kadry Naukowej "Żywność dzisiaj lokalna czy globalna? tradycyjna czy innowacyjna?" (s. 73). Poznań, 19-20.05.2022.
25. Zawadzka, A., Janczewska, A., **Dziedziński, M.**, Siwulski, M., Kubara, M., Kobus-Cisowska, J. (2022). Wpływ dodatku trzonów owocnika boczniaka ostrygowatego (Pleurotus ostreatus L.) na właściwości funkcjonalne kiełbasek wegańskich. W XXVI Sesja Naukowa Sekcji Młodej Kadry Naukowej "Żywność dzisiaj lokalna czy globalna? tradycyjna czy innowacyjna?" (s. 83). Poznań, 19-20.05.2022.

10. ZAŁĄCZNIKI

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji publikacji pt. „Pinus species as prospective reserves of bioactive compounds with potential use in functional food—Current state of knowledge." opublikowanej w czasopiśmie *Plants*.

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 70%. Dziedziński .....
(podpis)

2. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 15% Kobus .....
(podpis)

3. Dr hab. Inż. Barbara Stachowiak 15% Barbara Stachowiak .....
(podpis)

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji pt. „Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds" opublikowanej w czasopiśmie *Molecules*.

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 60%. Dziedziński .....
(podpis)

2. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 10% .....
(podpis)

3. Prof. UPP. dr hab. Daria Szymanowska 10% .....
(podpis)

4. Prof. UPP dr hab. inż. Kinga Stuper-Szablewska 10% .....
(podpis)

5. Dr inż. Marlena Baranowska 10% .....
(podpis)

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji pt. „Polyphenols composition, antioxidant and antimicrobial properties of Pinus sylvestris L. shoots extracts depending on different drying methods" opublikowanej w czasopiśmie *Emirates Journal of Food and Agriculture.*

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 60%. Dziedziński .....
(podpis)

2. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 10% .....
(podpis)

3. Prof. UPP. dr hab. Daria Szymanowska 10% .....
(podpis)

4. Prof. UPP dr hab. inż. Kinga Stuper-Szablewska 10% .....
(podpis)

5. Dr inż. Marlena Baranowska 10% .....
(podpis)

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji pt. „Antioxidant potential, mineral composition and inhibitory effects of conifer needle extract on hyaluronidase-prospects of application in functional food" opublikowanej w czasopiśmie *Journal of Elementology*

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 60%. ..... (podpis)
2. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 8% ..... (podpis)
3. Prof. UPP dr hab. inż. Kinga Stuper-Szablewska 8% ..... (podpis)
4. Prof. dr hab. Judyta Cielecka-Piontek 8% ..... (podpis)
5. Dr inż. Radosław Wilk 8% ..... (podpis)
6. Mgr Dominika Ludowicz 8% ..... (podpis)

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji pt. **„Supplementation of beer with Pinus sylvestris L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles"** opublikowanej w czasopiśmie *Electronic Journal of Biotechnology*.

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 55%. Dziedziński
(podpis)

2. Dr hab. Inż. Barbara Stachowiak 10% Barbara Stachowiak
(podpis)

3. Mgr Rafał Kozłowski 10% Kozłowski Rafał
(podpis)

4. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 7% Kobus
(podpis)

5. Prof. UPP dr hab. inż. Kinga Stuper-Szablewska 6%
(podpis)

6. Dr inż. Katarzyna Szambelan 6% Katarzyna Szambelan
(podpis)

7. Mgr inż. Barbara Górna 6% Barbara Górna
(podpis)

OŚWIADCZENIE O WKŁADZIE WSPÓŁAUTORÓW

Oświadczamy, że jesteśmy współautorami publikacji pt. „Antioxidant, sensory and functional properties of low-alcoholic IPA beer with *Pinus sylvestris* L. shoots addition fermented using unconventional yeast" opublikowanej w czasopiśmie *Open Chemistry*.

Jednocześnie wyrażam zgodę na włączenie przez mgr. inż. Marcina Dziedzińskiego ww. publikacji w postępowaniu o nadanie stopnia doktora w dziedzinie nauk rolniczych w dyscyplinie technologii żywności i żywienia.

Wkład w powstanie publikacji był następujący:

1. Mgr inż. Marcin Dziedziński 55%. Dziedziński .....
(podpis)

2. Dr hab. Inż. Barbara Stachowiak 15% Barbara Stachowiak .....
(podpis)

3. Prof. UPP dr hab. inż. Joanna Kobus-Cisowska 10% .....
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Review

Pinus Species as Prospective Reserves of Bioactive Compounds with Potential Use in Functional Food—Current State of Knowledge

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Abstract: The pine (*Pinus* L.) is the largest and most heteromorphic plant genus of the pine family (*Pinaceae* Lindl.), which grows almost exclusively in the northern hemisphere. The demand for plant-based remedies, supplements and functional food is growing worldwide. Although pine-based products are widely available in many parts of the world, they are almost absent as food ingredients. The literature shows the beneficial effects of pine preparations on human health. Despite the wide geographical distribution of pine trees in the natural environment, there are very few data in the literature on the widespread use of pine in food technology. This study aims to present, characterise and evaluate the content of phytochemicals in pine trees, including shoots, bark and conifer needles, as well as to summarise the available data on their health-promoting and functional properties, and the potential of their use in food and the pharmaceutical industry to support health. Various species of pine tree contain different compositions of bioactive compounds. Regardless of the solvent, method, pine species and plant part used, all pine extracts contain a high number of polyphenols. Pine tree extracts exhibit several described biological activities that may be beneficial to human health. The available examples of the application of pine elements in food are promising. The reuse of residual pine elements is still limited compared to its potential. In this case, it is necessary to conduct more research to find and develop new products and applications of pine residues and by-products.

Keywords: *Pinus*; pine; antioxidants; functional food; bioactive compounds

Citation: Dziedziński, M.; Kobus-Cisowska, J.; Stachowiak, B. *Pinus* Species as Prospective Reserves of Bioactive Compounds with Potential Use in Functional Food—Current State of Knowledge. *Plants* **2021**, *10*, 1306. https://doi.org/10.3390/plants10071306

Academic Editor: Petko Denev

Received: 4 June 2021
Accepted: 21 June 2021
Published: 28 June 2021

1. Introduction

Pinus (*Pinaceae*) is considered the largest genus of conifers, which includes more than 100 different species (Tables 1 and 2) [1].

Table 1. Taxonomic hierarchy of genus *Pinus* L. [2].

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Pinopsida
Subclass	Pinidae
Order	Pinales
Family	*Pinaceae*
Genus	*Pinus* L.

Table 2. Classification of subgenus *Pinus* [1].

Section *Pinus*			Section Trifoliae	
Subsection *Pinus*	**Subsection Pinaster**	**Subsection Contortae**	**Subsection Australes**	**Subsection Ponderosae**
P. densata, densiflora, hwangshanensis, kesiya, luchuensis, massoniana, merkusii, mugo, nigra, resinosa, sylvestris, tabuliformis, taiwanensis, thunbergii, tropicalis, uncinata, yunnanensis	*P. brutia, canariensis, halepensis, heldreichii, pinaster, pinea, roxburghii.*	*P. banksiana, clausa, contorta, virginiana;*	*P. attenuata, caribaea, cubensis, echinata, elliottii, glabra, greggii, herrerae, jaliscana, lawsonii, leiophylla, lumholtzii, muricata, occidentalis, oocarpa, palustris, patula, praetermissa, pringlei, pungens, radiata, rigida, serotina, taeda, tecunumanii, teocote*	*P. cooperi, coulteri, donnell-smithii, devoniana, douglasiana, durangensis, engelmannii, hartwegii, jeffreyi, maximinoi, montezumae, nubicola, ponderosa, pseudostrobus, sabineana, torreyana, washoensis.*

Pinus is a term first applied by Lineus in his work "Species Plantarum" for a group of 10 species, only five of which are currently included in this genus, i.e., *P. cembra, P. pinea*. *P. strobus, P. taeda* and *P. sylvestris* [3]. Because of the prevalence and morphological diversity of pines that can be found in many countries, many conflicting affiliations are known, particularly because many early affiliations to this genus were based on a very small number of morphological discriminants [3]. *Pinus* belongs to *Pinaceae* as a result of having shoot dimorphism, which includes short shoots (fascicles) that have one to eight narrow needles surrounded by bud scales at the base. Strong woody cone scales with the apical structure exposed after the first growing season (bump) and in the mature cone are also typical of the genus *Pinus*. Currently, *Pinus* is treated as a monophyletic taxon [1]. The subgenus *Pinus* (diploxylon or hard pines) has two fibrovascular bundles per needle, diverging pulvini at cataphyll bases ("fascicle breaks"), which usually have persistent sheaths. There are two to eight needles per fascicle and the position of the resin canals is polymorphic (septa; internal, medial external); the seed wings are articulated or oppressed [4]. In this subgenus, section *Trifoliae*, which is characterised by persistent fascicle sheaths, can be distinguished. Most species have cones with thick, woody scales that open at maturity; however, a few species have serotine pine cones. The section includes all North American hard pines, excluding *P. tropicalis* and *P. resinosa* [1]. The *Pinus* section has persistent fascicle sheaths. The number of needles ranges from one to three. External or medial resin canals are usually found [1]. Mature cones open at maturity (excluding *P. pinea*) and have thick scales. In most species, the seed wings are articulated; however, in *P. canariensis* and *P. roxburghii*, they have a decorative function. The section is widespread throughout Eurasia and the Mediterranean basin, as well as includes two species from the Americas: *P. resinosa* from eastern North America and *P. tropicalis* from western Cuba [1].

2. Nutritional Value and Mineral Content

Table 3 shows data on the nutritional value of different parts of trees of the genus *Pinus*. The nutritional value was identified in seeds, needles, bark and shoots.

Table 3. Nutritional value and mineral content.

Index	Species	Part of the Tree	Content	Reference
Energy value	*P. contorta* L.	needles	500 kcal/100 g	[5]
Energy value	*P. pinea* L.	seeds	583 kcal/100 g	[6]
Dry mass	*P. sylvestris* L.	shoots	13.98%	[7]
	P. taeda L.	stem	30.74%	[8]
		needles	1.55%	[8]
crude protein	*P. contorta* L.	needles	3.63%	[5]
crude protein	*P. pinea* L.	seeds	31.6 g/100 g	[6]
fat	*P. pinea* L.	seeds	44.9 g/100 g	[6]
triglycerides	*P. sylvestris* L.	inner bark	33.40 mg/g	[9]
		outer bark	1.71 mg/g	[9]
		conifer needles	10.3 µmol/g dry weight	[10]
Mono- and diglycerides of fatty acids		inner bark	2.26 mg/g	[9]
		outer bark	5.46 mg/g	[9]
		conifer needles	2.3 µmol/g dry weight	[10]
steryl esters		inner bark	1.54 mg/g	[9]
		outer bark	0.19 mg/g	[9]
free fatty acids		inner bark	0.63 mg/g	[9]
		outer bark	1.68 mg/g	[9]
		conifer needles	10.3 µmol/g	[10]
resin acids		inner bark	7.16 mg/g	[9]
		outer bark	2.39 mg/g	[9]
sterols and triterpenic alcohols		inner bark	4.50 mg/g	[9]
		outer bark	2.98 mg/g	[9]
fatty alcohols		inner bark	1.33 mg/g	[9]
		outer bark	1.25 mg/g	[9]
carbohydrates	*P. pinea* L.	seeds	13.3 g/100 g	[6]
total soluble sugar	*P. pinea* L.	seeds	5.15 g/100 g	[6]
reducing sugar	*P. pinea* L.	seeds	0.7 g/100 g	[6]
glucose	*P. sylvestris* L.	needles	121.8 µmol/g	[10]
fructose	*P. sylvestris* L.	needles	151.3 µmol/g	[10]
galactose/arabinose	*P. sylvestris* L.	needles	5.2 µmol/g	[10]
sucrose	*P. sylvestris* L.	needles	59.6 µmol/g	[10]
sucrose	*P. pinea* L.	seeds	4.3 g/100 g	[6]
raffinose/melibiose	*P. sylvestris* L.	needles	4.1 µmol/g	[10]
starch	*P. sylvestris* L.	needles	124.8 µmol/g	[10]
Na	*P. pinea* L.	seeds	11.7 g/100 g	[6]
Ca	*P. pinea* L.	seeds	13.8 mg/100 g	[6]
Ca	*P. sylvestris* L.	bark	0.38%	[11]
Ca	*P. sylvestris* L.	needles	0.53%	[12]

Table 3. *Cont.*

Index	Species	Part of the Tree	Content	Reference
Ca	*P. taeda* L.	stem	0.09%	[8]
Ca	*P. taeda* L.	needles	0.31%	[8]
K	*P. pinea* L.	seeds	713 mg/100 g	[6]
K	*P. sylvestris* L.	Needles	0.54%	[12]
K	*P. sylvestris* L.	bark	0.172%	[11]
K	*P. taeda* L.	stem	0.08%	[8]
K	*P. taeda* L.	needles	0.54%	[8]
Mg	*P. pinea* L.	seeds	325 mg/100 g	[6]
Mg	*P. sylvestris* L.	Needles	0.09%	[12]
Mg	*P. sylvestris* L.	bark	0.059	[11]
Mg	*P. taeda* L.	stem	0.14%	[8]
Mg	*P. taeda* L.	needles	0.18%	[8]
P	*P. pinea* L.	seeds	512 mg/100 g	[6]
S	*P. sylvestris* L.	Needles	0.095%	[12]
Fe	*P. pinea* L.	seeds	10.2 mg/100 g	[6]
Fe	*P. sylvestris* L.	Needles	61.7 µg/g	[12]
Mn	*P. pinea* L.	seeds	6.9 mg/100 g	[6]
Mn	*P. sylvestris* L.	Needles	275.6 µg/g.	[12]
Zn	*P. pinea* L.	seeds	6.4 mg/100 g	[6]
Zn	*P. sylvestris* L.	Needles	53.63 µg/g	[12]
Cu	*P. pinea* L.	seeds	1.5 mg/100 g	[6]
Cu	*P. sylvestris* L.	Needles	5.3 µg/g	[12]
Cu	*P. sylvestris* L.	bark	2.98 mg/kg	[11]
N	*P. sylvestris* L.	bark	0.49%	[11]
N	*P. taeda* L.	stem	0.35%	[8]
N	*P. taeda* L.	needles	1.39%	[8]
ascorbic acid	*P. pinea* L.	seeds	2.5 mg/100 g	[6]
ascorbic acid	*P. sylvestris* L.	shoots	29.3 mg/g	[7]
Thiamine	*P. pinea* L.	seeds	1.5%	[6]
Riboflavin	*P. pinea* L.	seeds	0.28%	[6]

The seeds have the highest energy value due to a high fat content [6]. The seeds also generally have the highest content of the tested nutrients, excluding vitamin C, which is higher in the conifer needles. The seeds of *P. pinea* can be a good source of Mg, P and especially Zn [6]. These seeds have higher zinc content than sesame seeds (approx. 4.5 mg/100 g) and seeds of some pumpkin species (0.54–1.31 mg/100 g), which are considered to be good dietary sources of zinc [13,14]. It is well known that different parts of plants have different nutritional content [15]. Seeds are generally lower in vitamins than the green parts of plants; however, they are higher in macronutrients, especially fats [16]. The uptake of mineral nutrients and their content in a plant depends not only on their content in the soil in the form available for plants, but also on the mutual quantitative ratio of individual mineral nutrients in the environment and on the afforestation level [17–20]. Other factors, such as soil pH, temperature, water supply, rainfall, access to sunlight, precipitation, weather and climate change, are also of great importance [21–23]. Nutrients, which can be

categorized as macro- and micronutrients, have a nutritional role in plants [24]. Macronutrients affect biochemical processes, physiological responses and yield quantity [17,25]. When it comes to macronutrients, their role in plant organisms includes many life processes that determine plant functioning [24,26]. Therefore, it is very difficult to clearly indicate a specific role of elements because they act in a complex way. The role of micronutrients, on the other hand, is more specific, as it is related to specific, well-defined life processes in the plant and to plant growth [27,28]. Nutrient deficiency results in various disorders in terms of the normal growth and development of the plant [29,30]. Some nutrients, because of their specific functions in the plant, may limit the growth of certain pathogens [31]. Those constituents include zinc, sulphur, calcium and potassium [32]. Plant raw materials are a good source of minerals in the diet. This includes brews such as tea brews, coffee brews and herbal mixtures. As indicated by the results of many works, pine shoots can also be a valuable raw material for the preparation of brews in nutrition [33,34]. Pine seeds were found to be a good source of magnesium—an electrolyte essential for many metabolic and biological processes in the body, including acting as a cofactor in over 300 enzyme reactions [35]. Pine seeds were also found to be high in phosphorus and zinc, which are key minerals in terms of metabolic processes and energy metabolism [36]. Both the outer and inner bark is rich in resinous acids. These compounds may be toxic and allergenic; however, a positive effect has also been shown—abietic acid, which is found mainly in the inner bark, can act as an inhibitor of testosterone 5α-reductase [37]. Testosterone reductase inhibitors are used for treatment of benign prostatic hyperplasia, prostate cancer and pattern hair loss [38].

3. Polyphenol Content

Polyphenols are chemical compounds found in herbs, vegetables and fruit that have a wide range of uses. Currently, more than 8000 phenolic compounds are known. They include flavonoids, tannins, phenolic acids and their derivatives such as polymers [39]. Polyphenols are essential secondary metabolites that allow plants to grow and develop. They also protect plants from insects and other factors [38,40,41]. Polyphenols found in plants are involved in functions related to sensory properties such as colour, bitterness and sourness [42,43]. The presence of benzene rings and hydroxyl groups is common to all polyphenols. However, they are very diverse and can be divided into several subgroups. There are different ways to categorise these compounds based on their source of origin, biological function or chemical structure [39]. Polyphenols can be divided into different categories. Classifications are frequently used according to the number of present phenolic rings and structural components, which combine these rings, by differentiating the molecules into phenolic acids, flavonoids, stilbenes and lignans [44,45]. Simple phenols and flavonoids correspond to most natural phenolic substances. Moreover, flavonoids belong to the most common group of these compounds. Their common order is C6–C3–C6, which corresponds to two aromatic rings (rings A and B) bonded to three carbon atoms to produce an oxidised heterocycle (ring C). As a result of the type of hydroxylation and differences in the chromate ring (C ring), flavonoids can be further divided into distinct subgroups, including anthocyanins, flavan-3-ols, flavones, flavanones and flavonols [46–48]. The demand for phenolic acids is very high in many industries because they are used as precursors to other important bioactive molecules that are regularly needed for therapeutic and cosmetic purposes, as well as for food industry. Phenolic acids are also commercially available as dietary supplements [49].

Various parts of a pine (needles, seeds, bark and cones) and different solvents can be used to extract polyphenols. The pine bark is the best-examined part. Although all pine extracts have significant amounts of polyphenols, their content in the extract depends on the solvent type, extraction method, plant part used or pine species (Table 4). This results from natural variability, such as genotype, crop differences and harvesting conditions, climate, soil type, etc. [49,50]. Polyphenols were found to reduce morbidity and slow the progression of cardiovascular, neurodegenerative and cancer diseases. The

mechanism of action of polyphenols is strongly associated with their antioxidant activity and reduction of reactive oxygen species in the human body [51,52]. Furthermore, the health-promoting properties of plant polyphenols include anti-inflammatory, anti-allergic, anti-atherosclerotic, anticoagulant and antimutagenic effects [53]. There are now pine tree preparations on the market, which are concentrated sources of polyphenols. The most popular pine tree preparation is an extract from *P. pinaster*—Pycnogenol® (Horphag Research Ltd., Geneva, Switzerland). The quality of this extract is defined in the United States Pharmacopeia (USP 28). Between 65% and 75% of Pycnogenol are procyanidins comprising catechin and epicatechin subunits with varying chain lengths. Other constituents include polyphenolic monomers, phenolic or cinnamic acids and their glycosides. According to many studies, the constituents of Pycnogenol are highly bioavailable [54]. The daily intake of polyphenols among the general population ranges from 0.1 to 1.0 g per day. Fruit, vegetables, herbs, spices, coffee, tea and wine are the main source of polyphenols [55,56].

Table 4. Polyphenol content.

Compound	Species	Part of the Tree	Content	Reference
gallic acid	*P. sylvestris* L.	shoots	208.38 ± 069 µg/g dw	[7]
2,5-dihydroxybenzoic acid			16.63 ± 0.54 µg/g dw	[7]
4-hydroxybenzoic acid			1084.92 ± 39.04 µg/g dw	[7]
caffeic acid			1502.03 ± 52.53 µg/g dw	[7]
syringic acid			145.44 ± 3.28 µg/g dw	[7]
p-coumaric acid			387.89 ± 15.83 µg/g dw	[7]
ferulic acid			2088.89 ± 56.89 µg/g dw	[7]
chlorogenic acid			518.25 ± 4.90 µg/g dw	[7]
sinapic acid			54.09 ± 2.06 µg/g dw	[7]
t-cinnamic acid			111.44 ± 3.4 µg/g dw	[7]
vanillic acid			0.46 ± 0.01 µg/g dw	[7]
salicylic acid			0.36 ± 0.00 µg/g dw	[7]
naringenin			1.59 ± 0.02 µg/g dw	[7]
vitexin			0.61 ± 0.01 µg/g dw	[7]
rutin			0.63 ± 0.02 µg/g dw	[7]
quercetin			0.98 ± 0.03 µg/g dw	[7]
apigenin			0.30 ± 0.01 µg/g dw	[7]
kaempferol			0.38 ± 0.01 µg/g dw	[7]
luteolin			0.30 ± 0.01 µg/g dw	[7]
protocatechuic acid	*P. radiata*	bark	46.2 ± 1.1 µg/mg	[57]
	P. sibirica	seeds	49.2 ± 0.5 mg/100 g dw	[58]
(+)-Catechin			52.5 ± 0.6 mg/100 g dw	[58]
vanillic acid			85.5 ± 1.0 mg/100 g dw	[58]
epigallocatechin gallate			47.0 ± 1.4 mg/100 g dw	[58]
syringic acid			101 ± 0.3 mg/100 g dw	[58]
()-epicatechin;			125 ± 3.1 mg/100 g dw	[58]
taxifolin			172 ± 3.1 mg/100 g dw	[58]
eriodictyol			383 ± 1.0 mg/100 g dw	[58]
(E)-cinnamic acid			12.2 ± 1.2 mg/100 g dw	[58]
naringenin			37.0 ± 2.1 mg/100 g dw	[58]

Table 4. *Cont.*

Compound	Species	Part of the Tree	Content	Reference
catechin	*P. sinaster*	bark	117.0 ± 8.0 mg/L	[59]
gallocatechin			16.8 ± 4.9 mg/L	[59]
taxifolin			447.7 ± 32.5 mg/L	[59]
quercetin			105.5 ± 2.7 mg/L	[59]
3,4 hydroxybenzoic acid			17.3 ± 2.4 mg/L	[59]
gallic acid			3.6 ± 0.7 mg/L	[59]
caffeic acid			20.6 ± 1.1 mg/L	[59]
o-coumaric acid			47.5 ± 25.3 mg/L	[59]
ferulic acid			47.2 ± 0.8 mg/L	[59]
rosmarinic acid			72.5 ± 4.0 mg/L	[59]
ellagic acid			402.2 ± 51.4 mg/L	[59]
naringin			173.4 ± 55.5 mg/L	[59]
apigenin			53.9 ± 0.1 mg/L	[59]
resveratrol			40.0 ± 0.4 mg/L	[59]
trans-ferulic acid	*P. radiata*	bark	5.9 ± 0.1 µg/mg	[57]
trans-caffeic acid			2.6 ± 0.1 µg/mg	[57]
()-epicatechin;			21.6 ± 1.7 µg/mg	[57]
(+)-Catechin			198.5 ± 6.4 µg/mg	[57]
cis-taxifolin			73.6 ± 2.7 µg/mg	[57]
trans-taxifolin			382.5 ± 12.1 µg/mg	[57]
quercetin			15.2 ± 1.0 µg/mg	[57]
quercetin, resin acid (abietic acid, neoabietic acid), taxifolin, catechin, quercetin derivative, taxifolin derivative, catechin and gallocatechin, kaempferol, rhamnetin isorhamnetin, myricetin, 3,4-dihydroxybenzoic acid, 3,4-dihydroxycinnamic acid, pinosylvin 3-methyl ether, dihydromonomethyl pinosylvin, resveratrol, glycoside, pinoresinol, secoisolariciresinol	*P. wallichiana and P. roxburghii, P. gerardiana*	stem and needle extract	presence found	[60,61]
1,5-diliydroxy-3,6,7-triniethoxy-8-allyloxyxanthone, 1-hydroxy-3,6-diinethoxy-2-β glucopyranoxanthone, friedelin, ceryl alcohol, b-sitosterol, taxifolin, quercetin, catechin, kaempferol, rhamnetin, 3,4-dihydroxybenzoic acid, 3,4-dihydroxycinnamic acid, pinosylvin, pinoresinol, resin acid, sterols, gallocatechin and tannins was found. hexacosyl ferulate	*P. roxburghii*	bark	presence found	[62,63]
12-hydroxydodecanoic acid, 14-hydroxytetradecanoic acid and 16-hydroxy-hexadecanoic acid		needle wax	presence found	[64]

Abbreviation dw—dry weight.

4. Essential Oils

Essential oils are volatile, natural, complex compounds with strong odours, which are generated by aromatic plants as secondary metabolites. They are usually obtained through steam or water distillation. Because of their known antiseptic, bactericidal, virucidal, fungicidal, medicinal and aromatic properties, they are used in the food industry and pharmacy to increase shelf life. They are also used as antibacterial, analgesic, sedative, anti-inflammatory, spasmolytic substances and local anaesthetics [65–67]. Most constituents of essential oils can be classified as lipophilic terpenoids, phenylpropanoids, or short-chain aliphatic hydrocarbon derivatives of low molecular weight; the former are the most common and characteristic. These include allylic, mono, bi- or tricyclic mono- and sesquiterpenoids from different classes that constitute the major part of essential oils, such as hydrocarbons, ketones, alcohols, oxides, aldehydes, phenols or esters [68,69]. Moreover, organic acids, phenols, coumarins, nitrogen and sulphuric substances are also found in essential oils. A single essential oil can have from 20 to 200 components, of which only one is ever dominant and gives a scent to the whole mixture of compounds. Variations in the composition of essential oils depend on environmental factors, plant varieties and the plant parts from which the oil is extracted [70]. Therefore, the chemical composition of oil is closely related to its storage conditions, as well as the environment in which the starting material was stored before its distillation. Since terpenes, i.e., α-pinene, are volatile and thermolabile, they are easily oxidised and hydrolysed [71–73]. The essential oil content is only a small percentage of the total weight of the plant. The oils can be found in plant cell tissue, glands or canals located in several parts of the plant (leaves, bark, roots, flowers, fruit, seeds). The presence of this mixture in living tissue is not fully explained. It is believed to be related to attracting insects that pollinate the plant or to repelling potential pests [74–76]. Pine essential oils are most frequently used as perfume and repellent ingredients. Turpentine is used for manufacturing many cosmetics, air fresheners and aromatherapy products [77–79]. The product that remains after distillation is the rosin that is a non-volatile fraction of oleoresins. It usually contains approx. 90% of resin acids and 10% of neutral components, monocarboxylic acids and diterpenoid acids, whereas the most common acids are abietic or pimaranic ones [80]. Rosin is used for manufacturing of adhesives, printer's inks, soldering fluxes, varnishes and sealing waxes. It is also used as a glazing agent in many food products, including medicines and chewing gums [81].

Pine essential oils contain more than 50 ingredients. Their concentrations vary depending on the plant variety, crop, distillation method and part of the plant (Table 5). The following compounds are found in the greatest quantity: α-pinene, β-pinene, β-phellandrene, β-caryophyllene, camphene, α-terpineol, germacrene D, bornyl acetate, citronellol, β-caryophyllene and tricyclene [82–84]. Alpha-pinene (α-pinene) and beta-pinene (β-pinene) belong to bicyclic monoterpenes, commonly found in various species of pine trees of the genus *Pinus* [85]. Studies have shown that these phytochemicals exhibit diverse biological activity, which contributes to their various uses and applications. They can be used as fungicides, flavours and fragrances, as well as antiviral and antimicrobial agents [86]. The uses of α- and β-pinene go beyond therapeutic and nutritional applications. They are versatile compounds that are used in polymer synthesis [87]. Pinenes are generally recognised as safe (GRAS); thus, they are recognised by the U.S. Food and Drug Administration (FDA) as compounds that can be used in food products [88].

Table 5. The composition of essential oils extracted from pine [82].

Part of the Plant	Bioactive Components	Average Concentration (%)
Needles	α-pinene	31.6
	β-pinene	13.8
	β-phellandrene	9.8
	germacrene D	9.2
	α-Terpineol	6.2
	camphene	7.7
	bornyl acetate	4.4
twigs	β-phellandrene	34.4
	α-pinene	17.7
	β-pinene	17.4
	germacrene D	6.5
	bornyl acetate	4.3
	camphene	3.2
	α-Terpineol	2.1
Needles and twigs	Tricyclene, Sabinene, Myrcene, 3-Carene, β-Z-Ocimene, γ-Terpinene, Terpinolene, E-Pinene hydrate, α-Campholenal, iso-3-Thujanol, Z-Verbenol, Borneol, Terpinene-4-ol, Myrtenal, E-Piperitol, Linalool acetate, α-Terpineol acetate, α-Copaene, β-Bourbonene, β-Elemene, β-Caryophyllene, β-Copaene, α-E-Bergamotene, α-Humulene, Z-Muurola-4(14),5-diene, γ-Cadinene, δ-Cadinene, α-Cadinene, E-Nerolidol, Germacrene-4-ol, Spathulenol, Caryophyllene oxide, Humulene epoxide II, Z-Cadin-4-en-7ol, Cubenol, α-Muurolol, α-Cadinol, Eudesma-4(15),7-diene-1-β-ol, Oplopanone, Cembrene	<1

5. Antioxidant Activity

Free radicals and other reactive oxygen species, such as superoxides, hydroxyl radicals and hydrogen peroxide, are generated by either exogenous substances or endogenous metabolic processes of the human body, or in food products, react very rapidly with DNA, lipids and proteins, causing cell damage. Antioxidants, whose action is based on their ability to donate hydrogen atoms to free radicals, are compounds that protect against them [89,90]. In recent years, the interest in natural antioxidants has increased, which resulted in an intensification of research on them in various scientific fields. As a result, numerous articles concerning natural antioxidants, including polyphenols, flavonoids, vitamins and volatile compounds, have been published. Various assays were developed to evaluate the antioxidant activity of plants and food ingredients [91–94]. The use of at least two different methods of testing the antioxidant activity of samples is a generally accepted good practice. A combination of electron- or free radical capture assays, such as DPPH, ABTS, ACA or FRAP, as well as lipid peroxidation assays, is also recommended [95–98].

Different parts of trees (bark, needles, shoots, seeds), as well as various extraction methods and solvents, were used in the study on antioxidant properties of trees from the *Pinus* genus. As a result, extracts were correlated with various components and, thus, differ-

ent antioxidant potentials, measured using multiple methods (Table 6). Several correlations may be observed. In the study of total polyphenol content using the Folin-Ciocalteu reagent, alcoholic extracts obtained from the tree bark, particularly from *P. radiata* (1610 mg of gallic acid equivalents/200 mL) and *P. brutia* (412.42 ± 7.56 mg gallic acid/g extract), adopting higher values compared to the aqueous extracts obtained from the shoots of *P. sylvestris* (0.86 ± 0.09 mg gallic acid/g dw) [7,99,100]. In free radical tests, which determined the value of EC50, the ethanolic extracts from *P. koraiensis* seeds (0.023 ± 0.004 mg/mL) and methanolic extracts from *P. bruti* bark (9.17 ± 0.13 µg/mL) assumed the lowest values of EC50, and thus, exhibited the strongest antioxidant activity [100,101]. Many existing studies explicitly state that the application of aqueous mixtures of water and organic solvents, such as ethanol, methanol, acetone, isopropanol or acetonitrile, significantly increases the antioxidant efficacy of many plant products [102]. In research studying the effect of solvent on the antioxidant activity of *P. densiflora* needle extracts using various concentrations of water and ethanol (0–100%), it was observed that 40% ethanolic needle extracts exhibited the highest radical scavenging capacity, followed by extracts containing 60%, 20%, 80%, 0% and 100% of ethanol, respectively [103]. Similar results were observed in the study on *P. densiflora* bark, which compared the content of phenolic compounds and antioxidant potential of extracts containing ethanol in the range of 0, 20, 40, 60, 80 or 100%, 20 or 40% of methanol, isopropanol and acetonitrile, as well as used acetone with distilled water (*v*/*v*) as extraction solvents [102]. Experiments revealed that bark extracts containing 20% of ethanol, 40% of ethanol and 20% of acetronile displayed the highest antioxidant potential and the highest content of phenolic compounds [102].

Table 6. Antioxidant properties of various *Pinus* species.

Method	Species	Material	Result	Reference
Total phenolic content	*P. koraiensis*	Seed 40% ethanolic extract	264 ± 10.52 mg of gallic acid equivalents/g	[101]
	P. pinaster	Bark ethanolic extract	890 mg of gallic acid equivalents/200 mL	[99]
	P. radiata	Bark ethanolic extract	1610 mg of gallic acid equivalents/200 mL	[99]
	P. cembra L.	Bark 80% aqueous methanol extract	299.3 ± 1.4 mg of gallic acid/g extract	[104]
	P. cembra L.	Needle 80% aqueous methanol extract	78.22 ± 0.44 mg of gallic acid/g extract	[104]
	P. sylvestris L.	Shoot aqueous extract	0.86 ± 0.09 mg of gallic acid/g dw	[7]
	P. sylvestris L.	Air-dried shoot 40% aqueous ethanol extract	13.4 ± 4.07 mg of gallic acid/g dw	[105]
	P. sylvestris L.	Vacuum-dried shoot 40% aqueous ethanol extract	8.34 ± 2.01 mg of gallic acid/g dw	[105]
	P. sylvestris L.	Freeze-dried shoot 40% aqueous ethanol extract	5.73 ± 2.55 mg of gallic acid/g dw	[105]
	P. brutia	Bark 80%aqueous methanol extract	412.42 ± 7.56 mg of gallic acid/g extract	[100]
OH scavenging activity EC50	*P. koraiensis*	Seed 40% ethanolic extract	0.391 ± 0.055 mg/mL	[101]
	P. brutia	Bark 80%aqueous methanol extract	0.5 ± 0.0 mg/mL	[100]
DPPH radical scavenging activity	*P. koraiensis*	Seed 40% aqueous ethanol extract	EC50 value 0.023 ± 0.004 mg/mL	[101]
	P. cembra L.	Bark 80% aqueous methanol extract	EC50 value 71.1 ± 0.5 µg/mL	[104]
	P. cembra L.	Needle 80% aqueous methanol extract	EC50 value 186.1 ± 1.7 µg/mL	[104]
	P. sylvestris L.	Shoot aqueous extract	200.94 ± 23.47 mg of gallic acid/g dw	[7]
	P. sylvestris L.	Air-dried shoot 40% aqueous ethanol extract	332.25 ± 10.49 dw µM Trolox/g dw	[105]

Table 6. *Cont.*

Method	Species	Material	Result	Reference
	P. sylvestris L.	Vacuum-dried shoot 40% aqueous ethanol extract	299.72 ± 15.97 dw µM Trolox/g dw	[105]
	P. sylvestris L.	Freeze-dried shoot 40% aqueous ethanol extract	339.00 ±19.61 dw µM Trolox/g dw	[105]
	P. radiata	Aqueous bark extract	36.3 ± 5.0% at 2.0 µg/mL	[57]
	P. brutia	Bark 80%aqueous methanol extract	1.47 ± 0.02 *Trolox equivalent* mg/mL	[100]
O2 inhibition activity	*P. sylvestris* L.	Vacuum-dried shoot 40% aqueous ethanol extract	8.34 ± 2.01 mg of gallic acid/g dw	[105]
ABTS radical cation scavenging assay	*P. sylvestris* L.	Freeze-dried shoot 40% aqueous ethanol extract	5.73 ± 2.55 mg of gallic acid/g dw	[105]
	P. cembra L.	Needle 80% aqueous methanol extract	0.3 ± 0.0 µM Trolox equivalent to 1 µg/mL extract	[104]
	P. radiata	Aqueous bark extract	55.1 ± 5.8% at 1.0 ug/mL	[57]
Reducing power assay EC50	*P. cembra* L.	Bark 80% aqueous methanol extract	26.0 ± 0.3 mg/mL	[104]
	P. cembra L.	Needle 80% aqueous methanol extract	104 ± 2 mg/mL	[104]
	P. brutia	Bark 80%aqueous methanol extract	9.17 ± 0.13 µg/mL	[100]
Ferrous ion chelating ability assay	*P. cembra* L.	Needle 80% aqueous methanol extract	EC50 = 1.755 ± 22 µg/mL	[104]
	P. sylvestris L.	Shoot aqueous extract	42.76 ± 5.7 µM $FeSO_4$/g dw	[7]
	P. sylvestris L.	Air-dried shoot 40% aqueous ethanol extract	37.79 ±3.64 µM $FeSO_4$/g dw	[105]
	P. sylvestris L.	Vacuum-dried shoot 40% aqueous ethanol extract	47.25 ±14.06 µM $FeSO_4$/g dw	[105]
	P. sylvestris L.	Freeze-dried shoot 40% aqueous ethanol extract	21.79 ± 4.36 µM $FeSO_4$/g dw	[105]
Superoxide anion	*P. radiata*	Aqueous bark extract	47.6 ± 5.8% at 23.0 ug/mL	[57]
	P. brutia	Bark 80%aqueous methanol extract	39.37 ± 0.85 µg/mL	[100]
Hydrogen peroxide	*P. radiata*	Aqueous bark extract	47.8 ± 12.3% at 8.0 ug/mL a	[57]
15-LO inhibition assay	*P. brutia*	Bark 80%aqueous methanol extract	EC50 = 22.47 ± 0.75 µg/mL	[100]

6. Pharmacological Properties

People around the world use herbal supplements and medicines due to their beneficial effects on human health [106]. Bark, needles, pollen and other parts of numerous pine species have been used for many years and proven to constitute excellent raw materials in the production of goods [107]. The first documented use of pine bark extracts dates back to 1535, when French explorer Jacques Cartier described events in which he and his crew avoided death from scurvy—a disease caused by vitamin C deficiency—by drinking pine bark brew. In 1951, French researcher Jacques Masquelier began studying herbal raw materials to identify their bioactive components. He was able to extract proanthocyanidins from the *P. pinaster* bark in the amount that could be used for manufacturing purposes [54]. Despite their acknowledged medicinal properties, the timber industry had regarded tree bark and shoots as inconvenient waste products; only in recent years have they been widely recognised as a rich source of natural polyphenols, containing potentially beneficial nutritional, health and medicinal properties [99]. Many standardised extracts of various pine species are currently used as dietary supplements and phytochemicals aiding in the

treatment of various diseases around the world, including chronic inflammation, circulatory disorders and asthma (Table 7).

Several in vitro, animal and human studies have indicated the prophylactic and therapeutic effects of extracts from various pine species [107–130]. In a systematic review, published by the Cochrane Collaboration, which included 27 RCTs evaluating the effects of supplements containing pine bark extracts on 10 different chronic diseases: asthma, Attention Deficit Hyperactivity Disorder, cardiovascular disease and risk factors, chronic venous insufficiency, diabetes, erectile dysfunction, female sexual dysfunction, osteoarthritis, osteopenia and traumatic brain injury, it was concluded that small sample sizes, a limited number of RCTs, variability in outcome measures and poor reporting of the RCTs included, rendered it impossible to draw definitive conclusions about the efficacy or safety of supplements containing pine bark extract [108]. However, the aforementioned review did not take into account many other studies, including particularly interesting research on skin health and protection. Both the study on photoprotective and anti-photoaging effects presented a positive influence of *P. pinaster* bark extracts [109,110]. Furthermore, the role of antioxidants from the pine extracts in neuroprotective activity may prove to be fundamental, as *P. radiata* bark extracts exhibited effectiveness in two cases of RCT [111,112]. Nervous system inflammation and oxidative stress are believed to be the most characteristic symptoms of Alzheimer's disease and play a key role in neurotoxicity. Thus, a suitable antioxidant strategy may improve the treatment of neurodegenerative diseases and dementia. Numerous studies have confirmed the neuroprotective effects of polyphenolic compounds, which protect neurons from the neurotoxin-induced injuries, as well as provide the ability to inhibit nervous system inflammations and the potential to advocate memory, learning and cognitive functions [113].

Table 7. Pharmacological properties of *Pinus*.

Activity	Material	Experimental Model	Result	Source
Antihypertensive	*P. densiflora Sieb. et Zucc.* extract	A group of Wistar-Kyoto rats—a normotensive group—was orally administered tap water. Four groups of spontaneously hypertensive rats were orally administered tap water, captopril (a positive control), 50 mg/kg/day of KRPBE *P. densiflora* bark extract (Korean red pine bark extract; KRPBE) and 150 mg/kg/day of KRPBE, respectively. The blood pressure of rats was measured once a week during the seven weeks of oral administration of drugs. After seven weeks, the researchers collected the rats' lungs, kidneys and serum, and subsequently determined the activity of angiotensin-converting enzyme (ACE), as well as the content of angiotensin II and malondialdehyde (MDA).	Blood pressure of rats served with captopril and KRPBE was significantly lower than that of the SHR control group. The activity of ACE, as well as the content of angiotensin II and MDA, was significantly lower in groups administered with captopril and KRPBE than those in the SHR control group.	[114]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
Anti-adipogenic	*P. densiflora* aqueous bark extract	Four-week-old male C57BL/6 mice were fed with regular feed (18% kcal from fat) or HFD (60% kcal from fat). Animals fed with HFD were additionally subjected to PineXol treatment at 10 or 50 mg/kg body weight (PX10 or PX50, respectively).	Compared to the HFD group, the PX50 group was characterised by statistically lower body weight and body fat mass ($p < 0.05$ and $p < 0.001$, respectively). In the PX50 group, concentrations of hepatic triglycerides, total cholesterol and low-density lipoprotein cholesterol were lower than those in the HFD group ($p < 0.01$). The levels of acetyl CoA carboxylase ($p < 0.01$), elongase of a very long chain of fatty acids 6 ($p < 0.01$), stearoyl CoA desaturase 1 ($p < 0.05$), microsomal triglyceride transfer protein ($p < 0.01$) and sterol regulatory element-binding protein 1 ($p < 0.05$) in the PX50 group were significantly lower compared to their respective levels in the HFD group. In the white adipose tissue, the levels of CCAAT enhancer-binding protein alpha ($p < 0.05$), peroxisome proliferator-activated receptor gamma ($p < 0.001$) and perilipin ($p < 0.01$) in the PX50 group were lower than those in the HFD group.	[115]
Hepatoprotective	*P. roxburghii* wood oil	The administration of *P. roxburghii* wood oil at 200, 300 and 400 mg/kg body weight was examined in terms of its hepatoprotective activity on rat liver damage induced by carbon tetrachloride and ethanol.	Noticeably high levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, malondialdehyde (MDA) and low levels of reduced glutathione (GSH) and total protein induced by hepatotoxins, significantly inclined towards adopting normal levels due to the wood oil administered at 200 and 300 mg/kg.	[117]
Hepatoprotective	*P. roxburghii* wood oil	The administration of *P. roxburghii* wood oil at 200, 300 and 400 mg/kg body weight was examined in terms of its hepatoprotective activity on rat liver damage induced by carbon tetrachloride and ethanol.	Noticeably high levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, malondialdehyde (MDA) and low levels of reduced glutathione (GSH) and total protein induced by hepatotoxins, significantly inclined towards adopting normal levels due to the wood oil administered at 200 and 300 mg/kg.	[117]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
Antidiabetic	*P. roxburghii* ethanolic bark extract	Rats were induced with diabetes through alloxan injection (120 mg/kg body weight). Control rats were either healthy and untreated, or induced with diabetes, untreated and provided only with distilled water. The acute effect of ethanolic extract was evaluated by administering 100, 300 and 500 mg/kg body weight p.o. of the extract to normoglycemic rats. In the chronic model, the ethanolic extract was administered to normal and alloxan-induced, diabetic rats at 100, 300 and 500 mg/kg body weight p.o. per day for 21 days. Levels of blood glucose and the values of body weight were monitored at specific intervals using different biochemical parameters.	Statistical data indicated a significant ($p < 0.01$) increase in the body weight, as well as a decrease in the level of blood glucose, glycated haemoglobin, total cholesterol and serum triglycerides. The level of HDL cholesterol was significantly ($p < 0.01$) increased in rats administered with the extract.	[116]
Antidyslipidemic	*P. roxburghii* needles, hexane (B), chloroform (C), n-butanol soluble (D) and n-butanol insoluble (E) fractions.	Dyslipidemic hamsters were divided into six groups and fed with five solvent fractions (A, B, C, D and E) of *P. roxburghii* needles.	Extract from *P. roxburghii* needles exhibited the significant potential to decrease the level of the plasma lipid profile, as well as having a beneficial effect on the HDL-C and its ratio with total cholesterol in a dyslipidemic hamster model.	[118]
Analgesic	*P. roxburghii* Sarg. stem bark ethanolic extract	Analgesic activity was evaluated using acetic acid-induced writhing and tail immersion tests in Swiss albino mice.	Alcoholic extract from *Pinus roxburghii* Sarg. (at 100, 300 and 500 mg/kg) significantly and dependently reduced the number of abdominal constrictions induced in mice by administering a 1% solution of acetic acid. This dose-dependent protective effect reached a maximum pain inhibition of 80.95% at 500 mg/kg.	[119]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
Hepatoprotective	*P. roxburghii* wood oil	The administration of *P. roxburghii* wood oil at 200, 300 and 400 mg/kg body weight was examined in terms of its hepatoprotective activity on rat liver damage induced by carbon tetrachloride and ethanol.	Noticeably high levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, malondialdehyde (MDA) and low levels of reduced glutathione (GSH) and total protein induced by hepatotoxins, significantly inclined towards adopting normal levels due to the wood oil administered at 200 and 300 mg/kg.	[117]
Anticonvulsant	*P. roxburghii* alcoholic extract	Anticonvulsant activity was evaluated by means of maximal electroshock (MES) and pentylenetetrazole-induced (PTZ) seizures in Wistar albino rats at various doses (i.e., 100, 300 and 500 mg/kg).	In the MES-induced seizure model, AEPR at 300 and 500 mg/kg body weight significantly reduced all phases of convulsion ($p < 0.01$). In the PTZ-induced seizure model, administration of the extract at 300 and 500 mg/kg half an hour before the injection of PTZ significantly delayed the onset of clonic seizures ($p < 0.01$).	[120]
Anti-viral (HIV-1)	*P. pinaster* ssp. atlantica extract (Pycnogenol)	The inhibitory effect of the extract on virus binding to MT-4 cells was examined by infecting the MT-4 cells with IIIB-env-Hiv-1 in the presence or absence of extract.	Addition of the compound at the time of injection resulted in a dose-dependent inhibition of the cytopathic effect, as well as a dose-dependent reduction in p24.	[121]
Anti-viral (Epstein-Barr virus)	*P. massoniana* aqueous bark extract	Inhibition of the immediate-early viral gene transpiration by the extract was assessed by transient transfection assay.	*P. massoniana* bark extract (PMBE) at 60 microg/mL or a higher dose, inhibits the expression of the Epstein-Barr virus (EBV) lytic proteins, such as Rta, Zta and EA-D. The EBV lytic cycle was blocked by the inhibition of the immediate-early gene transcription.	[122]
Wound healing	Methanol and *P. longifolia roxburghii* aqueous leave extracts	Extracts were examined in terms of wound healing properties on excision and incision wound models in Wistar albino rats.	Both extracts exhibited significant wound healing activity. However, the rate of wound contraction and epithelialisation was faster in groups administered with methanol extract.	[123]
Anti-cancer	*P. roxburghii* essential oil	The essential oil was tested against human cancer cell lines, i.e., cultured HCT-116 (colon cancer), KBM-5 (myelogenous leukaemia), U-266 (multiple myeloma cells), MiaPaCa-2 (pancreatic cancer cells), A-549 (lung carcinoma cells) and SCC-4 (squamous cell carcinoma) cell lines by means of the MTT assay.	The percentage inhibition of PREO activity was found to be concentration-dependent. U-266 exhibited maximum inhibition of 83%, while HCT-116, SCC4, MiaPaCa-2, A-549 and KBM-5 manifested 71, 69, 73, 73 and 76% of inhibition, respectively.	[124]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
Hepatoprotective	*P. roxburghii* wood oil	The administration of *P. roxburghii* wood oil at 200, 300 and 400 mg/kg body weight was examined in terms of its hepatoprotective activity on rat liver damage induced by carbon tetrachloride and ethanol.	Noticeably high levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, malondialdehyde (MDA) and low levels of reduced glutathione (GSH) and total protein induced by hepatotoxins, significantly inclined towards adopting normal levels due to the wood oil administered at 200 and 300 mg/kg.	[117]
	Petroleum ether, ethyl acetate, chloroform and *P. roxburghii* Sarg. ethanolic extract	Effect of *Pinus roxburghii* Sarg. extracts on the growth of human IMR32 neuroblastoma cancer cell line was studied using the SRB assay.	Petroleum ether and chloroform extracts were the only extracts that exhibited anticancer activity.	[125]
Cardio-protective	*P. pinaster* ssp. atlantica extract (Pycnogenol)	Twenty-three patients with coronary artery disease (CAD) completed this randomised, double-blind, placebo-controlled cross-over study. Apart from the standard cardiovascular therapy, patients received Pycnogenol (200 mg/day) for 8 weeks followed by the placebo, or vice versa. At a baseline and after each treatment period, the endothelial function, assessed in a non-invasive manner via flow-mediated dilatation (FMD) of the brachial artery using high-resolution ultrasound, biomarkers of oxidative stress and inflammation, platelet adhesion and 24 h blood pressure monitoring were evaluated.	In CAD patients, treatment with Pycnogenol was associated with an improvement of FMD from 5.3 ± 2.6 to 7.0 ± 3.1 ($p < 0.0001$), while no change was observed in case of placebo (5.4 ± 2.4 to 4.7 ± 2.0; $p = 0.051$). Isoprostane—which influences the oxidative stress index—significantly decreased from 0.71 ± 0.09 to 0.66 ± 0.13 after treatment with Pycnogenol, while no change was observed in the group treated with placebo (mean difference 0.06 pg/mL with an associated 95% CI (0.01, 0.11), $p = 0.012$). Inflammation markers, platelet adhesion and blood pressure levels did not change following the treatment with Pycnogenol or placebo.	[126]
Hepatoprotective	*P. roxburghii* wood oil	The administration of *P. roxburghii* wood oil at 200, 300 and 400 mg/kg body weight was examined in terms of its hepatoprotective activity on rat liver damage induced by carbon tetrachloride and ethanol.	Noticeably high levels of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, malondialdehyde (MDA) and low levels of reduced glutathione (GSH) and total protein induced by hepatotoxins, significantly inclined towards adopting normal levels due to the wood oil administered at 200 and 300 mg/kg.	[117]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
Neuroprotective	*P. densiflora* aqueous bark extract	Neuroprotective effect (anticholinesterase activity) was determined using the AChE and BChE assays while intracellular oxidative stress was evaluated using the fluorescent assay using DCFH-DA on neuronal PC-12 cells.	Pretreatment of PC-12 cells with Kextract decreased the oxidative stress in a dose-dependent manner compared to cells exposed solely to oxidative stress. Inhibition of AChE and BChE occurred at 10 µg/mL and 100 µg/mL in TE values—approx. 68.3 nM and 15.1 nM for the inhibition of AChE and BChE, respectively.	[127]
	P. roxburghii Sarg. methanolic extract	The in vitro cell viability activity of *P. roxburghii* was assessed using the PC-12 cell lines. The in vivo neuroprotective activity of *P. roxburghii* was tested on Wistar albino rats (both sexes). ICV-STZ (3 mg/kg, bilateral) was administered to induce a memory deficit.	*P. roxburghii* exhibited significant cell viability at 10, 50 and 100 µg/mL in an in vitro assay on PC-12 cell lines. In the in vivo activity, ICV-STZ significantly deteriorated memory, cognition, tissue oxidative stress and the AchE activity. *P. roxburghii* (at 100, 200 and 300 mg/kg p.o.) and donepezil (at 3 mg/kg, p.o.) significantly ($p < 0.05$) reversed the behavioural changes in rats when tested in a morris water maze and elevated plus maze. Increased levels of lipid peroxidation, AchE activity and decreased the level of glutathione were significantly ($p < 0.05$) antagonised by *P. roxburghii*, similarly to the case of donepezil in rat brain.	[128]
	P. radiata bark	Sixty adults who sustained a mild TBI 3–12 months before recruitment and were experiencing persistent cognitive difficulties (CFQ score > 38), were randomised in order to receive enzogenol (1000 mg/day) or a corresponding dose of placebo for 6 weeks. Subsequently, all participants received enzogenol for a further 6 weeks, followed by placebo for 4 weeks. Compliance, side-effects, cognitive failures, working and episodic memory, post-concussive symptoms and mood were evaluated at baseline, as well as in the 6th, 12th and 16th week.	Enzogenol was found to be safe and well-tolerated. Trend and breakpoint analyses revealed a significant reduction in cognitive failures after 6 weeks (mean CFQ score, 95% CI, Enzogenol versus placebo 6.9 (10.8 to 4.1)). Improvements in the frequency of self-reported cognitive failures were estimated to continue until the 11th week before stabilising.	[111]

Table 7. *Cont.*

Activity	Material	Experimental Model	Result	Source
	P. radiata	During the period of 5 weeks, the participants (42 males aged 50–65) were supplemented either with Enzogenol combined with vitamin C, or vitamin C only. A battery of computerised cognitive tests was administered while cardiovascular and haematological parameters were assessed before and after supplementation.	The speed of the response to the spatial working memory and immediate recognition tasks improved after supplementation with Enzogenol combined with vitamin C, whereas supplementation with vitamin C alone did not induce any improvement. A trend in the reduction of systolic blood pressure was observed in patients supplemented with Enzogenol combined with vitamin C, but not with vitamin C alone. The blood safety parameters remained unchanged.	[112]
Photoprotective	*P. pinaster*	A total of 21 volunteers were administered oral supplementation of Pycnogenol: 1.10 mg/kg body weight (b. wt.)/day (d) for the first 4 weeks and 1.66 mg/kg b. wt./d for the following 4 weeks. The minimal erythema dose (MED) was measured twice before the supplementation (baseline MED), once after the first 4 weeks of supplementation and the last time at the end of the study.	An increase in MED was observed after supplementation with 1.10 mg/kg b. wt./d of PBE for 4 weeks (mean MED 5 34.62 mJ/cm^2, 95% CI 5 from 31.87 to 37.37). A supplementation with 1.66 mg/kg b. wt./d of PBE for the last 4 weeks of the study caused an even further increase in MED (mean MED 5 39.62 mJ/cm^2, 95% CI 5, from 37.51 to 41.73).	[109]
Anti-photoaging	*P. pinaster*	A total of 112 women with mild to moderate skin photoaging symptoms were randomised to either take part in a 12-week open trial regimen of 100 mg PBE supplementation once a day or to be in a parallel-group—a trial regimen of 40 mg PBE supplementation once a day.	A significant decrease in clinical grading of skin photoaging scores was observed during both 100 mg and 40 mg of PBE daily supplementation regimens. Furthermore, a significant reduction in the pigmentation of age spots was demonstrated using skin colour measurements.	[110]

Abbreviations: KRPBE—Korean red pine bark extract; SHR—spontaneously hypertensive rats; ACE—angiotensin-converting enzyme; MDA—malondialdehyde; HFD—high fat diet, PX—PineXol; GSH—glutathione; AEPR—alcoholic extract of bark of *Pinus roxburghii* Sarg.; MES—maximal electroshock; PTZ—pentylenetetrazole; EBV—Epstein-Barr virus; PREO—*P. roxburghii* essential oil; MED—minimal erythema dose; CAD—coronary artery disease; FMD—flow-mediated dilatation; AChE—acetylcholinesterase; BChE—butyrylcholinesterase; ICV-STZ—Intracerebroventricular Streptozotocin Injections; CFQ—Cognitive Failures Questionnaire; b. wt./d—body weight/day; PBE—pine bark extract.

7. Antimicrobial Activity

The increasing incidence of infectious diseases, severe side effects related to the intake of many antibiotics and the development of antibiotic resistance substantiate the growing interest in the identification of new antimicrobial compounds, both natural and synthetic agents [131–133]. Plant resin has been applied to treat diseases in folk medicine for thousands of years. It was also used in the pharmaceutical industry before the introduction of modern antibiotics. Many of the secondary metabolites of trees adopt a protective function against predators and pathogenic microorganisms. The antimicrobial activity of extracts, oils and resins from trees of the *Pinus* genus may be related to various organic

compounds, such as alkaloids, phenols and terpenes (Tables 4 and 5) [82,134]. The discovery of biological effects of the *Pinus* spp. compounds suggests that they may be applied in the creation of environmentally friendly and biocompatible pharmaceuticals.

The most common human pathogen, colonising one-third of healthy people throughout the world, is *Staphylococcus aureus* [135]. *S. aureus* is also an etiologic agent contributing to the development of many human infections, including pneumonia, meningitis, toxic shock syndrome, bacteremia and endocarditis. *S. aureus* is further known for its rapidly advancing resistance to antibiotics [136–139]. The studies proved that extracts and essential oils from *P. cembra*, *P. koraiensis*, *P. brutia*, *P. densiflora* and *P. sylvestris* inhibit the growth of many *S. aureus* strains, including: ATCC 25923, 25923, 503, 29213, ATCC BAA-977 and ATCC 13565 (Table 8) [104,140–142]. Trees from the *Pinus* species display properties aiding in the fight against many strains of various bacteria. The highest inhibition was observed in *M. luteus* NRRL B-4375, *Proteus vulgaris* ATCC 13315, *Shigella flexneri* AT CC 12026 and *Streptococcus faecalis* ATCC 19433 [141,142]. *Shigella flexneri* is a gram-negative bacterium causing the most contagious bacterial shigellosis. Shigellosis generates 1.1 million deaths and more than 164 million cases each year. The majority of said cases involve children in developing countries. Pathogenesis of *S. flexneri* is based on its ability to invade and replicate within the colonic epithelium, leading to severe inflammation and destruction of the epithelium itself [143]. Despite intensive research, conducted for over 60 years using various vaccination strategies, a safe and effective vaccine is not yet available [144]. Numerous studies indicate that plant secondary metabolites can inhibit the spread of phytopathogens, by acting both as antimicrobial agents and elicitors of other defensive responses. Many of the aforementioned metabolites negatively affect the clinically relevant pathogens and their use as "antibiotic enhancers" or "virulence attenuators" fighting against infectious diseases in humans is promising [145].

Table 8. Commercial pharmacological products from pine.

Name of Formulation	Plant Part Used	Pharmacological Activity Declared by the Manufacturer	References
Polyherbal oil extract	Oleoresin of *P. roxburghii*	Analgesic and anti-inflammatory	[129]
Rumalaya gel	Resin from *P. roxburghii*	Lowers the joint and bone pain associated with various orthopedic ailments	[119]
Pycnogenol®	*P. pinaster bark*	Antimicrobial activity and treatment of asthma, Attention Deficit Hyperactivity Disorder, chronic venous insufficiency, diabetes, erectile disorders and osteoarthritis	[108]
Oligopin®	*P. pinaster bark*	Cardiovascular and vein health, antioxidant, treatment of male sexual disorders and ADHD (Attention Deficit Hyperactivity Disorder)	[130]
PineXol®	*P. densiflora* bark	Anti-inflammatory agent, enhances blood circulation and improves skin conditions	[146]
Flavangenol®	*P. maritima* bark	Lowers blood pressure and improves glycemic control, plasma lipoprotein profile, body weight, antioxidative capacity, level of anti-inflammatory markers and liver function tests	[147]
Enzogenol®	*P. radiata* bark	Antioxidant, anti-inflammatory, neuroprotective and anti-diabetic properties.	[148]

Compounds extracted from the trees of the *Pinus* genus presented in many studies exhibited different levels of antimicrobial activity against yeast, gram-positive and gram-negative bacteria, which validates the traditional application of these substances [140]. Additionally, such extracts, oils and resins display the insecticidal, phytotoxic and antioxidant potential [141]. Therefore, it is necessary to conduct research aided by biological studies with recovery, identification and testing of a single compound and/or multiple compounds to determine its/their biological effects [142].

8. Food Application of *Pinus*

There is an increasing demand for health-promoting plant products all over the world [149]. Today, conifer shoots are virtually unused as a food ingredient, despite their common availability in many parts of the world. An exception is a common juniper, whose berry-like cones are a valued seasoning in Europe [150]. Pine shoot products, such as pine shoot syrup, pine shoot-based beer and herbal teas are available on the market. Despite its many potential applications, currently, the shoot products are not very popular [151].

To date, there has been little research on the use of pine tree elements in food products (Table 9). However, current literature indicates a possible application of such ingredients in beverages, dairy products, meat products or even bread. The addition of *P. pinaster* extracts increases the antioxidant potential of juices and dairy products. With regard to juices, polyphenols derived from pine extracts may also have a negative, inhibitory effect on the microflora [151–154]. Moreover, in terms of sensory experience, kefir enriched with pine bud syrup was assessed higher than the control sample, which indicates that it may also serve as an ingredient providing flavour and aroma [151]. In the case of the addition of pine extract to bread and meat, the substance acted as a shelf life extender by inhibiting the growth of bacteria and oxidisation of fats [155,156]. Moreover, pine extracts can be possibly applied in the future as additives and preservatives, as they are commercially sold as dietary supplements. Many of these extracts are listed on the Everything Added to Food in the United States (EAFUS) database that the Food and Drug Administration (FDA) approved as food additives or affirmed as Generally Recognised as Safe (GRAS) [157].

Table 9. Application of *Pinus* in food products.

Food Application	Material Used	Application Result	References
Fruit juices supplementation	*P. pinaster* Ait bark extract	Fresh fruit juices enriched with PBE exhibited the highest inhibitory effect on the growth of pathogenic intestinal bacteria, primarily *E. coli* and *Enterococcus faecalis*. The in vitro digestion process reduced the antibacterial effect of juices on the majority of pathogenic bacteria by approx. 10%.	[152]
		ROS production increased in the inflamed cells exposed to digested commercial red fruit juice (86.8 ± 1.3%) in comparison with the fresh juice (77.4 ± 0.8%) and increased in the inflamed cells exposed to digested enriched red fruit juice (82.6 ± 1.6%) in comparison with the fresh enriched juice (55.8 ± 6%)	[158]
		Following the in vitro digestion, the level of detectable phenolic compounds (expressed as gallic acid equivalent) was higher in both pineapple and red fruit juices enriched with Pycnogenol than non-enriched commercial juices (155.6 mg/100 mL vs 94.6 mg/100 mL and 478.5 mg/100 mL vs 406.9 mg/100 mL respectively). Increased antioxidant activity (measured by 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and oxygen radical absorbance capacity (ORAC) methods) was observed in digested enriched juices, contrary to the same samples before digestion. Undigested, enriched with Pycnogenol pineapple juice displayed a higher antiproliferative effect between the 24th and 72nd hour of incubation in comparison with the non-enriched juice.	[153]

Table 9. *Cont.*

Food Application	Material Used	Application Result	References
	P. brutia, *P. pinea* bark extracts, Pycnogenol®.	The paper shows that juices enriched with pine bark extracts exhibit higher antioxidant capacities and ascorbic acid contents compared to the control group, thereby providing improved functionality.	[154]
Yoghurt supplementation	French marine bark extract	Addition of Pycnogenol neither significantly affected the growth of microorganisms nor caused any modifications in nutritional parameters during the storage of yoghurt. Data indicate that neither the content of total polyphenol nor selected phenolic substances (catechin, epicatechins, chlorogenic acid and caffeic acid) was affected during the shelf life. In conclusion, these results indicate Pycnogenol as a valuable ingredient for the enrichment of yoghurt preparations.	[159]
	P. nigra cones	This study used yoghurt samples to identify the LAB strains generated by the pine cone addition and determined the physicochemical properties of these samples. The genotypic identification revealed that in yoghurt samples, *Streptococcus* thermophilus strains were the main force conducting the fermentation process, while Lactobacillus plantarum strains appeared in three yoghurt samples as an adjunct culture. The time of pine cones collection significantly affected the physicochemical properties of yoghurt.	[160]
Kefir	Pine bud syrup	The pine bud syrup used to enrich kefir contains a lot of polyphenols and terpenes, as well as exhibiting a high antioxidant activity. The addition of pine bud syrup resulted in an increase in total solids, as well as a decrease in the content of fat, proteins and pH levels. The kefir sample containing 10% pine bud syrup was the most appreciated by the sensory panel. Its overall acceptability score was higher (6.71 points) than that of the regular kefir (5.57 points). The addition of 10% pine bud syrup improved the texture and consistency of regular kefir.	[151]
Meat	Pine bark extract (Pycnogenol)	The pine bark extract (Pycnogenol®) significantly improved the oxidative stability of cooked beef and reduced the hexanal content by 73% after 3 days of refrigerated storage.	[155]
Tea	Pine needles	Supplementation of pine needle extract at 1, 2, 4 and 8% in the control diet and mixed groups significantly decreased the weight gain and visceral fat mass in comparison with the corresponding values of the control group.	[34]

Table 9. *Cont.*

Food Application	Material Used	Application Result	References
Beer	*P. sylvestris* needles	The addition of needles increases the beer gustatory properties and decreases the methanol content. The content of ascorbic acid in ready-made drinks amounts to 3.52 mg/100 g. The antioxidant activity of elaborated beer is 178.1 C/100 g and determines its high biological value. In the study, the influence of beer enriched with needle extract was evaluated concerning the antioxidant system of organisms of biological objects. Under acute pathological conditions, a beer with needle extract decreases its oxidative influence on brains of the biological objects.	[161]
Bread	Fermented pine needle extract syrup	Bread with a higher content of pine needle extract syrup demonstrated a slower increase of bread hardening during the storage period, suggesting a slowdown of bread retrogradation. The addition of pine needle extract syrup in bread dough also inhibited the growth of aerobic bacteria and moulds on the bread surface (by 0.8~24 in log (CFU/g) during the 4-day storage). The use of concentration higher than 11% initially gave the bread a strong, fine needle flavour, which disappeared after 2 days. Generally, the addition of pine needle extract syrup had no negative effect on the quality (including sensory) of bread. Therefore, the addition of needle extract syrup could improve storage stability and extend the shelf life of bread.	[156]

Abbreviations: PBE—pine bark extract; ABTS—2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; ORAC—oxygen radical absorbance capacity; LAB—lactic acid bacteria; CFU—colony-forming unit.

9. Conclusions

Residues and by-products constitute an important source of industrially significant biocomponents. Various species of pine tree contain different compositions of bioactive compounds. However, even though the pine bark extracts are commercially available, there is no universal method of extraction that is suitable for all phenols. Depending on the ultimate goal of extraction, an individual examination should be performed to ensure the most appropriate extraction procedure. Regardless of the solvent, method, pine species and plant part used, all pine extracts contain a high number of polyphenols. Nevertheless, individual compounds are characterised by different concentrations, types and levels of their bioactivity. There are few studies on the identification and even fewer studies presenting the quantitative determination of individual polyphenols contained in pine extracts. Pine tree extracts exhibit several described biological activities that may be beneficial to human health. The available examples of the application of pine elements in food are promising. Pine tree extracts, syrups and other intermediates may be components that impart functional properties, extend the shelf life and assign desirable qualities to food products. Pine extracts and oils exhibit great potential as formulation ingredients for food, cosmeceutical and pharmaceutical industries. The reuse of residual pine elements is still limited compared to its potential. In this case, it is necessary to conduct more research to find and develop new products and applications of pine residues and by-products.

Author Contributions: Conceptualisation, writing—original draft preparation, M.D.; writing—review and editing, J.K.-C.; supervision, B.S. All authors have read and agreed to the published version of the manuscript.

Funding: The authors would like to acknowledge funding support from the Folwark Wąsowo Ltd. (Wąsowo, Poland). The research was also supported by statutory funds of the Department of Gastronomy Sciences and Functional Foods of the Poznań University of Life Sciences, grant number 506.751.03.00.

Acknowledgments: Many thanks to Magda and Piotr Wieła (the owners of Folwark Wąsowo, Poland) who inspired to take up this research topic.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Gernandt, D.S.; López, G.G.; García, S.O.; Liston, A. Phylogeny and Classification of *Pinus*. *Taxon* **2005**, *54*, 29–42. [CrossRef]
2. Gifford, E.M.; Foster, A.S. *Morphology and Evolution of Vascular Plants*, 3rd ed.; W. H. Freeman: New York, NY, USA, 1989; ISBN 978-0-7167-1946-5.
3. Richardson, D.M. *Ecology and Biogeography of Pinus*; Cambridge University Press: Cambridge, UK, 2000; ISBN 978-0-521-78910-3.
4. Farjon, A. *Pines: Drawings and Descriptions of the Genus Pinus*, 2nd ed.; Brill: Buckinghamshire, UK, 2018; ISBN 978-90-474-1516-9.
5. Boag, D.A.; Kiceniu, J.W. Protein and Caloric Content of Lodge Pole Pine Needles. *For. Chron.* **1968**, *44*, 28–31. [CrossRef]
6. Nergiz, C.; Dönmez, İ. Chemical Composition and Nutritive Value of *Pinus pinea* L. Seeds. *Food Chem.* **2004**, *86*, 365–368. [CrossRef]
7. Dziedzinski, M.; Kobus-Cisowska, J.; Szymanowska, D.; Stuper-Szablewska, K.; Baranowska, M. Identification of Polyphenols from Coniferous Shoots as Natural Antioxidants and Antimicrobial Compounds. *Molecules* **2020**, *25*, 3527. [CrossRef] [PubMed]
8. Angel, H.Z.; Priest, J.S.; Stovall, J.P.; Oswald, B.P.; Weng, Y.; Williams, H.M. Individual Tree and Stand-Level Carbon and Nutrient Contents across One Rotation of Loblolly Pine Plantations on a Reclaimed Surface Mine. *New For.* **2019**, *50*, 733–753. [CrossRef]
9. Anäs, E.; Ekman, R.; Holmbom, B. Composition of Nonpolar Extractives in Bark of Norway Spruce and Scots Pine. *J. Wood Chem. Technol.* **1983**, *3*, 119–130. [CrossRef]
10. Fischer, C.; Höll, W. Food Reserves of Scots Pine (*Pinus sylvestris* L.). *Trees* **1991**, *5*, 187–195. [CrossRef]
11. Miranda, I.; Gominho, J.; Mirra, I.; Pereira, H. Chemical Characterization of Barks from Picea Abies and *Pinus sylvestris* after Fractioning into Different Particle Sizes. *Ind. Crop. Prod.* **2012**, *36*, 395–400. [CrossRef]
12. Bajorek-Zydroń, K.; Krzaklewski, W.; Pietrzykowski, M. Ocena zaopatrzenia sosny zwyczajnej (*Pinus sylvestris* L.) w składniki pokarmowe w warunkach zwałowiska zewnętrznego KWB "Bełchatów.". *Górnictwo i Geoinżynieria* **2007**, *31*, 67–74.
13. Bamigboye, A.; Okafor, A.; Adepoju, O. Proximate and Mineral Composition of Whole and Dehulled Nigerian Sesame Seed. *Afr. J. Food Sci. Technol.* **2010**, *1*, 71–75.
14. Kulczyński, B.; Gramza-Michałowska, A. The Profile of Secondary Metabolites and Other Bioactive Compounds in *Cucurbita pepo* L. and *Cucurbita moschata* Pumpkin Cultivars. *Molecules* **2019**, *24*, 2945. [CrossRef]
15. Edelman, M.; Colt, M. Nutrient Value of Leaf vs. *Seed. Front. Chem.* **2016**, *4*. [CrossRef]
16. Sayeed, M.A.; Ali, M.A.; Sohel, F.I.; Khan, G.R.M.A.M.; Yeasmin, M.S. Physico-Chemical Characteristics of Mesua Ferrea Seed Oil and Nutritional Composition of Its Seed and Leaves. *Bull. Chem. Soc. Ethiop.* **2004**, *18*. [CrossRef]
17. Szulc, P.; Barłóg, P.; Ambroży-Deręgowska, K.; Mejza, I.; Kobus-Cisowska, J. In-Soil Application of NP Mineral Fertilizer as a Method of Improving Nitrogen Yielding Efficiency. *Agronomy* **2020**, *10*, 1488. [CrossRef]
18. Szulc, P.; Ambroży-Deręgowska, K.; Mejza, I.; Kobus-Cisowska, J.; Ligaj, M. The Role of Agrotechnical Factors in Shaping the Protein Yield of Maize (*Zea mays* L.). *Sustainability* **2020**, *12*, 6833. [CrossRef]
19. Usowicz, B.; Lipiec, J. Spatial Variability of Soil Properties and Cereal Yield in a Cultivated Field on Sandy Soil. *Soil Tillage Res.* **2017**, *174*, 241–250. [CrossRef]
20. Jelonek, T.; Gzyl, J.; Arasimowicz-Jelonek, M.; Tomczak, A.; Remlein, A. The influence of the selected ratios of trees stability on the wall thickness of tracheids in the scots pine (*Pinus sylvestris* L.). *Acta Sci. Pol. Silvarum Colendarum Ratio et Ind. Lignaria* **2016**, *15*, 13–21. [CrossRef]
21. Köhler, I.H.; Huber, S.C.; Bernacchi, C.J.; Baxter, I.R. Increased Temperatures May Safeguard the Nutritional Quality of Crops under Future Elevated CO_2 Concentrations. *Plant J.* **2019**, *97*, 872–886. [CrossRef]
22. Borreani, G.; Tabacco, E.; Schmidt, R.J.; Holmes, B.J.; Muck, R.E. Silage Review: Factors Affecting Dry Matter and Quality Losses in Silages. *J. Dairy Sci.* **2018**, *101*, 3952–3979. [CrossRef]
23. Kyriacou, M.C.; Leskovar, D.I.; Colla, G.; Rouphael, Y. Watermelon and Melon Fruit Quality: The Genotypic and Agro-Environmental Factors Implicated. *Sci. Hortic.* **2018**, *234*, 393–408. [CrossRef]
24. Jakubus, M.; Graczyk, M. Microelement Variability in Plants as an Effect of Sewage Sludge Compost Application Assessed by Different Statistical Methods. *Agronomy* **2020**, *10*, 642. [CrossRef]
25. Castellini, M.; Stellacci, A.M.; Tomaiuolo, M.; Barca, E. Spatial Variability of Soil Physical and Hydraulic Properties in a Durum Wheat Field: An Assessment by the BEST-Procedure. *Water* **2019**, *11*, 1434. [CrossRef]
26. Wen, J.; Ji, H.; Sun, N.; Tao, H.; Du, B.; Hui, D.; Liu, C. Imbalanced Plant Stoichiometry at Contrasting Geologic-Derived Phosphorus Sites in Subtropics: The Role of Microelements and Plant Functional Group. *Plant Soil* **2018**, *430*, 113–125. [CrossRef]

27. Tripathi, D.K.; Singh, V.P.; Chauhan, D.K.; Prasad, S.M.; Dubey, N.K. Role of Macronutrients in Plant Growth and Acclimation: Recent Advances and Future Prospective. In *Improvement of Crops in the Era of Climatic Changes: Volume 2*; Ahmad, P., Wani, M.R., Azooz, M.M., Phan Tran, L.-S., Eds.; Springer: New York, NY, USA, 2014; pp. 197–216. ISBN 978-1-4614-8824-8.
28. Klimek, K.; Jelonek, T.; Tomczak, A. The effect of tree ageing processes on selected physical properties of xylem in Scots pine (*Pinus sylvestris* L.). *Acta Sci. Pol. Silvarum Colendarum Ratio et Ind. Lignaria* **2018**, *17*, 353–360.
29. Guo, W.; Nazim, H.; Liang, Z.; Yang, D. Magnesium Deficiency in Plants: An Urgent Problem. *Crop J.* **2016**, *4*, 83–91. [CrossRef]
30. Broadley, M.; Brown, P.; Cakmak, I.; Rengel, Z.; Zhao, F. Chapter 7—Function of Nutrients: Micronutrients. In *Marschner's Mineral Nutrition of Higher Plants*, 3rd ed.; Marschner, P., Ed.; Academic Press: San Diego, CA, USA, 2012; pp. 191–248. ISBN 978-0-12-384905-2.
31. Cabot, C.; Martos, S.; Llugany, M.; Gallego, B.; Tolrà, R.; Poschenrieder, C. A Role for Zinc in Plant Defense Against Pathogens and Herbivores. *Front. Plant Sci.* **2019**, *10*. [CrossRef]
32. Khan, M.R.; Khan, M.M. Plants Response to Diseases in Sulphur Dioxide Stressed Environment. *Plant Pathol. J.* **2011**, *10*, 1–12. [CrossRef]
33. Kim, K.-Y.; Chung, H.-J. Flavor Compounds of Pine Sprout Tea and Pine Needle Tea. *J. Agric. Food Chem.* **2000**, *48*, 1269–1272. [CrossRef]
34. Jeon, J.-R.; Kim, J.-Y.; Lee, K.-M.; Cho, D.-H. Anti-Obese Effects of Mixture Contained Pine needle, Black Tea and Green Tea Extracts. *Appl. Biol. Chem.* **2005**, *48*, 375–381.
35. Glasdam, S.-M.; Glasdam, S.; Peters, G.H. The Importance of Magnesium in the Human Body. In *Advances in Clinical Chemistry*; Elsevier: Amsterdam, The Netherlands, 2016; Volume 73, pp. 169–193. ISBN 978-0-12-804690-6.
36. Williams, M.H. Dietary Supplements and Sports Performance: Minerals. *J. Int. Soc. Sports Nutr.* **2005**, *2*, 43. [CrossRef]
37. Roh, S.-S.; Park, M.-K.; Kim, Y. Abietic Acid from Resina Pini of *Pinus* Species as a Testosterone 5α-Reductase Inhibitor. *J. Health Sci.* **2010**, *56*, 451–455. [CrossRef]
38. Aggarwal, S.; Thareja, S.; Verma, A.; Bhardwaj, T.R.; Kumar, M. An Overview on 5α-Reductase Inhibitors. *Steroids* **2010**, *75*, 109–153. [CrossRef]
39. Tsao, R. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients* **2010**, *2*, 1231–1246. [CrossRef]
40. Ren, T.; Zheng, P.; Zhang, K.; Liao, J.; Xiong, F.; Shen, Q.; Ma, Y.; Fang, W.; Zhu, X. Effects of GABA on the Polyphenol Accumulation and Antioxidant Activities in Tea Plants (*Camellia sinensis* L.) under Heat-Stress Conditions. *Plant Physiol. Biochem.* **2021**, *159*, 363–371. [CrossRef]
41. Ghahremani, A.; Ghasemi Pirbalouti, A.; Mozafari, H.; Habibi, D.; Sani, B. Phytochemical and Morpho-Physiological Traits of Mullein as a New Medicinal Crop under Different Planting Pattern and Soil Moisture Conditions. *Ind. Crop. Prod.* **2020**, *145*, 111976. [CrossRef]
42. Soares, S.; Brandão, E.; Guerreiro, C.; Soares, S.; Mateus, N.; de Freitas, V. Tannins in Food: Insights into the Molecular Perception of Astringency and Bitter Taste. *Molecules* **2020**, *25*, 2590. [CrossRef] [PubMed]
43. Debnath-Canning, M.; Unruh, S.; Vyas, P.; Daneshtalab, N.; Igamberdiev, A.U.; Weber, J.T. Fruits and Leaves from Wild Blueberry Plants Contain Diverse Polyphenols and Decrease Neuroinflammatory Responses in Microglia. *J. Funct. Foods* **2020**, *68*, 103906. [CrossRef]
44. Manach, C.; Scalbert, A.; Morand, C.; Rémésy, C.; Jiménez, L. Polyphenols: Food Sources and Bioavailability. *Am. J. Clin. Nutr.* **2004**, *79*, 727–747. [CrossRef]
45. Manasa, V.; Padmanabhan, A.; Anu Appaiah, K.A. Utilization of Coffee Pulp Waste for Rapid Recovery of Pectin and Polyphenols for Sustainable Material Recycle. *Waste Manag.* **2021**, *120*, 762–771. [CrossRef] [PubMed]
46. Cutrim, C.S.; Cortez, M.A.S. A Review on Polyphenols: Classification, Beneficial Effects and Their Application in Dairy Products. *Int. J. Dairy Technol.* **2018**, *71*, 564–578. [CrossRef]
47. Wang, H.; Wang, C.; Zou, Y.; Hu, J.; Li, Y.; Cheng, Y. Natural Polyphenols in Drug Delivery Systems: Current Status and Future Challenges. *Giant* **2020**, *3*, 100022. [CrossRef]
48. Debelo, H.; Li, M.; Ferruzzi, M.G. Processing Influences on Food Polyphenol Profiles and Biological Activity. *Curr. Opin. Food Sci.* **2020**, *32*, 90–102. [CrossRef]
49. Ferreira-Santos, P.; Zanuso, E.; Genisheva, Z.; Rocha, C.M.R.; Teixeira, J.A. Green and Sustainable Valorization of Bioactive Phenolic Compounds from *Pinus* By-Products. *Molecules* **2020**, *25*, 2931. [CrossRef]
50. Bindes, M.M.M.; Cardoso, V.L.; Reis, M.H.M.; Boffito, D.C. Maximisation of the Polyphenols Extraction Yield from Green Tea Leaves and Sequential Clarification. *J. Food Eng.* **2019**, *241*, 97–104. [CrossRef]
51. Gorzynik-Debicka, M.; Przychodzen, P.; Cappello, F.; Kuban-Jankowska, A.; Marino Gammazza, A.; Knap, N.; Wozniak, M.; Gorska-Ponikowska, M. Potential Health Benefits of Olive Oil and Plant Polyphenols. *Int. J. Mol. Sci.* **2018**, *19*, 686. [CrossRef] [PubMed]
52. Dzah, C.S.; Duan, Y.; Zhang, H.; Authur, D.A.; Ma, H. Ultrasound-, Subcritical Water- and Ultrasound Assisted Subcritical Water-Derived Tartary Buckwheat Polyphenols Show Superior Antioxidant Activity and Cytotoxicity in Human Liver Carcinoma Cells. *Food Res. Int.* **2020**, *137*, 109598. [CrossRef]
53. Gabaston, J.; Richard, T.; Cluzet, S.; Palos Pinto, A.; Dufour, M.-C.; Corio-Costet, M.-F.; Mérillon, J.-M. *Pinus pinaster* Knot: A Source of Polyphenols against Plasmopara Viticola. *J. Agric. Food Chem.* **2017**, *65*, 8884–8891. [CrossRef] [PubMed]

54. D'Andrea, G. Pycnogenol: A Blend of Procyanidins with Multifaceted Therapeutic Applications? *Fitoterapia* **2010**, *81*, 724–736. [CrossRef] [PubMed]
55. Han, X.; Shen, T.; Lou, H. Dietary Polyphenols and Their Biological Significance. *Int. J. Mol. Sci.* **2007**, *8*, 950–988. [CrossRef]
56. Kobus-Cisowska, J.; Szymanowska, D.; Szczepaniak, O.M.; Gramza-Michałowska, A.; Kmiecik, D.; Kulczyński, B.; Szulc, P.; Górnaś, P. Composition of Polyphenols of Asparagus Spears (Asparagus Officinalis) and Their Antioxidant Potential. *Ciência Rural* **2019**, *49*. [CrossRef]
57. Ku, S.; Mun, S.P. Antioxidant Properties of Monomeric, Oligomeric, and Polymeric Fractions in Hot Water Extract from *Pinus radiata* Bark. *Wood Sci. Technol.* **2007**, *42*, 47–60. [CrossRef]
58. Lantto, T.A.; Dorman, H.J.D.; Shikov, A.N.; Pozharitskaya, O.N.; Makarov, V.G.; Tikhonov, V.P.; Hiltunen, R.; Raasmaja, A. Chemical Composition, Antioxidative Activity and Cell Viability Effects of a Siberian Pine (*Pinus* Sibirica Du Tour) Extract. *Food Chem.* **2009**, *112*, 936–943. [CrossRef]
59. Ferreira-Santos, P.; Genisheva, Z.; Pereira, R.N.; Teixeira, J.A.; Rocha, C.M.R. Moderate Electric Fields as a Potential Tool for Sustainable Recovery of Phenolic Compounds from *Pinus pinaster* Bark. *ACS Sustain. Chem. Eng.* **2019**, *7*, 8816–8826. [CrossRef]
60. Coppen, J.J.W.; Robinson, J.M.; Kaushal, A.N. Composition of Xylem Resin from *Pinus* Wallichiana and P. Roxburghii. *Phytochemistry* **1988**, *27*, 2873–2875. [CrossRef]
61. Willför, S.; Ali, M.; Karonen, M.; Reunanen, M.; Arfan, M.; Harlamow, R. Extractives in Bark of Different Conifer Species Growing in Pakistan. *Holzforschung* **2009**, *63*, 551–558. [CrossRef]
62. Beri, R.M. Chemical Constituents of the Bark of *Pinus roxburghii* Sargent. *Indian J. Chem.* **1970**, 469–470.
63. Rawat, U.; Srivastava, B.; Semwal, S.; Sati, O.P. Xanthones from *Pinus roxburghii*. *J. Indian Chem. Soc.* **2006**, *83*, 391–392. [CrossRef]
64. Iqbal, Z.; Fatima, A.; Khan, S.; Rehman, Z.; Mehmud, S. GC-MS Studies of Needles Essential Oil of *Pinus roxburghaii* and Their Antimicrobial Activity From. *Electron. J. Environ. Agric. Food Chem.* **2010**, 9.
65. Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological Effects of Essential Oils—A Review. *Food Chem. Toxicol.* **2008**, *46*, 446–475. [CrossRef]
66. Zaccardelli, M.; Roscigno, G.; Pane, C.; Celano, G.; Di Matteo, M.; Mainente, M.; Vuotto, A.; Mencherini, T.; Esposito, T.; Vitti, A.; et al. Essential Oils and Quality Composts Sourced by Recycling Vegetable Residues from the Aromatic Plant Supply Chain. *Ind. Crop. Prod.* **2021**, *162*, 113255. [CrossRef]
67. Zhang, L.; Ye, M.; Shi, Y.; Zhu, H.; Chi, L.; Pan, C.; Xu, Y.; Zheng, X.; Xiang, H.; Li, C. Phytochemical Components and Biological Activities of Essential Oils from Three Selected Medicinal Plants. *Ind. Crop. Prod.* **2021**, *160*, 113127. [CrossRef]
68. Turek, C.; Stintzing, F.C. Stability of Essential Oils: A Review. *Compr. Rev. Food Sci. Food Saf.* **2013**, *12*, 40–53. [CrossRef]
69. Sounouvou, H.T.; Toukourou, H.; Catteau, L.; Toukourou, F.; Evrard, B.; Van Bambeke, F.; Gbaguidi, F.; Quetin-Leclercq, J. Antimicrobial Potentials of Essential Oils Extracted from West African Aromatic Plants on Common Skin Infections. *Sci. Afr.* **2021**, e00706. [CrossRef]
70. Park, C.H.; Park, Y.E.; Yeo, H.J.; Chun, S.W.; Baskar, T.B.; Lim, S.S.; Park, S.U. Chemical Compositions of the Volatile Oils and Antibacterial Screening of Solvent Extract from Downy Lavender. *Foods* **2019**, *8*. [CrossRef]
71. Tisserand, R.; Young, R. *Essential Oil Safety—E-Book: A Guide for Health Care Professionals*; Elsevier Health Sciences: Philadelphia, PA, USA, 2013; ISBN 978-0-7020-5434-1.
72. Marques, A.P.S.; Bonfim, F.P.G.; Santos, D.G.P.O.; da Paz Lima, M.; Semir, J.; Martins, E.R.; Zucchi, M.I.; Hantao, L.W.; Sawaya, A.C.H.F.; Marques, M.O.M. Chemical Diversity of Essential Oils from the Brazilian Medicinal Plant Lychnophora Pinaster Mart from Different Environments. *Ind. Crop. Prod.* **2020**, *156*, 112856. [CrossRef]
73. Ramos, Y.J.; de BritoMachado, D.; de Queiroz, G.A.; Guimarães, E.F.; e Defaveri, A.C.; de Lima Moreira, D. Chemical Composition of the Essential Oils of Circadian Rhythm and of Different Vegetative Parts from Piper Mollicomum Kunth—A Medicinal Plant from Brazil. *Biochem. Syst. Ecol.* **2020**, *92*, 104116. [CrossRef]
74. Sharifi-Rad, J.; Sureda, A.; Tenore, G.C.; Daglia, M.; Sharifi-Rad, M.; Valussi, M.; Tundis, R.; Sharifi-Rad, M.; Loizzo, M.R.; Ademiluyi, A.O.; et al. Biological Activities of Essential Oils: From Plant Chemoecology to Traditional Healing Systems. *Molecules* **2017**, *22*. [CrossRef]
75. Armijos, C.; Matailo, A.; Bec, N.; Salinas, M.; Aguilar, G.; Solano, N.; Calva, J.; Ludeña, C.; Larroque, C.; Vidari, G. Chemical Composition and Selective BuChE Inhibitory Activity of the Essential Oils from Aromatic Plants Used to Prepare the Traditional Ecuadorian Beverage Horchata Lojana. *J. Ethnopharmacol.* **2020**, *263*, 113162. [CrossRef] [PubMed]
76. Gaire, S.; Lewis, C.D.; Booth, W.; Scharf, M.E.; Zheng, W.; Ginzel, M.D.; Gondhalekar, A.D. Bed Bugs, Cimex Lectularius L., Exhibiting Metabolic and Target Site Deltamethrin Resistance Are Susceptible to Plant Essential Oils. *Pestic. Biochem. Physiol.* **2020**, *169*, 104667. [CrossRef]
77. Kandi, S.; Godishala, V.; Rao, P.; Ramana, K.V. Biomedical Significance of Terpenes: An Insight. *Biomed. Biotechnol.* **2015**, *3*, 8–10.
78. Ali, B.; Al-Wabel, N.A.; Shams, S.; Ahamad, A.; Khan, S.A.; Anwar, F. Essential Oils Used in Aromatherapy: A Systemic Review. *Asian Pac. J. Trop. Biomed.* **2015**, *5*, 601–611. [CrossRef]
79. Tadtong, S.; Kamkaen, N.; Watthanachaiyingcharoen, R.; Ruangrungsi, N. Chemical Components of Four Essential Oils in Aromatherapy Recipe. *Nat. Prod. Commun.* **2015**, *10*, 1934578X1501000673. [CrossRef]
80. Karlberg, A.-T. Colophony. In *Handbook of Occupational Dermatology*; Kanerva, L., Wahlberg, J.E., Elsner, P., Maibach, H.I., Eds.; Springer: Berlin, Germany, 2000; pp. 509–516. ISBN 978-3-662-07677-4.

81. Silori, G.; Kushwaha, N.; Kumar, V. Essential Oils from Pines: Chemistry and Applications. In *Essential Oil Research, Trends in Biosynthesis, Analytics, Industrial Applications and Biotechnological Production*; Springer: Cham, Swizerland, 2019; pp. 275–297.
82. Hajdari, A.; Mustafa, B.; Nebija, D.; Selimi, H.; Veselaj, Z.; Breznica, P.; Quave, C.L.; Novak, J. Essential Oil Composition of *Pinus peuce* Griseb. Needles and Twigs from Two National Parks of Kosovo. Available online: https://www.hindawi.com/journals/tswj/2016/5393079/ (accessed on 26 January 2021).
83. Koukos, P.K.; Papadopoulou, K.I.; Patiaka, D.T.; Papagiannopoulos, A.D. Chemical Composition of Essential Oils from Needles and Twigs of Balkan Pine (*Pinus peuce* Grisebach) Grown in Northern Greece. *J. Agric. Food Chem.* **2000**, *48*, 1266–1268. [CrossRef] [PubMed]
84. Ustun, O.; Sezik, E.; Kurkcuoglu, M.; Baser, K.H.C. Study of the Essential Oil Composition of *Pinus sylvestris* from Turkey. *Chem. Nat. Compd.* **2006**, *42*, 26–31. [CrossRef]
85. Borges, R.S.; Ortiz, B.L.S.; Pereira, A.C.M.; Keita, H.; Carvalho, J.C.T. Rosmarinus Officinalis Essential Oil: A Review of Its Phytochemistry, Anti-Inflammatory Activity, and Mechanisms of Action Involved. *J. Ethnopharmacol.* **2019**, *229*, 29–45. [CrossRef]
86. Salehi, B.; Upadhyay, S.; Erdogan Orhan, I.; Kumar Jugran, A.; Jayaweera, S.L.D.; Dias, D.A.; Sharopov, F.; Taheri, Y.; Martins, N.; Baghalpour, N.; et al. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules* **2019**, *9*. [CrossRef]
87. Thomsett, M.R.; Moore, J.C.; Buchard, A.; Stockman, R.A.; Howdle, S.M. New Renewably-Sourced Polyesters from Limonene-Derived Monomers. *Green Chem.* **2019**, *21*, 149–156. [CrossRef]
88. Almirall, M.; Montaña, J.; Escribano, E.; Obach, R.; Berrozpe, J.D. Effect of D-Limonene, Alpha-Pinene and Cineole on in Vitro Transdermal Human Skin Penetration of Chlorpromazine and Haloperidol. *Arzneimittelforschung* **1996**, *46*, 676–680.
89. Park, Y.S.; Jeon, M.H.; Hwang, H.J.; Park, M.R.; Lee, S.-H.; Kim, S.G.; Kim, M. Antioxidant Activity and Analysis of Proanthocyanidins from Pine (*Pinus densiflora*) Needles. *Nutr. Res. Pract.* **2011**, *5*, 281–287. [CrossRef]
90. Wei, Q.-Y.; Zhou, B.; Cai, Y.-J.; Yang, L.; Liu, Z.-L. Synergistic Effect of Green Tea Polyphenols with Trolox on Free Radical-Induced Oxidative DNA Damage. *Food Chem.* **2006**, *96*, 90–95. [CrossRef]
91. Kinyua Muthuri, L.; Nagy, L.; Nagy, G. Chronopotentiometric Method for Assessing Antioxidant Activity: A Reagentless Measuring Technique. *Electrochem. Commun.* **2021**, *122*, 106907. [CrossRef]
92. Zhu, H.; Qiu, Y.; Gong, D.; Wang, J.; Sun, G. A Novel Method for Quality Consistency Evaluation of Yankening Tablet by Multi-Wavelength Fusion Profiling Combined with Overall Components Dissolution Method and Antioxidant Activity Analysis. *J. Pharm. Biomed. Anal.* **2021**, *196*, 113910. [CrossRef]
93. Kusznierewicz, B.; Staroszczyk, H.; Malinowska-Pańczyk, E.; Parchem, K.; Bartoszek, A. Novel ABTS-Dot-Blot Method for the Assessment of Antioxidant Properties of Food Packaging. *Food Packag. Shelf Life* **2020**, *24*, 100478. [CrossRef]
94. Juárez-Gómez, J.; Ramírez-Silva, M.T.; Guzmán-Hernández, D.S.; Romero-Romo, M.; Palomar-Pardavé, M. Novel Electrochemical Method to Evaluate the Antioxidant Capacity of Infusions and Beverages, Based on in Situ Formation of Free Superoxide Radicals. *Food Chem.* **2020**, *332*, 127409. [CrossRef] [PubMed]
95. Moon, J.-K.; Shibamoto, T. Antioxidant Assays for Plant and Food Components. *J. Agric. Food Chem.* **2009**, *57*, 1655–1666. [CrossRef]
96. Naji, K.; Thamer, F.; Dauqan, M.; Alshaibi, Y.; Dsouza, M. Ferric-Bipyridine Assay: A Novel Spectrophotometric Method for Measurement of Antioxidant Capacity. *Heliyon* **2020**, *6*. [CrossRef]
97. Pastor, F.T.; Šegan, D.M.; Gorjanović, S.Ž.; Kalušević, A.M.; Sužnjević, D.Ž. Development of Voltammetric Methods for Antioxidant Activity Determination Based on Fe(III) Reduction. *Microchem. J.* **2020**, *155*, 104721. [CrossRef]
98. dos Reis Teixeira, A.; Teixeira, R.R.; Ribeiro, I.M.L.; Pereira, W.L.; Manhabosco, T.M.; de Brito, A.C.F.; Oliveira, L.A.M.; Coelho Nogueira, K. de O.P. Association of Electroanalytical and Spectrophotometric Methods to Evaluate the Antioxidant Activity of Isobenzofuranone in Primary Cultures of Hippocampal Neurons. *Toxicol. In Vitro* **2020**, *68*, 104970. [CrossRef]
99. Jerez, M.; Selga, A.; Sineiro, J.; Torres, J.L.; Núñez, M.J. A Comparison between Bark Extracts from *Pinus pinaster* and *Pinus radiata*: Antioxidant Activity and Procyanidin Composition. *Food Chem.* **2007**, *100*, 439–444. [CrossRef]
100. Cretu, E.; Karonen, M.; Salminen, J.-P.; Mircea, C.; Trifan, A.; Charalambous, C.; Constantinou, A.I.; Miron, A. In Vitro Study on the Antioxidant Activity of a Polyphenol-Rich Extract from *Pinus brutia* Bark and Its Fractions. *J. Med. Food* **2013**, *16*, 984–991. [CrossRef]
101. Su, X.-Y.; Wang, Z.-Y.; Liu, J.-R. In Vitro and in Vivo Antioxidant Activity of *Pinus koraiensis* Seed Extract Containing Phenolic Compounds. *Food Chem.* **2009**, *117*, 681–686. [CrossRef]
102. Venkatesan, T.; Choi, Y.-W.; Kim, Y.-K. Impact of Different Extraction Solvents on Phenolic Content and Antioxidant Potential of *Pinus densiflora* Bark Extract. Available online: https://www.hindawi.com/journals/bmri/2019/3520675/ (accessed on 27 January 2021).
103. Venkatesan, T.; Choi, Y.-W.; Kim, Y.-K. Effect of an Extraction Solvent on the Antioxidant Quality of *Pinus densiflora* Needle Extract. *J. Pharm. Anal.* **2019**, *9*, 193–200. [CrossRef]
104. Apetrei, C.L.; Tuchilus, C.; Aprotosoaie, A.C.; Oprea, A.; Malterud, K.E.; Miron, A. Chemical, Antioxidant and Antimicrobial Investigations of *Pinus cembra* L. Bark and Needles. *Molecules* **2011**, *16*, 7773–7788. [CrossRef]
105. Dziedziński, M.; Kobus-Cisowska, J.; Szymanowska-Powałowska, D.; Stuper-Szablewska, K.; Baranowska, M. Polyphenols Composition, Antioxidant and Antimicrobial Properties of *Pinus sylvestris* L. Shoots Extracts Depending on Different Drying Methods. *Emir. J. Food Agric.* **2020**, 229–237. [CrossRef]
106. Iravani, S.; Zolfaghari, B. Pharmaceutical and Nutraceutical Effects of *Pinus pinaster* Bark Extract. *Res. Pharm. Sci.* **2011**, *6*, 1–11.

107. Li, Y.-Y.; Feng, J.; Zhang, X.-L.; Cui, Y.-Y. Pine Bark Extracts: Nutraceutical, Pharmacological, and Toxicological Evaluation. *J. Pharmacol. Exp. Ther.* **2015**, *353*, 9–16. [CrossRef] [PubMed]
108. Robertson, N.U.; Schoonees, A.; Brand, A.; Visser, J. Pine Bark (*Pinus* Spp.) Extract for Treating Chronic Disorders. *Cochrane Database Syst. Rev* **2020**. [CrossRef]
109. Saliou, C.; Rimbach, G.; Moini, H.; McLaughlin, L.; Hosseini, S.; Lee, J.; Watson, R.R.; Packer, L. Solar Ultraviolet-Induced Erythema in Human Skin and Nuclear Factor-Kappa-B–Dependent Gene Expression in Keratinocytes Are Modulated by a French Maritime Pine Bark Extract. *Free. Radic. Biol. Med.* **2001**, *30*, 154–160. [CrossRef]
110. Furumura, M.; Sato, N.; Kusaba, N.; Takagaki, K.; Nakayama, J. Oral Administration of French Maritime Pine Bark Extract (Flavangenol®) Improves Clinical Symptoms in Photoaged Facial Skin. *Clin. Interv. Aging* **2012**, *7*, 275–286. [CrossRef]
111. Theadom, A.; Mahon, S.; Barker-Collo, S.; McPherson, K.; Rush, E.; Vandal, A.C.; Feigin, V.L. Enzogenol for Cognitive Functioning in Traumatic Brain Injury: A Pilot Placebo-Controlled RCT. *Eur. J. Neurol.* **2013**, *20*, 1135–1144. [CrossRef]
112. Pipingas, A.; Silberstein, R.B.; Vitetta, L.; Rooy, C.V.; Harris, E.V.; Young, J.M.; Frampton, C.M.; Sali, A.; Nastasi, J. Improved Cognitive Performance after Dietary Supplementation with a *Pinus radiata* Bark Extract Formulation. *Phytother. Res.* **2008**, *22*, 1168–1174. [CrossRef] [PubMed]
113. Spagnuolo, C.; Napolitano, M.; Tedesco, I.; Moccia, S.; Milito, A.; Russo, G.L. Neuroprotective Role of Natural Polyphenols. *Curr. Top. Med. Chem.* **2016**, *16*, 1943–1950. [CrossRef]
114. Kim, K.J.; Hwang, E.-S.; Kim, M.-J.; Park, J.-H.; Kim, D.-O. Antihypertensive Effects of Polyphenolic Extract from Korean Red Pine (*Pinus densiflora* Sieb. et Zucc.) Bark in Spontaneously Hypertensive Rats. *Antioxidants* **2020**, *9*. [CrossRef]
115. Ahn, H.; Go, G.-W. *Pinus densiflora* Bark Extract (PineXol) Decreases Adiposity in Mice by Down-Regulation of Hepatic De Novo Lipogenesis and Adipogenesis in White Adipose Tissue. *J. Microbiol. Biotechnol.* **2017**, *27*, 660–667. [CrossRef] [PubMed]
116. Kaushik, P.; Khokra, S.L.; Kaushik, D. Evaluation of Antidiabetic Potential of *Pinus roxburghii* Bark Extract in Alloxan Induced Diabetic Rats. *J. Pharmacogn. Nat. Prod.* **2015**, *1*, 1–5. [CrossRef]
117. Khan, I.; Singh, V.; Chaudhary, A.K. Hepatoprotective Activity of *Pinus roxburghii* Sarg. Wood Oil against Carbon Tetrachloride and Ethanol Induced Hepatotoxicity. *Bangladesh J. Pharmacol.* **2012**, *7*, 94–99. [CrossRef]
118. Puri, A.; Srivastava, A.K.; Singhal, B.; Mishra, S.K.; Srivastava, S.; Lakshmi, V. Antidyslipidemic and Antioxidant Activity of *Pinus roxburghii* Needles. *Med. Chem. Res.* **2011**, *20*, 1589–1593. [CrossRef]
119. Kaushik, D.; Kumar, A.; Kaushik, P.; Rana, A.C. Analgesic and Anti-Inflammatory Activity of *Pinus roxburghii* Sarg. Available online: https://www.hindawi.com/journals/aps/2012/245431/ (accessed on 19 December 2020).
120. Kaushik, D.; Kumar, A.; Kaushik, P.; Rana, A.C. Anticonvulsant Activity of Alcoholic Extract of Bark of *Pinus roxburghii* Sarg. *J. Chin. Integr. Med.* **2012**, *10*, 1056–1060. [CrossRef]
121. Feng, W.Y.; Tanaka, R.; Inagaki, Y.; Saitoh, Y.; Chang, M.O.; Amet, T.; Yamamoto, N.; Yamaoka, S.; Yoshinaka, Y. Pycnogenol, a Procyanidin-Rich Extract from French Maritime Pine, Inhibits Intracellular Replication of HIV-1 as Well as Its Binding to Host Cells. *Jpn. J. Infect. Dis.* **2008**, *61*, 279–285.
122. Xu, S.; Zhang, S.; Wang, X.; Gao, Y.; Qin, X.; Wu, K. Effect of *Pinus massoniana* Lamb. Bark Extract on Lytic Cycle of Epstein-Barr Virus. *Indian J. Exp. Biol.* **2012**, *50*, 708–713.
123. Kinger, H.K.; Dutt, K.R.; Saini, V.; Sheeja, E.; Gupta, V.B.; Deb, L. Wound Healing Activity of *Pinus longifolia* Roxb. *Plant Arch.* **2006**, *6*, 651–652.
124. Sajid, A.; Manzoor, Q.; Iqbal, M.; Tyagi, A.K.; Sarfraz, R.A.; Sajid, A. *Pinus roxburghii* Essential Oil Anticancer Activity and Chemical Composition Evaluation. *EXCLI J.* **2018**, *17*, 233–245. [CrossRef]
125. Kaushik, P.; Khokra, S.; Rana, A.C.; Kaushik, D. Evaluation of Anticancer Activity of *Pinus roxburghii* Sarg. Against IMR-32 Human Neuroblastoma Cancer Cell Line. *Int. J. Pharm. Clin. Res.* **2015**, *7*, 105–108.
126. Enseleit, F.; Sudano, I.; Périat, D.; Winnik, S.; Wolfrum, M.; Flammer, A.J.; Fröhlich, G.M.; Kaiser, P.; Hirt, A.; Haile, S.R.; et al. Effects of Pycnogenol on Endothelial Function in Patients with Stable Coronary Artery Disease: A Double-Blind, Randomized, Placebo-Controlled, Cross-over Study. *Eur. Heart J.* **2012**, *33*, 1589–1597. [CrossRef] [PubMed]
127. Kim, J.-W.; Im, S.; Jeong, H.-R.; Jung, Y.-S.; Lee, I.; Kim, K.J.; Park, S.K.; Kim, D.-O. Neuroprotective Effects of Korean Red Pine (*Pinus densiflora*) Bark Extract and Its Phenolics. *J. Microbiol. Biotechnol.* **2018**, *28*, 679–687. [CrossRef] [PubMed]
128. Sharma, A.; Goyal, R. In-Vitro Neuronal Cell Proliferation and in-Vivo Neuroprotective Activity of *Pinus roxburghii* for Memory and Cognition. *Alzheimer's Dement.* **2020**, *16*, e038337. [CrossRef]
129. Azmat, A.; Ahmed, K.; Ahmed, M.; Tariq, B. Antinocciceptive Effects of Poly Herbal Oil Extract. *Pak. J. Phamacol.* **2006**, *23*, 1–7.
130. Panahande, S.B.; Maghbooli, Z.; Hossein-nezhad, A.; Qorbani, M.; Moeini-Nodeh, S.; Haghi-Aminjan, H.; Hosseini, S. Effects of French Maritime Pine Bark Extract (Oligopin®) Supplementation on Bone Remodeling Markers in Postmenopausal Osteopenic Women: A Randomized Clinical Trial. *Phytother. Res.* **2019**, *33*, 1233–1240. [CrossRef]
131. Fair, R.J.; Tor, Y. Antibiotics and Bacterial Resistance in the 21st Century. *Perspect. Med. Chem.* **2014**, *6*, 25–64. [CrossRef]
132. Brecha, F.S.; Ozanne, E.M.; Esplin, J.; Stoddard, G.J.; Nirula, R.; Huang, L.C.; Cohan, J.N. Patient Willingness to Accept Antibiotic Side Effects to Reduce SSI After Colorectal Surgery. *J. Surg. Res.* 2020. [CrossRef]
133. Lai, C.-S.; Ponnusamy, Y.; Lim, G.-L.; Ramanathan, S. Antibacterial, Antibiofilm and Antibiotic-Potentiating Effects of a Polyphenol-Rich Fraction of Dicranopteris Linearis (Burm.f.). *Underw. J. Herb. Med.* **2021**, *25*, 100419. [CrossRef]
134. Virjamo, V.; Julkunen-Tiitto, R. Quality and Quantity of Piperidine Alkaloids in Needles and Bark of Scots Pine (*Pinus sylvestris*) Seedlings. *Phytochem. Lett.* **2018**, *26*, 106–109. [CrossRef]

135. Tong, S.Y.C.; Davis, J.S.; Eichenberger, E.; Holland, T.L.; Fowler, V.G. Staphylococcus Aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. *Clin. Microbiol. Rev.* **2015**, *28*, 603–661. [CrossRef]
136. Mun, S.-H.; Joung, D.-K.; Kim, Y.-S.; Kang, O.-H.; Kim, S.-B.; Seo, Y.-S.; Kim, Y.-C.; Lee, D.-S.; Shin, D.-W.; Kweon, K.-T.; et al. Synergistic Antibacterial Effect of Curcumin against Methicillin-Resistant Staphylococcus Aureus. *Phytomedicine* **2013**, *20*, 714–718. [CrossRef]
137. Craft, K.M.; Nguyen, J.M.; Berg, L.J.; Townsend, S.D. Methicillin-Resistant Staphylococcus Aureus (MRSA): Antibiotic-Resistance and the Biofilm Phenotype. *Med. Chem. Commun.* **2019**, *10*, 1231–1241. [CrossRef]
138. Haaber, J.; Penadés, J.R.; Ingmer, H. Transfer of Antibiotic Resistance in Staphylococcus Aureus. *Trends Microbiol.* **2017**, *25*, 893–905. [CrossRef] [PubMed]
139. Safarpoor Dehkordi, F.; Gandomi, H.; Basti, A.A.; Misaghi, A.; Rahimi, E. Phenotypic and Genotypic Characterization of Antibiotic Resistance of Methicillin-Resistant Staphylococcus Aureus Isolated from Hospital Food. *Antimicrob. Resist. Infect. Control* **2017**, *6*, 104. [CrossRef]
140. Lee, J.-H.; Yang, H.-Y.; Lee, H.-S.; Hong, S.-K. Chemical Composition and Antimicrobial Activity of Essential Oil from Cones of *Pinus koraiensis*. *J. Microbiol. Biotechnol.* **2008**, *18*, 497–502.
141. Ulukanli, Z.; Karabörklü, S.; Bozok, F.; Ates, B.; Erdogan, S.; Cenet, M.; Karaaslan, M.G. Chemical Composition, Antimicrobial, Insecticidal, Phytotoxic and Antioxidant Activities of Mediterranean *Pinus brutia* and *Pinus pinea* Resin Essential Oils. *Chin. J. Nat. Med.* **2014**, *12*, 901–910. [CrossRef]
142. Park, J.-S.; Lee, G.-H. Volatile Compounds and Antimicrobial and Antioxidant Activities of the Essential Oils of the Needles of *Pinus densiflora* and *Pinus thunbergii*: Bioactivity of Pine Needle Essential Oil. *J. Sci. Food Agric.* **2011**, *91*, 703–709. [CrossRef]
143. Jennison, A.V.; Verma, N.K. Shigella Flexneri Infection: Pathogenesis and Vaccine Development. *FEMS Microbiol. Rev.* **2004**, *28*, 43–58. [CrossRef] [PubMed]
144. Ashkenazi, S.; Cohen, D. An Update on Vaccines against Shigella. *Ther. Adv. Vaccines* **2013**, *1*, 113–123. [CrossRef]
145. González-Lamothe, R.; Mitchell, G.; Gattuso, M.; Diarra, M.S.; Malouin, F.; Bouarab, K. Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens. *Int. J. Mol. Sci.* **2009**, *10*, 3400. [CrossRef]
146. Lee, Y.; Han, O.; Choi, H.-S.; Lee, B.-Y.; Chung, H.-J.; Lee, O.-I. Antioxidant and Anti-Adipogenic Effects of PineXol ®. *Korean J. Food Sci. Technol.* **2013**, *45*, 97–103. [CrossRef]
147. Yang, H.; Chowdhury, V.S.; Han, G.; Zhang, R.; Furuse, M. Flavangenol Regulates Gene Expression of HSPs, Anti-Apoptotic and Anti-Oxidative Factors to Protect Primary Chick Brain Cells Exposed to High Temperature. *J. Therm. Biol.* **2019**, *81*, 1–11. [CrossRef]
148. Shand, B.; Strey, C.; Scott, R.; Morrison, Z.; Gieseg, S. Pilot Study on the Clinical Effects of Dietary Supplementation with Enzogenol, a Flavonoid Extract of Pine Bark and Vitamin C. *Phytother. Res.* **2003**, *17*, 490–494. [CrossRef] [PubMed]
149. Varelas, V.; Langton, M. Forest Biomass Waste as a Potential Innovative Source for Rearing Edible Insects for Food and Feed—A Review. *Innov. Food Sci. Emerg. Technol.* **2017**, *41*, 193–205. [CrossRef]
150. Šojić, B.; Tomović, V.; Jokanović, M.; Ikonić, P.; Džinić, N.; Kocić-Tanackov, S.; Popović, L.; Tasić, T.; Savanović, J.; Šojić, N.Ž. Antioxidant Activity of *Juniperus communis* L. Essential Oil in Cooked Pork Sausages. *Czech J. Food Sci.* **2017**, *35*, 189–193. [CrossRef]
151. Semeniuc, C.A.; Rotar, A.; Stan, L.; Pop, C.R.; Socaci, S.; Mireşan, V.; Muste, S. Characterization of Pine Bud Syrup and Its Effect on Physicochemical and Sensory Properties of Kefir. *CyTA J. Food* **2016**, *14*, 213–218. [CrossRef]
152. López-Nicolás, R.; González-Bermúdez, C.A.; Ros-Berruezo, G.; Frontela-Saseta, C. Influence of in Vitro Gastrointestinal Digestion of Fruit Juices Enriched with Pine Bark Extract on Intestinal Microflora. *Food Chem.* **2014**, *157*, 14–19. [CrossRef] [PubMed]
153. Frontela-Saseta, C.; López-Nicolás, R.; González-Bermúdez, C.A.; Peso-Echarri, P.; Ros-Berruezo, G.; Martínez-Graciá, C.; Canali, R.; Virgili, F. Evaluation of Antioxidant Activity and Antiproliferative Effect of Fruit Juices Enriched with Pycnogenol®in Colon Carcinoma Cells. The Effect of In Vitro Gastrointestinal Digestion. *Phytother. Res.* **2011**, *25*, 1870–1875. [CrossRef]
154. Yesil Celiktas, O.; Isleten, M.; Vardar-Sukan, F.; Oyku Cetin, E. In Vitro Release Kinetics of Pine Bark Extract Enriched Orange Juice and the Shelf Stability. *Br. Food J.* **2010**, *112*, 1063–1076. [CrossRef]
155. Ahn, J.; Grün, I.U.; Fernando, L.N. Antioxidant Properties of Natural Plant Extracts Containing Polyphenolic Compounds in Cooked Ground Beef. *J. Food Sci.* **2002**, *67*, 1364–1369. [CrossRef]
156. Choi, D.-M.; Chung, S.-K.; Lee, D.-S. Shelf Life Extension of Steamed Bread by the Addition of Fermented Pine Needle Extract Syrup as an Ingredient. *J. Korean Soc. Food Sci. Nutr.* **2007**, *36*, 616–621. [CrossRef]
157. Ahn, J.; Grün, I.U.; Mustapha, A. Effects of Plant Extracts on Microbial Growth, Color Change, and Lipid Oxidation in Cooked Beef. *Food Microbiol.* **2007**, *24*, 7–14. [CrossRef]
158. Frontela-Saseta, C.; López-Nicolás, R.; González-Bermúdez, C.A.; Martínez-Graciá, C.; Ros-Berruezo, G. Anti-Inflammatory Properties of Fruit Juices Enriched with Pine Bark Extract in an in Vitro Model of Inflamed Human Intestinal Epithelium: The Effect of Gastrointestinal Digestion. *Food Chem. Toxicol.* **2013**, *53*, 94–99. [CrossRef] [PubMed]
159. Ruggeri, S.; Straniero, R.; Pacifico, S.; Aguzzi, A.; Virgili, F. French Marine Bark Extract Pycnogenol as a Possible Enrichment Ingredient for Yogurt. *J. Dairy Sci.* **2008**, *91*, 4484–4491. [CrossRef]
160. Sert, D.; Mercan, E.; Dertli, E. Characterization of Lactic Acid Bacteria from Yogurt-like Product Fermented with Pine Cone and Determination of Their Role on Physicochemical, Textural and Microbiological Properties of Product. *LWT* **2017**, *78*, 70–76. [CrossRef]
161. Penkina, N.; Tatar, L.; Kolesnyk, V.; Karbivnycha, T.; Letuta, T. the Study of Beer Quality with the Reduced Toxic Effect. *EUREKA Life Sci.* **2017**, 35–43. [CrossRef]

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Identification of Polyphenols from Coniferous Shoots as Natural Antioxidants and Antimicrobial Compounds

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Academic Editors: Ana Barros and Irene Gouvinhas
Received: 1 July 2020; Accepted: 22 July 2020; Published: 1 August 2020

Abstract: Currently, coniferous shoots are almost absent as a food ingredient despite their wide availability in many parts of the world. The aim of the study was to assess and compare the composition of selected plant metabolites, evaluate the antioxidant and antimicrobial properties of selected shoots collected in 2019 from the arboretum in Zielonka (Poland), including individual samples from *Picea abies* L. (PA), *Larix decidua* Mill (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC). The present work has shown that aqueous extracts obtained from tested shoots are a rich source of phenols such as caffeic acid, ferulic acid, chlorogenic acid, 4-hydroxybenzoic acid and many others. Obtained extracts exhibit antioxidant and antimicrobial properties in vitro. The highest sum of the studied phenolic compounds was found in the PA sample (13,947.80 µg/g dw), while the lowest in PS (6123.57 µg/g dw). The samples were particularly rich in ferulic acid, chlorogenic acid and 4-hydroxybenzoic acid. The highest values regarding the Folin-Ciocâlteu reagent (FCR) and ferric reducing ability of plasma (FRAP) reducing ability tests, as well as the total flavonoid content assay, were obtained for the LD sample, although the LD (14.83 mg GAE/g dw) and PM (14.53 mg GAE/g dw) samples did not differ statistically in the FCR assay. With respect to free radical quenching measurements (DPPH), the PA (404.18-µM Trolox/g dw) and JC (384.30-µM Trolox/g dw) samples had the highest radical quenching ability and did not differ statistically. Generally, extracts obtained from PA and PS showed the highest antimicrobial activity against tested Gram-negative bacteria, Gram-positive bacteria and fungi.

Keywords: bioactive compounds; phytochemicals; antioxidant and antimicrobial properties; polyphenols; coniferous trees; shoots

1. Introduction

The demand for plant health products is increasing worldwide [1]. Currently, coniferous shoots are almost absent as a food ingredient despite their wide availability in many parts of the world. The exceptions are the common juniper, whose berry-like cones are a spice valued in Europe and pine shoots [2]. Products with pine shoots are available on the market, including pine shoot syrup, beer made with pine shoots or herbal teas. Despite this, products with shoots are currently not very

popular [3,4]. However, these raw materials were often used in folk medicine in the past, among others in ancient Rome or traditional Chinese and Islamic medicine. Bark, shoots and resins were used as a panacea for, e.g., diseases of the urinary tract, digestive tract, nervous system, respiratory and skin diseases [5–7]. Research carried out in recent years has confirmed that compounds present in conifer shoots exhibit therapeutic effects, and shoots are rich sources of polyphenols and have antioxidant properties [8,9]. Coniferous shoots are a particularly rich source of terpenoid hydrocarbon, pinene, in the form of alpha and beta isomers, which as one of the main components of the resin belongs to water-insoluble fraction. Alpha- and beta-pinene may serve as precursors of aromatic compounds in food production, they are also components of renal and hepatic drugs [9]. In rodent studies, alpha-pinene showed, inter alia, gastroprotective, analgesic and anti-convulsive properties; it has also exerted therapeutic effects in some cancers and allergies [10–13]. Compounds present in conifers also show antioxidant and reducing effects [14]. New studies have indicated that functional foods with antioxidant potential can be crucial in maintaining health. Excessive increase in free radical concentration due to endogenous and exogenous factors in the body may be conducive to damage to, i.e., biologic structures such as DNA, lipid membranes and proteins [15]. Cell oxidation–reduction homeostasis is one of the most important elements regulating the body's functions at the molecular level, its disorders can promote dysfunctions, especially in terms of enzymatic activity [16].

Currently, the state of knowledge about the properties and applications of coniferous components is incomplete, thus far no experiments have been conducted on the use of the most popular coniferous trees in food, and these raw materials are a promising component not only of drugs or dietary supplements, but also functional food, which is currently one of the fastest-growing food market segments [17].

The aim of the study was to assess and compare the composition of selected plant metabolites, evaluate the antioxidant and antimicrobial properties of selected shoots samples from various conifers, including *Picea abies* L. (PA), *Larix decidua* Mill (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC).

2. Results

2.1. Shoot Commodity Assessment

The tested shoots samples differed visually significantly (Figure 1). As shown in the Table 1, needles obtained from the shoots of individual trees were of different shapes and dimensions, the longest needles had the PS sample (54.84), while the shortest the JC sample (8.74). Shoots differed in color measured in the CIELab space. The PM sample had the highest brightness (L* = 33.74) while the PA sample was the darkest (L* = 26.09). In terms of parameter L*, the JC, LD and PS samples did not differ statistically. The values a* and b* expressed the color in the range from green to red and from blue to yellow, respectively; the highest value for the parameter a* was found in the PA sample (8.26), the lowest in JC (−5.25). The highest b* value was found in the JC sample (24.48), the lowest for PS (10.73). Samples differed significantly in terms of dry weight except for the PM and PA samples. The top-level dry weight content, significantly higher than in the other samples, was recorded for the PS sample. Extracts from dried shoots did not differ significantly in osmolality. The PA sample freezing point was the lowest (0.09), but the differences between the samples were small.

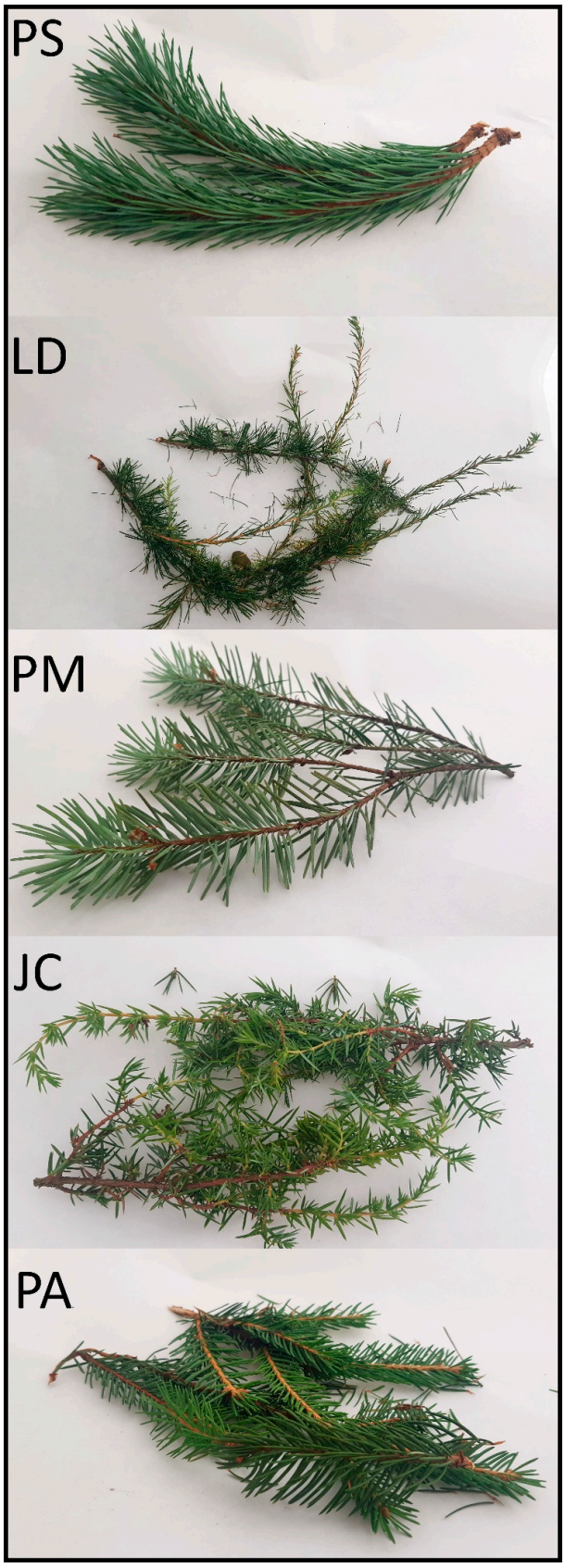

Figure 1. Fresh conifer shoots. Abbreviations: *Picea abies* L. (PA), *Larix decidua* Mill (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus* communis L. (JC).

Table 1. Commodity assessment of tested coniferous shoots.

Parameter		*Pseudotsuga menziesii*	*Juniperus communis* L.	*Larix decidua* Mill	*Pinus sylvestris* L.	*Picea abies* L.
Needle Shape		Flattened, cylindrical	Wide, flattened diamonds	Thin, filamentous	filamentous	Elongated diamond
Needle Length (mm)		23.27 ^{a} ± 2.99	8.74 ^{b} ± 3.08	25.31 ^{c} ± 5.26	54.84 ^{d} ± 9.91	14.32 ^{e} ± 2.91
Color	L*	33.74 ^{a} ± 2.50	38.83 ^{b} ± 0.35	29.31 ^{c} ± 0.14	30.33 ^{c} ± 0.78	26.09 ^{d} ± 0.56
	a*	−0.18 ^{a} ± 0.99	−5.25 ^{b} ± 0.20	2.14 ^{c} ± 0.22	−1.14 ^{a} ± 1.19	8.26 ^{d} ± 0.91
	b*	13.43 ^{a} ± 0.18	24.48 ^{b} ± 0.21	15.28 ^{c} ± 0.71	10.73 ^{d} ± 0.23	14.82 ^{c} ± 0.36
Dry Weight (%)		13.25 ^{a} ± 0.35	10.95 ^{b} ± 0.35	16.16 ^{c} ± 0.49	32.22 ^{d} ± 0.49	13.98 ^{a} ± 0.49
Extract Osmolality (mOsm/kg H_2O)		0.04 ^{a} ± 0.00	0.04 ^{b} ± 0.00	0.05 ^{c} ± 0.00	0.02 ^{d} ± 0.00	0.05 ^{e} ± 0.00
Freezing Temperature (°C)		−0.08 ± 0.00	−0.07 ± 0.00	−0.09 ± 0.00	−0.04 ± 0.00	−0.09 ± 0.00

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

2.2. Phytochemical Shoot Content

There was a wide variation for other phenolic acids tested (Table 2). The highest sum of the studied phenolic compounds was found in the PA sample (13,947.8 µg/g dw), while the lowest in PS (6123.57 µg/g dw). The samples were particularly rich in caffeic acid, ferulic acid, chlorogenic acid and 4-hydroxybenzoic acid. The highest variation was found in the case of ferulic acid, where in the PM sample it was 5002.20 µg/g dw and 1129.85 µg/g dw for PA. Salicylic acid, naringenin, vitexin, rutin, quercetin, apigenin, kaempferol, and luteolin were present in very low concentrations in all samples.

Table 2. HPLC analysis of phenolic compounds in tested conifer shoot extracts.

Phenolic Acid (µg/g dw)	LD	JC	PM	PS	PA
Gallic Acid	10.86 ^{b} ± 0.48	994.72 ^{c} ± 47.49	57.04 ^{d} ± 1.27	208.38 ^{e} ± 069	695.88 ^{f} ± 5.29
2,5-Dihydroxobenzoic Acid	130.11 ^{b} ± 6.8	25.55 ^{c} ± 0.15	7.18 ^{c} ± 0.16	16.63 ^{c} ± 0.54	62.43 ^{d} ± 1.95
4-Hydroxybenzoic Acid	622.99 ^{b} ± 24.61	22.96 ^{b} ± 1.44	1148.62 ^{b} ± 23.72	1084.92 ^{b} ± 39.04	4014.44 ^{c} ± 58.25
Caffeic Acid	2994.35 ^{b} ± 104.77	5999.36 ^{c} ± 156.04	1499.61 ^{d} ± 36.84	1502.03 ^{d} ± 52.53	5094.84 ^{e} ± 228.14
Syringic Acid	139.15 ^{b} ± 3.89	50.12 ^{c} ± 1.79	113.97 ^{d} ± 4.2	145.44 ^{b} ± 3.28	301.96 ^{e} ± 9.55
***p*-Coumaric Acid**	298.03 ^{b} ± 6.58	82.49 ^{c} ± 4.26	68.75 ^{c} ± 3.39	387.89 ^{d} ± 15.83	168.58 ^{a} ± 10.89
Ferulic Acid	3708.83 ^{b} ± 127.71	1379.03 ^{c} ± 14.44	5002.20 ^{d} ± 212.87	2088.89 ^{e} ± 56.89	1129.85 ^{f} ± 31.1
Chlorogenic Acid	501.97 ^{b} ± 22.84	2093.81 ^{c} ± 34.93	984.09 ^{d} ± 23.28	518.25 ^{b} ± 4.90	4534.29 ^{e} ± 227.15
Sinapic Acid	43.61 ^{b} ± 1.64	214.18 ^{c} ± 3.68	6.86 ^{a} ± 0.18	54.09 ^{b} ± 2.06	1172.00 ^{d} ± 24.37
***t*-Cinnamic Acid**	819.74 ^{b} ± 29.33	127.53 ^{a} ± 1.91	55.86 ^{c} ± 2.63	111.44 ^{a} ± 3.4	781.83 ^{d} ± 40.05
Vanillic Acid	0.33 ^{b} ± 0.00	0.47 ^{c} ± 0.01	0.95 ^{d} ± 0.02	0.46 ^{c} ± 0.01	1.56 ^{e} ± 0.01
Salicylic acid	0.36 ^{b} ± 0.00	0.75 ^{c} ± 0.01	1.04 ^{d} ± 0.01	0.36 ^{b} ± 0.00	0.34 ^{a} ± 0.01
Naringenin	1.00 ^{b} ± 0.02	1.03 ^{c} ± 0.08	1.04 ^{b} ± 0.02	1.59 ^{d} ± 0.02	1.42 ^{e} ± 0.06
Vitexin	0.53 ^{b} ± 0.00	1.11 ^{c} ± 0.02	0.78 ^{d} ± 0.02	0.61 ^{e} ± 0.01	0.30 ^{f} ± 0.00
Rutin	0.52 ^{b} ± 0.01	1.14 ^{c} ± 0.02	0.73 ^{d} ± 0.03	0.63 ^{e} ± 0.02	0.31 ^{f} ± 0.01
Quercetin	0.63 ^{b} ± 0.00	0.64 ^{b} ± 0.01	1.38 ^{c} ± 0.06	0.98 ^{d} ± 0.03	1.24 ^{e} ± 0.04
Apigenin	0.62 ^{b} ± 0.02	0.30 ^{a} ± 0.01	0.30 ^{a} ± 0.00	0.30 ^{a} ± 0.01	0.31 ^{a} ± 0.01
Kaempferol	0.30 ^{a} ± 0.01	0.31 ^{a} ± 0.00	0.33 ^{b} ± 0.01	0.38 ^{c} ± 0.01	0.36 ^{d} ± 0.01
Luteolin	0.30 ^{a} ± 0.01	0.30 ^{a} ± 0.01	0.31 ^{a} ± 0.01	0.30 ^{a} ± 0.01	0.30 ^{a} ± 0.01
Total Content	9274.23	10,995.8	8951.04	6123.57	13,947.80

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Larix decidua* Mill (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC).

2.3. Antioxidant and Antiradical Extract Properties

Antioxidant and antiradical properties of extracts obtained from coniferous shoots were examined using spectrophotometric methods (Table 3). The highest values regarding the FCR and FRAP reducing ability tests, as well as the total flavonoid content assay, were obtained for the LD sample, although the LD (14.83 mg GAE/g dw) and PM (14.53 mg GAE/g dw) samples did not differ statistically in the FCR assay. With respect to free radical quenching measurements (DPPH), the PA (404.18-µM Trolox/g dw) and JC (384.30-µM Trolox/g dw) samples had the highest radical quenching ability and did not differ statistically.

Table 3. Radical scavenging and antioxidant properties of tested conifers shoots using spectrophotometric methods.

Species	DPPH (μM Trolox/g dw)	FRAP (μM $FeSO_4$/g dw)	FCR (mg GAE/g dw)	Total Flavonoid Content (mg QE/g dw)
***Picea abies* L.**	404.18 ^{a} ± 10.15	15.37 ^{a} ± 2.55	13.30 ^{a} ± 0.55	3.54 ^{a} ± 0.19
***Pinus sylvestris* L.**	200.94 ^{b} ± 23.47	42.76 ^{b} ± 5.7	0.86 ^{b} ± 0.09	8.29 ^{b} ± 0.94
Pseudotsuga menziesii	269.55 ^{c} ± 6.31	5.43 ^{a} ± 1.58	14.53 ^{c} ± 0.64	7.46 ^{c} ± 0.27
***Juniperus communis* L.**	384.30 ^{a} ± 10.88	62.88 ^{c} ± 0.36	8.25 ^{d} ± 1.01	6.34 ^{d} ± 0.09
***Larix decidua* Mill**	326.93 ^{d} ± 21.21	147.94 ^{d} ± 21.86	14.83 ^{c} ± 0.30	9.90 ^{e} ± 0.12

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($p \leq 0.05$). Abbreviations: Folin-Ciocâlteu reagent (FCR), the ferric reducing ability of plasma (FRAP), 2,2-diphenyl-1-picrylhydrazyl. (DPPH), gallic acid equivalents (GAE), quercetin equivalents (QE).

2.4. Antimicrobial Screening

The effect of water extracts from against indicator microorganisms of both Gram-positive and Gram-negative bacteria as well as mold and yeast was studied. The results obtained are summarized in Table 4. The highest antimicrobial activity was shown for PA extract against *P. aeruginosa* (32 mm), while the lowest activity was shown for PM extract against *S. aureus* (2 mm) and *L. fermentum* (3 mm). Generally, extracts obtained from PA and PS showed the highest antimicrobial activity, against Gram-negative bacteria, Gram-positive bacteria and against fungi, but the growth inhibition zone is significantly smaller in case of fungi than in tested bacteria.

Table 4. Antimicrobial properties of tested conifer shoots.

Microorganism		PM	JC	LD	PS	PA
		Growth Inhibition Zone (mm)				
Gram-Negative Bacteria						
1	*Klebsiella pneumoniae* ATCC 31,488	8 ^{a} ± 2	12 ^{b} ± 2	5 ^{c} ± 1	22 ^{d} ± 2	28 ^{e} ± 3
2	*Salmonella enteritidis* ATCC 13076	6 ^{a} ± 1	9 ^{b} ± 2	3 ^{c} ± 1	16 ^{d} ± 2	29 ^{e} ± 3
3	*Pseudomonas aeruginosa* ATCC 27853	11 ^{a} ± 2	10 ^{a} ± 2	9 ^{b} ± 2	27 ^{c} ± 3	32 ^{d} ± 2
4	*Acinetobacter baumannii* ATCC 19606	8 ^{a} ± 1	11 ^{b} ± 2	3 ^{c} ± 1	20 ^{d} ± 2	26 ^{e} ± 3
Gram-Positive Bacteria						
5	*Enterococcus faecium* ATCC 27270	4 ^{a} ± 1	12 ^{b} ± 2	9 ^{c} ± 1	18 ^{d} ± 2	19 ^{d} ± 2
6	*Staphylococcus aureus* ATCC 25923	2 ^{a} ± 0	15 ^{b} ± 3	11 ^{c} ± 2	19 ^{d} ± 2	22 ^{e} ± 2
7	*Lactobacillus fermentum* ATCC 14932	3 ^{a} ± 1	17 ^{b} ± 3	13 ^{c} ± 2	13 ^{c} ± 2	29 ^{d} ± 3
8	*Clostridium butyricum* ATCC 860	9 ^{a} ± 2	16 ^{b} ± 2	10 ^{c} ± 1	17 ^{d} ± 2	28 ^{e} ± 3
9	*Listeria monocytogenes* ATCC 19,115	8 ^{a} ± 2	15 ^{b} ± 2	7 ^{a} ± 1	19 ^{c} ± 2	25 ^{d} ± 3
10	*Bacillus coagulans* GBI-30, 6086	7 ^{a} ± 2	12 ^{b} ± 1	10 ^{c} ± 2	19 ^{d} ± 2	21 ^{e} ± 3
Fungi						
11	*Candida utilis* ATCC 9950	3 ^{a} ± 1	5 ^{b} ± 1	3 ^{a} ± 1	6 ^{c} ± 1	8 ^{d} ± 2
12	*Aspergillus* sp.	4 ^{a} ± 1	4 ^{a} ± 1	6 ^{b} ± 1	5 ^{c} ± 1	5 ^{c} ± 1
13	*Fusarium* sp.	2 ^{a} ± 0	5 ^{b} ± 1	2 ^{a} ± 0	5 ^{a} ± 1	9 ^{b} ± 2

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a line are not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Larix decidua* Mill (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC).

Conducted correlation analysis of antioxidant assays, antimicrobial parameters and phenolic compound showed a small number of statistically significant correlations between different assays and compounds (Figure 2). The strongest statistically significant negative correlations are observed between FCR assay and inhibition of *Aspergillus* sp., between total flavonoid content assay and chlorogenic and sinapic acid content. Strongest statistically significant positive correlations are observed between DPPH assay and caffeic acid content.

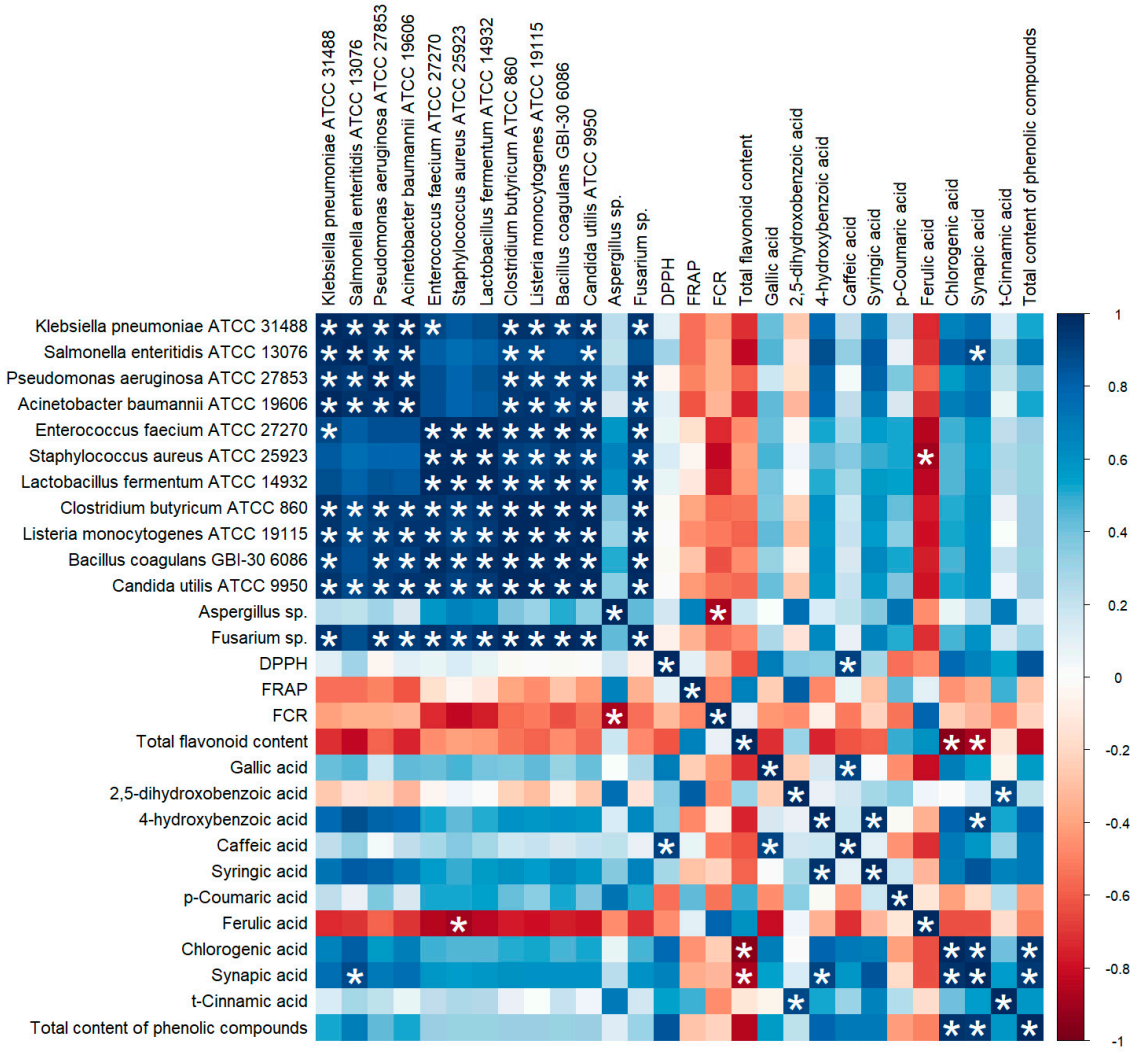

Figure 2. Correlation matrix. The intensity of the color is proportional to the correlation coefficient; white asterisk indicates a significantly statistical correlation ($p < 0.05$).

3. Discussion

The use of raw materials such as coniferous shoots can contribute to the development of a functional food sector. Food enriched with ingredients containing active phytochemicals allows reducing the incidence of diseases, which is desirable [18,19]. Thus, the production of inexpensive, natural health-promoting products is of great importance in the current socio-economic situation of many countries in the world, especially in the context of the incidence of civilization diseases [20]. Many products perceived as health-promoting are known in natural medicine, including coniferous shoots, which are not currently widely used in this field. In this publication, individual samples of shoots of selected popular conifers, i.e., *Picea abies* L.; *Larix decidua* Mill; *Pinus sylvestris* L.; *Pseudotsuga menziesii*; *Juniperus communis* L. were characterized. It was shown that the content of polyphenolic compounds depended on the species, which significantly contributed to the antioxidant capacity.

Visual perception is extremely important for consumers when selecting food products in terms of attractiveness and perception of health-promoting properties The use of plant raw materials as ingredients in food products can affect not only the taste and aroma but also their color, thus is important to assess raw materials in this respect [21]. In the present study, it was noticed that the PA sample, which was the darkest, i.e., with the lowest parameter L*, was characterized by, among others, the highest total content of phenolic compounds measured by HPLC and the strongest ability to

quench free DPPH radicals. A relationship between phenolic compounds and color hue was observed, i.e., in studies on honey, where darker samples were richer in these compounds [22]. However, it should be noted that some compounds, including anthocyanins and lycopene, can give similar color to raw materials, but they differ significantly in antioxidant capacity. The relationship between color and antioxidant content should not be generalized to all raw materials, but it can be stated that differences in color usually mean different content of bioactive compounds [23].

The tested shoots, in addition to the color, differed noticeably in length, shape and dry matter content, which had an impact on the content of bioactive compounds in the extracts. In the current study, all raw materials were dried under the same conditions, although earlier studies indicated that the drying process significantly affected the content of bioactive compounds as well as antioxidant and antimicrobial properties of coniferous shoots [8]. In the present study, distilled water was used for extraction, which allowed to obtain a high content of phenolic compounds in extracts that had high antioxidant potential, although water is not the most effective solvent. As many authors have shown earlier for vegetable raw materials, such as *Pistacia terebinthus* L. or *Limnophila aromatica*, methanol and acetone are much more effective solvents, allowing obtaining extracts with higher antioxidant potential and a higher concentration of bioactive compounds [24]. However, evaluation of the efficiency of water extraction allows us to determine, from the point of view of practical application, whether raw materials could be components of functional products in the future, i.e., infusions, beverages, smoothies or yogurts [25].

The extracts obtained differed significantly in the content of phenolic compounds, however, 4-hydroxybenzoic acid, caffeic acid, ferulic acid and chlorogenic acid were dominant among most the trees studied. Conducted statistical analysis showed strong positive correlation between total content of phenolic compounds and sinapic and chlorogenic acid, but strong negative correlations between these compounds and total flavonoid content assay. This trend may be caused because the amounts of phenolic acids are higher than flavonoids and used methods in present study may not have covered the measurement of all individual phenolic compounds [26] In study of cones and berries from 16 different Turkish coniferous species authors used 95% acetone as an extractant and also identified many phenolic compounds, such as 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, catechin, the total amount of phenolics was determined in range of 60–6390 μg/g dw depending on the species, which is lower than in present study [27]. On the other hand, in study conducted on needles from Norway spruce, authors used 95% ethanol for extraction and detected the same compounds as in present study, i.e., chlorogenic acid, gallic acid, kaempferol, quercetin but in concentrations below the quantification limit of about 0.02 μmol/g dw. The dominant compound, similarly to the previously mentioned study was catechin, which was not tested for in this study [28], Phenolic compounds play protective functions in plants, but they can have health-promoting effects in the human body as well as positively affect the quality and safety of food products. The study of Raitanen et al. demonstrated that tannins from pine and spruce bark protected meat snacks against fat oxidation without affecting smell and taste [29]. Many studies have demonstrated the antioxidant activity of phenolic compounds, recent findings also point to possible genoprotective and neuroprotective effects, as well as those reducing the risk of metabolic diseases, cardiovascular diseases and cancers [30]. All of the tested extracts also showed antioxidant and reducing properties demonstrated by DPPH, FRAP and FCR methods. The highest DPPH inhibition was demonstrated for the PA and JC samples, they also had the highest total phenolic content. Studies by other authors have confirmed the strong antioxidant activity of extracts obtained from juniper and pine, although there is no such data on other conifers [31]. Differences between the samples were significant in the FRAP and FCR tests. It is concluded that differences between individual methods may be due to different reactivity of individual compounds in the extracts, including compounds that have not been tested in this publication. FCR and FRAP are non-specific tests for phenolic compounds and other compounds, e.g., ascorbic acid, may also influence the result [32]. Conducted correlation analysis also shows small amount of statistically significant correlations between assays and identified compounds, which may indicate that obtained extracts

are complex mixture of compounds and should be evaluated further. Strong positive correlations were observed between DPPH and total phenol content, as well as total flavonoid content and FRAP assay, but they were not statistically significant. In the study of different types of honey derived from Mount Olympus in Greece, authors observed similar effect, which can be explained by fact, that the antioxidant properties depend not on the quantity, but mainly on the chemical composition of polyphenols and other factors may affect results of conducted assays, such as the concentration of mineral contents, organic acids, amino acids [33]. Lack of correlation between conducted antiradical ant antioxidant assays can be explained by various complex mechanisms of neutralizing free radicals, e.g., Brand-Williams et al. and Tagashira et al. found no correlation between the content of phenolic acids and their antioxidant activity [34,35].

All extracts were characterized by antimicrobial properties in the screening test, where the strongest were pine and spruce extracts. In study on antimicrobial activity of aqueous extracts of *Juniperus phoenicea*, *Pistacia atlantica* and *Oudneya africana* tested extracts of *Juniperus* exhibited similar bacterial inhibition against *Listeria ivanovii* RBL30, *Listeria innocua* RBL29 and *Listeria monocytogenes* LSD530 [36]. In study of essential oils from *Pinus halepensis* Mill., essential oils showed strong inhibiting activity against *L. monocytogenes* and *Klebsiella pneumoniae,* There is much evidence in the literature for the antimicrobial effect of pine and juniper, however, tested properties are usually found in concentrated essential oils or ethanol extracts [37]. Pearson's correlation test revealed strong positive correlation between sinapic acid content and inhibition of *Salmonella enteritidis* ATCC 13,076. In the literature there are mentions about antimicrobial effects of phenolic compounds on *Salmonella* bacteria, notwithstanding sinapic acid is not widely recognized as antimicrobial compound, but many phenolic compounds may exhibit significant antibacterial activity, however mechanisms of antibacterial action of phenolic compounds are not yet fully deciphered [38,39]. Correlation analysis showed also statistically significant negative correlations between inhibition of *Staphylococcus aureus* ATCC 25,923 and ferulic acid content, *Aspergillus* spp. Inhibition and FCR assay. Different authors also observed negative correlations or lack of thereof between FCR assay and antimicrobial properties, therefore synergistic or antagonistic interactions between compounds cannot be fully explained by a simple linear relation [33,40].

4. Materials and Methods

4.1. Materials

The study material consisted of shoots samples from six different coniferous trees: *Picea abies* L. (PA), *Larix decidua* Mill; (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC) collected in 2019 from the arboretum in Zielonka (Poland, 17°06'33" E, 52°06'33" N), a part of the Forest Experimental Department of Poznan University of Life Sciences. The shoots were collected in August and subjected to natural air-drying at 21 °C for 72 h. The dried needles were sampled from three different shoots, crushed in a Grindomix GM 200 (Retsch GmbH, Haan, Germany) for 15 s at a rate of 500 rpm at 21 °C to a particle size of 0.5–0.9 mm.

4.2. Methods

4.2.1. Extraction

Water extracts were obtained by mixing 5 g of raw material with 150 mL of distilled water. The samples were shaken in a water bath for 30 min at 80 °C at constant amplitude. Extracts were decanted and filtered using Whatman No. 4 paper three times. The obtained extracts were stored at −21 °C for no more than two weeks before further analyses. Each measurement and analysis for each extract were conducted in triplicate.

4.2.2. Commodity Assessment, Color and Osmolality of Extracts

Shoots were subjected to commodity assessment in terms of characteristics and shape in the laboratory in daylight. Needle dimensions were determined by measuring the length of 100 needles of each species using a caliper and calculating the average. Dry weight of the shoots was determined using Sartorius MA 30 (Sartorius AG, Goettingen, Germany), where 1 g of the sample was incinerated at 130 °C for 30 min.

Colors of leaf extracts were measured. Color measurement was run in the L* a* b* CEN unit system using a CM-5 spectrometer (Konica Minolta, Tokyo, Japan) according to the methodology described by the device producer. D 65 was applied as a source of light and color temperature was 6504 K. The observation angle of the standard colorimetric observer was 10°. Measurements for each sample was repeated five times. Instrument calibration was performed using the black pattern.

4.2.3. HPLC Determination of Phenolic Acids and Flavonols

Extracts were evaporated to dryness in a stream of nitrogen. Next they 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 was heated in a water bath at 95 °C for 30 min. After cooling (approx. 20 min) test tubes was 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 (2 × 2 mL). 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 × 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 analyses samples were dissolved in 1 mL methanol. Phenolic compounds analysis was performed using an Acquity H class UPLC system equipped with a Acquity PDA detector (Waters Corp, Milford, MA, USA). Chromatographic separation was performed on an Acquity UPLC® BEH C_{18} column (100 mm × 2.1 mm, particle size—1.7 µm) (Watersy, Dublin, 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 λ = 320 nm and 280 nm and finally expressed as mg/100 g dw of sample. Compounds were identified by comparing the retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of the standard to the analyzed samples and repeated analysis. Detection level was 1 µg/g. Retention times for phenolic acids were as follows: 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, vanillic acid—16.80 min, sinapic acid—17.10 min, ferulic acid—17.50 min, salicylic acid—17.85 min., *t*- cinnamic acid—19.50 min Retention times for flavonoids were as follows: vitexin—1.10 min, apigenin—8.00 min, kaempferol—11.00 min., luteolin—16.90 min., quercetin—17.00 min, naringenin—17.50 min, rutin—17.90 min [30].

4.2.4. Folin-Ciocâlteu Reagent Assay

Reducing capacity of the obtained extracts was determined using the Folin-Ciocâlteu reagent (FCR) and the method of Kobus-Cisowska et al. with minor modifications [41]. Reducing capacity was expressed as mg of gallic acid (Sigma-Aldrich, Steinheim, Germany) equivalents (GAE) per 1 g (mg/1 g) of dry mass. The standard curve in the range of 0–500 mg/mL of gallic acid was used.

4.2.5. Total Flavonoid Content

Total flavonoid content was determined using a procedure described by Meda et al. [42]. Total flavonoid content was determined using a standard curve with quercetin (Sigma-Aldrich, Germany) concentration in range of 1–100 μg/mL. The mean of three readings was used and expressed as mg of quercetin equivalents QE/1 g raw material.

4.2.6. Ferric Reducing Antioxidant Power Assay

The antioxidant properties of water extracts were determined using the ferric reducing/antioxidant power assay (FRAP method) according to the procedure described by O'Sullivan et al. [43]. The calibration curve was constructed using $FeSO_4 \cdot 7H_2O$ in concentrations of 100–1000 μM. Samples were incubated for 30 min and the absorbance was measured at 593 nm (Metertech SP880, Metertech Inc., Taipei Taiwan). Data were expressed as μM $FeSO_4$/g dry mass.

4.2.7. DPPH Radical Scavenging Activity

DPPH inhibition capacity was investigated according to the procedure described by Szczepaniak et al. [44]. The calibration curve was prepared using Trolox standard solution in concentrations of 100–1000 μM. The decrease in DPPH absorbance was measured at 517 nm according to the blank. Inhibition capacity of DPPH was expressed as μM Trolox/g dw.

4.2.8. Antimicrobial Screening

Indicator microorganisms *Klebsiella pneumoniae* ATCC 31,488, *Salmonella enteritidis* ATCC 13,076, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Enterococcus* faecium ATCC 27,270, *Staphylococcus aureus* ATCC 25,923, *Lactobacillus fermentum* ATCC 14,932, *Clostridium butyricum* ATCC 860, *Listeria monocytogenes* ATCC 19,115, *Bacillus coagulans*, GBI-30, 6086, *Candida utilis* ATCC 9950, *Aspergillus* spp. and *Fusarium* spp. were transferred to test tubes containing Mueller-Hinton (for bacteria), yeast extract sucrose (for yeast), potato dextrose (for mold) medium. They were cultured at 37 °C for 24 h. Next, 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 150 μL of aqueous extracts of coniferous shoots. 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).

4.2.9. Statistical Analysis

All assays were conducted in triplicate and the results were expressed as mean ± SD. One-way ANOVA was used to analyze statistical differences between different extracts in terms of phenolic compound contents and different antioxidant assays with the least significant difference (LSD). A *p*-value less than 0.05 was considered to be statistically significant. Correlation analysis was conducted using Pearson parametric correlation test. Statistical analyses were calculated using Statistica 13.3 software (TIBCO, Palo Alto, CA, USA) and RStudio (RStudio PBC, 1.3.1056, Boston, MA, USA).

5. Conclusions

The present work has shown that aqueous extracts obtained from sample shoots of *Picea abies* L., *Larix decidua* Mill, *Pinus sylvestris* L., *Pseudotsuga menziesii* and *Juniperus communis* L. exhibit antioxidant and antimicrobial properties in vitro. They are characterized by a high content of

phenolic compounds—among others—4-hydroxybenzoic acid, caffeic acid, ferulic acid and chlorogenic acid, i.e., compounds considered to have a wide spectrum of pro-health effects. Shoots of the studied conifers differ from each other in terms of physical parameters as well as antioxidant and antimicrobial properties; however, the results of the research allow to conclude that after further necessary analyses, i.e., cytotoxicity and sensory tests, these raw materials could potentially be used as components of functional food with programmed health-promoting properties, as antioxidant ingredients and those extending the shelf life of the products.

Author Contributions: Conceptualization and methodology: J.K.-C., M.D.; formal analysis: M.D., D.S., K.S.-S.; resources: M.B.; writing—original draft preparation: M.D.; supervision: J.K.-C. All authors have read and agreed to the published version of the manuscript.

Funding: The publication was co-financed within the framework of the Ministry of Science and Higher Education program as "Regional Initiative Excellence" in years 2019–2022, project number 005/RID/2018/19 and by statutory funds of the Department of Gastronomy Sciences and Functional Foods of Poznan University of Life Sciences, Grant Number 506.751.03.00.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Varelas, V.; Langton, M. Forest biomass waste as a potential innovative source for rearing edible insects for food and feed—A review. *Innov. Food Sci. Emerg. Technol.* **2017**, *41*, 193–205. [CrossRef]
2. Šojić, B.; Tomović, V.; Jokanović, M.; Ikonić, P.; Džinić, N.; Kocić-Tanackov, S.; Popović, L.; Tasić, T.; Savanović, J.; Šojić, N.Ž. Antioxidant activity of Juniperus communis L. essential oil in cooked pork sausages. *Czech. J. Food Sci.* **2017**, *35*, 189–193. [CrossRef]
3. Penkina, N.; Tatar, L.; Kolesnyk, V.; Karbivnycha, T.; Letuta, T. The study of beer quality with the reduced toxic effect. *Eureka Life Sci.* **2017**, 35–43. [CrossRef]
4. Semeniuc, C.A.; Rotar, A.; Stan, L.; Pop, C.R.; Socaci, S.; Mireşan, V.; Muste, S. Characterization of pine bud syrup and its effect on physicochemical and sensory properties of kefir. *CyTa-J. Food* **2016**, *14*, 213–218. [CrossRef]
5. Akaberi, M.; Boghrati, Z.; Amiri, M.S.; Khayyat, M.H.; Emami, S.A. A review of conifers in Iran: Chemistry, biology and their importance in traditional and modern medicine. *Curr. Pharm. Des.* **2020**. [CrossRef]
6. Bradley, W.G.; Holm, K.N.; Tanaka, A. An orally active immune adjuvant prepared from cones of Pinus sylvestris, enhances the proliferative phase of a primary T cell response. *BMC Complementary Altern. Med.* **2014**, *14*, 163. [CrossRef]
7. Glaser, A.; Zhao, G. Utilization of Scots Pine oil in chinese medicine. *Int. J. Tradit. Chin. Med.* **2012**, *34*, 229–231.
8. Dziedziński, M.; Kobus-Cisowska, J.; Powałowska, D.S.-; Szablewska, K.S.-; Baranowska, M. Polyphenols composition, antioxidant and antimicrobial properties of Pinus sylvestris L. shoots extracts depending on different drying methods. *Emir. J. Food Agric.* **2020**, *32*, 229–237. [CrossRef]
9. Salehi, B.; Upadhyay, S.; Erdogan Orhan, I.; Kumar Jugran, A.; LD Jayaweera, S.; A Dias, D.; Sharopov, F.; Taheri, Y.; Martins, N.; Baghalpour, N.; et al. Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature. *Biomolecules* **2019**, *9*, 738. [CrossRef]
10. Matsuo, A.L.; Figueiredo, C.R.; Arruda, D.C.; Pereira, F.V.; Scutti, J.A.B.; Massaoka, M.H.; Travassos, L.R.; Sartorelli, P.; Lago, J.H.G. α-Pinene isolated from Schinus terebinthifolius Raddi (Anacardiaceae) induces apoptosis and confers antimetastatic protection in a melanoma model. *Biochem. Biophys. Res. Commun.* **2011**, *411*, 449–454. [CrossRef] [PubMed]
11. Nam, S.-Y.; Chung, C.; Seo, J.-H.; Rah, S.-Y.; Kim, H.-M.; Jeong, H.-J. The therapeutic efficacy of α-pinene in an experimental mouse model of allergic rhinitis. *Int. Immunopharmacol.* **2014**, *23*, 273–282. [CrossRef] [PubMed]
12. Pinheiro, M.D.A.; Magalhães, R.M.; Torres, D.M.; Cavalcante, R.C.; Mota, F.S.X.; Oliveira Coelho, E.M.A.; Moreira, H.P.; Lima, G.C.; Araújo, P.C.D.C.; Cardoso, J.H.L.; et al. Gastroprotective effect of alpha-pinene and its correlation with antiulcerogenic activity of essential oils obtained from Hyptis species. *Pharm. Mag.* **2015**, *11*, 123–130. [CrossRef]

13. Zamyad, M.; Abbasnejad, M.; Esmaeili-Mahani, S.; Mostafavi, A.; Sheibani, V. The anticonvulsant effects of Ducrosia anethifolia (Boiss) essential oil are produced by its main component alpha-pinene in rats. *Arq. Neuropsiquiatr.* **2019**, *77*, 106–114. [CrossRef] [PubMed]
14. Emami, S.A.; Shahani, A.; Hassanzadeh Khayyat, M. Antioxidant Activity of Leaves and Fruits of Cultivated Conifers in Iran. *Jundishapur J. Nat. Pharm. Prod.* **2013**, *8*, 113–117. [CrossRef] [PubMed]
15. El-Demerdash, F.M.; Tousson, E.M.; Kurzepa, J.; Habib, S.L. Xenobiotics, Oxidative Stress, and Antioxidants. *Oxid. Med. Cell Longev.* **2018**, *2018*, 9758951. [CrossRef] [PubMed]
16. Sies, H.; Berndt, C.; Jones, D.P. Oxidative Stress. *Annu. Rev. Biochem.* **2017**, *86*, 715–748. [CrossRef]
17. Burdock, G.A.; Carabin, I.G. Chapter 10—Breaking down the barriers to functional foods, supplements, and claims1. In *Nutraceutical and Functional Food Regulations in the United States and Around the World (Third Edition)*; Bagchi, D., Ed.; Academic Press: Cambridge, MA, USA, 2019; pp. 121–146. ISBN 978-0-12-816467-9.
18. Asgary, S.; Rastqar, A.; Keshvari, M. Functional Food and Cardiovascular Disease Prevention and Treatment: A Review. *J. Am. Coll. Nutr.* **2018**, *37*, 429–455. [CrossRef]
19. Górecka, D.; Wawrzyniak, A.; Jędrusek-Golińska, A.; Dziedzic, K.; Hamułka, J.; Kowalczewski, P.Ł.; Walkowiak, J. Lycopene in tomatoes and tomato products. *Open Chem.* **2020**, *18*, 752–756. [CrossRef]
20. Saklayen, M.G. The Global Epidemic of the Metabolic Syndrome. *Curr. Hypertens Rep.* **2018**, *20*, 12. [CrossRef]
21. Zhan, L.; Ma, Y.; Zhang, C.; Li, L.; Pang, L.; Huang, X. Antioxidants and Antioxidant Capacity in Leafy, Stem, and Fruit Vegetables Including 50 Species. *J. Food Eng. Technol.* **2018**, *7*, 8.
22. Al-Farsi, M.; Al-Amri, A.; Al-Hadhrami, A.; Al-Belushi, S. Color, flavonoids, phenolics and antioxidants of Omani honey. *Heliyon* **2018**, *4*, e00874. [CrossRef] [PubMed]
23. Cömert, E.D.; Mogol, B.A.; Gökmen, V. Relationship between color and antioxidant capacity of fruits and vegetables. *Curr. Res. Food Sci.* **2020**, *2*, 1–10. [CrossRef]
24. Durak, M.Z.; Uçak, G. Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish Pistacia terebinthus L. extracts. *Turk. J. Agric* **2015**, *39*, 10–19. [CrossRef]
25. Dziedziński, M.; Szczepaniak, O.; Telichowska, A.; Kobus-Cisowska, J. Antioxidant capacity and cholinesterase inhibiting properties of dietary infusions with Humulus lupulus. *J. Elem.* **2020**, *25*, 657–673. [CrossRef]
26. Sant'ana, L.D.; Ferreira, A.B.B.; Lorenzon, M.C.A.; Berbara, R.L.L.; Castro, R.N. Correlation of Total Phenolic and Flavonoid Contents of Brazilian Honeys with Colour and Antioxidant Capacity. *Int. J. Food Prop.* **2014**, *17*, 65–76. [CrossRef]
27. Kilic, A.; Hafizoglu, H.; Tümen, I.; Dönmez, I.E.; Sivrikaya, H.; Hemming, J. Phenolic extractives of cones and berries from Turkish coniferous species. *Eur. J. Wood Prod.* **2011**, *69*, 63–66. [CrossRef]
28. Ganthaler, A.; Stöggl, W.; Mayr, S.; Kranner, I.; Schüler, S.; Wischnitzki, E.; Sehr, E.M.; Fluch, S.; Trujillo-Moya, C. Association genetics of phenolic needle compounds in Norway spruce with variable susceptibility to needle bladder rust. *Plant. Mol. Biol.* **2017**, *94*, 229–251. [CrossRef]
29. Raitanen, J.-E.; Järvenpää, E.; Korpinen, R.; Mäkinen, S.; Hellström, J.; Kilpeläinen, P.; Liimatainen, J.; Ora, A.; Tupasela, T.; Jyske, T. Tannins of Conifer Bark as Nordic Piquancy—Sustainable Preservative and Aroma? *Molecules* **2020**, *25*, 567. [CrossRef]
30. Szczepaniak, O.; Ligaj, M.; Kobus-Cisowska, J.; Tichoniuk, M.; Dziedziński, M.; Przeor, M.; Szulc, P. The Genoprotective Role of Naringin. *Biomolecules* **2020**, *10*, 700. [CrossRef]
31. El Jemli, M.; Kamal, R.; Marmouzi, I.; Zerrouki, A.; Cherrah, Y.; Alaoui, K. Radical-Scavenging Activity and Ferric Reducing Ability of Juniperus thurifera (L.), J. oxycedrus (L.), J. phoenicea (L.) and Tetraclinis articulata (L.). *Adv. Pharm. Sci.* **2016**, *2016*, 6392656. [CrossRef]
32. Górnaś, P.; Dwiecki, K.; Siger, A.; Tomaszewska-Gras, J.; Michalak, M.; Polewski, K. Contribution of phenolic acids isolated from green and roasted boiled-type coffee brews to total coffee antioxidant capacity. *Eur. Food Res. Technol.* **2016**, *242*, 641–653. [CrossRef]
33. Stagos, D.; Soulitsiotis, N.; Tsadila, C.; Papaeconomou, S.; Arvanitis, C.; Ntontos, A.; Karkanta, F.; Adamou-Androulaki, S.; Petrotos, K.; Spandidos, D.A.; et al. Antibacterial and antioxidant activity of different types of honey derived from Mount Olympus in Greece. *Int. J. Mol. Med.* **2018**, *42*, 726–734. [CrossRef] [PubMed]
34. Brand-Williams, W.; Cuvelier, M.E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lwt -Food Sci. Technol.* **1995**, *28*, 25–30. [CrossRef]

35. Tagasgira, M.; Watanabe, M.; Uemitsu, N. Antioxidative Activity of Hop Bitter Acids and Their Analogues. *Biosci. Biotechnol. Biochem.* **1995**, *59*, 740–742. [CrossRef] [PubMed]
36. Hammami, R.; Zouhir, A.; Hamida, J.B.; Neffati, M.; Vergoten, G.; Naghmouchi, K.; Fliss, I. Antimicrobial properties of aqueous extracts from three medicinal plants growing wild in arid regions of Tunisia. *Pharm. Biol.* **2009**, *47*, 452–457. [CrossRef]
37. Ait-Ouazzou, A.; Lorán, S.; Arakrak, A.; Laglaoui, A.; Rota, C.; Herrera, A.; Pagán, R.; Conchello, P. Evaluation of the chemical composition and antimicrobial activity of Mentha pulegium, Juniperus phoenicea, and Cyperus longus essential oils from Morocco. *Food Res. Int.* **2012**, *45*, 313–319. [CrossRef]
38. Puupponen-Pimiä, R.; Nohynek, L.; Hartmann-Schmidlin, S.; Kähkönen, M.; Heinonen, M.; Määttä-Riihinen, K.; Oksman-Caldentey, K.-M. Berry phenolics selectively inhibit the growth of intestinal pathogens. *J. Appl. Microbiol.* **2005**, *98*, 991–1000. [CrossRef]
39. Bouarab-Chibane, L.; Forquet, V.; Lantéri, P.; Clément, Y.; Léonard-Akkari, L.; Oulahal, N.; Degraeve, P.; Bordes, C. ntibacterial Properties of Polyphenols: Characterization and QSAR (Quantitative Structure–Activity Relationship) Models. *Front. Microbiol.* **2019**, *10*. [CrossRef]
40. Combarros-Fuertes, P.; Estevinho, L.M.; Dias, L.G.; Castro, J.M.; Tomás-Barberán, F.A.; Tornadijo, M.E.; Fresno-Baro, J.M. Bioactive Components and Antioxidant and Antibacterial Activities of Different Varieties of Honey: A Screening Prior to Clinical Application. *J. Agric. Food Chem.* **2019**, *67*, 688–698. [CrossRef]
41. Kobus-Cisowska, J.; Szulc, P.; Szczepaniak, O.; Dziedziński, M.; Szymanowska, D.; Szymandera-Buszka, K.; Goryńska-Goldmann, E.; Gazdecki, M.; Telichowska, A.; Ligaj, M. Variability of Hordeum vulgare L. Cultivars in Yield, Antioxidant Potential, and Cholinesterase Inhibitory Activity. *Sustainability* **2020**, *12*, 1938. [CrossRef]
42. Meda, A.; Lamien, C.E.; Romito, M.; Millogo, J.; Nacoulma, O.G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem.* **2005**, *91*, 571–577. [CrossRef]
43. O'Sullivan, A.M.; O'Callaghan, Y.C.; O'Connor, T.P.; O'Brien, N.M. Comparison of the Antioxidant Activity of Commercial Honeys, Before and After In-Vitro Digestion. *Pol. J. Food Nutr. Sci.* **2013**, *63*, 167–171. [CrossRef]
44. Szczepaniak, O.M.; Ligaj, M.; Kobus-Cisowska, J.; Maciejewska, P.; Tichoniuk, M.; Szulc, P. Application for novel electrochemical screening of antioxidant potential and phytochemicals in Cornus mas extracts. *CyTa-J. Food* **2019**, *17*, 781–789. [CrossRef]

Sample Availability: Samples of the *Picea abies* L., *Larix decidua* Mill, *Pinus sylvestris* L., *Pseudotsuga menziesii* and *Juniperus communis* L. shoots are available from the authors.

Emirates Journal of Food and Agriculture. 2020. 32(3): 229-237
doi: 10.9755/ejfa.2020.v32.i3.2080
http://www.ejfa.me/

RESEARCH ARTICLE

Polyphenols composition, antioxidant and antimicrobial properties of *Pinus sylvestris* L. shoots extracts depending on different drying methods

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ABSTRACT

Scots pine (*Pinus sylvestris L.*) shoots have been increasingly commonly used as functional food or its component, the shoots contain various active components, unknown from other raw materials. The objective of the study was to assess the influence of the drying method on the content of bioactive compounds and antioxidative and antimicrobial activity of pine shoots. It was demonstrated that freeze drying (PSL), vacuum drying (PSP) and natural drying (PSN) have significant impact on the physicochemical properties, content of bioactive compounds and antioxidative activity of the prepared ethanol-water extracts. The content of the studied compounds varied significantly in the tested shoots. In spectrophotometric testing the highest total flavonoid content was demonstrated in the PSP sample, at 5.51 mg quercetin/g dw. On the other hand, the reducing capacity was as follows: PSN > PSP > PSL in the range from 13.4 to 5.73 mg gallic acid/g dw. However in assay conducted using HPLC methods the highest content of polyphenols characterized extract from freeze-dried raw material (9151.15 μg/g), followed by vacuum-dried (8264.57 μg/g), and the lowest content of phenolic compounds was found in convection-dried shoots (7621.76 μg/g). The studied extracts demonstrated antioxidative properties, both in ferric reducing antioxidant power assay (FRAP) as well as in free radical quenching measurement (DPPH). All of the studied extracts demonstrated antimicrobial and fungicidal properties, and they were particularly efficient in the case of gram-negative bacteria.

Keywords: Antioxidant properties; Drying conditions; Microbial growth; *Pinus sylvestris* L.; Polyphenols

INTRODUCTION

Researchers are currently looking natural sources of health-promoting compounds that could be used in food. The growing interest in food products characterized by high antioxidative potential results from the efficiency of these compounds in prevention of diseases associated with systemic oxidative stress, i.e. cardiovascular diseases, tumors, neurodegenerative disorders and metabolic disturbances (Roleira et al., 2015). As a result of new reports on the role of antioxidants, products rich in these compounds (especially of vegetable origin) have become increasingly desirable on the market, although numerous cultures have traditionally used vegetable products for hundreds and even thousands of years to treat various diseases, as well as to support health, stemming from the traditional use, abundant availability and low price, as well as the belief in the efficiency of these preparations (Ramana et al., 2018). Obtaining extracts at a large scale requires use of various drying methods in order to fix raw materials and retain their bioactive properties. Traditional and thus natural and the least expensive raw material drying methods include sun drying and air drying. Dehydration under the impact of these factors, due to the prolonged duration, possible contaminants and limited efficiency may not be used for numerous raw materials (Villalobos et al., 2016). Modern drying methods that are gaining popularity include, among others, microwave drying, vacuum drying, freeze drying, which may help enable retaining high quality of considerable amount of bioactive compounds, while optimizing parameters significant in economic terms. These methods largely enable minimizing surface overheating, and reduce the drying time.

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Received: 22 December 2019; **Accepted:** 11 March 2020

Scots pine is a long-lived coniferous tree of high economic significance, whose shoots and leaves are characterized by high content of bioactive compounds. These trees comprise 68% of Polish forests and have been traditionally used to produce liqueurs, ointments and syrups considered to have therapeutic properties (Bączek et al, 2017). Extracts from bark of *Pinus sylvestris* showed antioxidant activity in AAPH and DPPH assays as well as reducing and metal chelating properties (Nazari et al., 2013; Sokół-Łętowska et al., 2007). It has been demonstrated that the shoots and leaves of these trees contain, among others, essential oils rich in monoterpenes, i.e. alpha-pinene, beta-pinene, delta-3-carene, beta-myrcene, limonene, p-cymene, exhibiting strong antibacterial, antifungal, antiviral and antioxidative properties (Ács et al., 2018). Currently, the majority of researchers focus on the use of isolated essential oils, although the extracts alsho exhibit therapeutic potential, for example as chemopreventive or chemotherapeutic agent (Hoai et al., 2015; Nicolato et al., 2009). Recently there were attempts to use pine's components in food fortification, fore example in kefir and beer. Such products show higher antioxidant capacity, better storage stability and can positively influence quality of product (Penkina et al., 2017; Semeniuc et al., 2016). Currentliterature contains data on the use of shoots in food technology, as well as in pharmacy, yet the mode in which drying would influence the products' activity has not yet been studied. The objective of the study was to determine antioxidative and antimicrobial properties of *Pinus Sylvestris* L. shoots and impact of different drying methods on physicochemical, antimicrobial and antioxidative properties of Scots pine shoot extracts. Therefore, in the present study the shoots were dried via freeze drying, natural-air drying and vacuum drying and evaluated by physical, chemical and microbiological assays.

MATERIALS AND METHODS

Material

The study material consisted of *Pinus sylvestris* L. shoots collected in 2019 from the arboretum in Zielonka (Poland, 17°06'33"E, 52°06'33"N), a part of the Forest Experimental Department of Poznan University of Life Sciences. The shoots were collected in August and fixed using three different drying methods: (1) freeze drying (PSL) for 48h in an Alpha 1-2 LSC freeze drier (Christ, Germany); (2) vacuum drying (PSP) at 60 °C under the pressure of 470 mbar for 48h in a VO29 drier (Memmert, Germany); (3) natural-air drying (PSN) at 21 °C for 72h. The dried needles were crushed in a Grindomix GM 200 by Retsch (Haan, Germany) for 15 seconds at a rate of 500 rpm at 21 °C to a particle size of 0.5 - 0.9 mm.

Extraction

Ethanol-water extract (40%) was obtained by mixing 5 g of raw material with 150 mL of solvent (Sigma-Aldrich, Germany). The samples were shaken in a water bath for 15 min at 21 ◦C at constant amplitude. The extract was decanted and filtered using Whatman No. 4 paper three times. Obtained supernatants were stored at -21 °C for no more than two weeks before further analyses. Each measurements and analysis for each extract were conducted in triplicate.

Density and extraction yield

Extractable yield was measured and calculated according to methodology by Pham et al (Pham et al., 2015) and expressed as % dried extract. Density was measured by pipetting 1 ml of extract with automatic single channel pipette (Thermo Fisher Scientific, USA) on weighing vessel and weighed using laboratory scale, expressed in g/ml.

Instrumental analysis of color

The color of the extracts (reflectance values: L*, a* and b*) was measured using CM-5 spectrometer (Konica Minolta, Japan) according to methodology described by the device producer. L*, a*, b* values were determined using Illuminant D65 and an observer angle of 10°, color temperature equaled 6504 K.

Ascorbic acid content

Vitamin C (ascorbic acid) content was determined according to the modified method described by Ajila et al. (Ajila et al., 2007). The absorbance drop was calculated. Ascorbic acid was used as a standard. The calibration curve was performed using AA standard solutions. The linearity of the curve coefficient – r^2 equaled 0.98. The final results are given in mg AA/g dw.

Folin–Ciocalteu reagent assay

Reducing capacity was determined using Folin-Ciocalteu reagent (FCR) of the obtained extracts was determined using the method of Kobus-Cisowska et al. with minor modifications (Kobus-Cisowska et al., 2020). The reducing capacity was 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.

Total flavonoid content

The total flavonoid content was determined using the procedure described by Meda et al. (Meda et al., 2005). The total flavonoid content was determined using a standard curve with quercetin (Sigma–Aldrich, Germany) as the standard. The mean of three readings was used and expressed as mg of quercetin equivalents QE/1 g of raw material.

Content of flavonols and phenolic acids

The procedure was based on the method published by Kobus et al. (Kobus-Cisowska et al., 2019b).

Content of chlorophylls and carotenoids

The carotenoids and chlorophyll were determined using the standard method (Kobus-Cisowska et al., 2019b) and expressed in mg/g of dry product.

Ferric reducing/antioxidant power assay

The antioxidant properties of the water-ethanol extracts were determined using a ferric reducing/antioxidant power assay (FRAP method) according to procedure described by O'Sullivan et al. (O'Sullivan et al., 2013). A calibration curve was constructed using $FeSO_4$ $7H_2O$. Samples were incubated for 30 min and the absorbance was measured at 593 nm (Metertech SP880, Taiwan). Data were expressed as μM $FeSO_4$/g dry mass.

DPPH radical scavenging activity

The inhibition capacity of DPPH was investigated according to the procedure performed by Szczepaniak et al. (Szczepaniak et al., 2019). A calibration curve was prepared using a Trolox standard solution. The decrease in DPPH absorbance (A) was measured at 517 nm according to the blank (A'). The inhibition capacity of the DPPH radical was calculated and expressed as a percentage.

Antimicrobial activity

Antimicrobial activity of extracts was measured according to Kobus-Cisowska et al. as the inhibition of the growth of the indicator microorganisms: *Klebsiella pneumoniae* ATCC 31488, *Salmonella enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC 19606, *Enterococcus faecium* ATCC 27270, *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25923, *Lactobacillus fermentum* ATCC 14932, *Clostridium butyricum* ATCC 860, *Listeria monocytogenes* ATCC 19115, *Bacillus coagulans,* GBI-30, 6086, *Candida utillis* ATCC 9950, *Aspergillus* sp., *Fusarium* sp. (Kobus-Cisowska et al., 2019b). Bacteriostatic properties were determined by measuring the diameter of the growth inhibition zone (indicator strain growth limitation).

Statistical analysis

All assays were conducted in triplicates and results expressed as mean ± SD. One way ANOVA testing was used to analyze statistical differences amongst the various extracts for phenolic compound contents and different antioxidant assays with least significant difference (LSD). The P value less than 0.05 was assumed as a level of significance. Correlations between the content of components and antioxidant attributes were determined by Pearson's correlation coefficients. Additionally, the analysis of the principal components was used (PCA). Statistical analyses were calculated using Statistica 13.3 software (Statsoft, Poland).

RESULTS

Extraction yield and physical properties of extracts

Extraction yield, density, pH and extract color were examined and are presented in Table 1. The yield level was as follows: PSL>PSP>PSN, these values correlated with pH, but did not correlate with extract density. In the case of material dried using freeze drying, high yield of 32.05% was obtained, for the natural-air-dried raw material in room temperature, a 10-fold lower yield was obtained. The density remained at a similar level between all samples, with the highest density (0.973 g/ml) characterizing PSL sample, whereas lowest PSP (0.932). The indicative of hydrogen ion concentration was similar for PSL and PSP samples (4.07 and 4.15, respectively), indicating their acidity, whereas higher for PSN, demonstrating its more alkaline pH. The extracts differed significantly in terms of color measured in the CIELab space. The PSL sample had the highest lightness value (L^* = 75.01), whereas PSN was the darkest sample (L^* = 62.03). The a^* and b^* values indicating colors in the range from yellow and blue, also differed significantly between the samples, showing the impact of drying on the content of pigments in the extracts.

Content of ascorbic acid, flavonoids, chlorophylls and carotenoids

The content of the studied compounds varied significantly in the tested shoots. The highest content of vitamin C characterized PSP sample (59.12 mg/g), where this value was close to 3-fold higher than in PSN sample (20.96 mg/g) and as much as 7.5 fold higher than in PSL sample (7.81 mg/g), although the difference was not statistically significant. The highest concentration of total chlorophyll and carotenoids was observed in the extract of air-dried

Table 1: Yield of *Pinus sylvestris* L. needles extraction.

Sample	pH	Density (g/ml)	Extract yield (%)	Color		
				*L**	*a**	*b**
PSN	5,48^a±0,00	0,961	3,21^a±0,01	62,03^a±0,05	5,76^a±0,11	61,68^a±0,15
PSL	4,07^b±0,02	0,973	32,05^b±6,66	75,01^b±0,01	1,08^b±0,01	26,66^b±0,36
PSP	4,15^c±0,00	0,932	20,84^c±1,60	70,32^c±0,27	2,22^c±0,05	57,41^c±0,03

Results are mean values of three determinations ± standard deviation. Values sharing the same letter in a column are not significantly different ($p \leq 0.05$).
PSN – air-dried needles of *Pinus sylvestris* L.; PSP – vacuum-dried needles of *Pinus sylvestris* L; PSL – freeze-dried needles of *Pinus sylvestris* L

shoots (PSN), respectively 126.11 mg/g dw and 49.34 mg/g dw, the lowest content of these compounds was exhibited by the extract from vacuum-dried leaves (PSP). The difference of total chlorophyll concentration was not statistically significant between PSN and PSP, and between PSP and PSL. In terms of total flavonoid content the values are analogous to the content of ascorbic acid, the highest content was demonstrated in the PSP sample, at 5.51 mg quercetin/g dw, in case of PSN and PSL samples the content of flavonoids was similar and amounted to 4.44 and 4.22 mg quercetin/g dw and the difference between these samples was not statistically significant. These values show that drying has a significant impact on the content of individual compounds in the raw material.

Content of flavonols and phenolic acids by HPLC

The content of polyphenols and phenolic acids depended on the applied drying method. The highest content of polyphenols characterized extract from freeze-dried raw material (9151.15 µg/g), followed by vacuum-dried (8264.57 µg/g), and the lowest content of phenolic compounds was found in convection-dried shoots (7621.76 µg/g). The most abundant phenolic compound found in the extracts was naringenin (3221.25 – 3868.04 µg/g), ferrulic (1222.65- 1380.69µg/g) and caffeic acid (954.89 - 1432.17 µg/g). In addition, high contents of hydroxybenzoic, chlorogenic, coumaric and vanillic acid have been identified in the extracts.

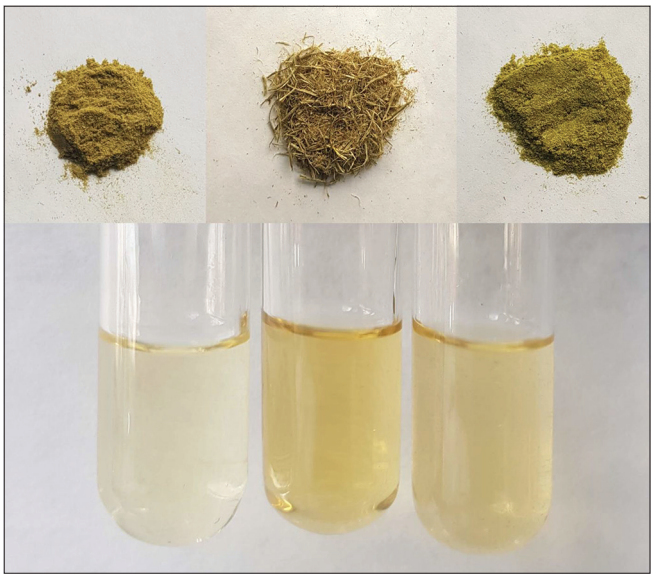

Fig 1. Ground dried shoots and of *Pinus sylvestris* L. and extracts. Order of samples from left: PSL, PSN, PSP

Antioxidative properties

The studied extracts demonstrated high antioxidative properties, both in ferric reducing antioxidant power assay (FRAP) as well as in free radical quenching measurement (DPPH) and Folin-Cicoalteau reagent assay (FCR). In the case of Fe^{3+} reduction to $Fe^{2+,}$ the most pronounced activity was shown by the PSP extract (47.25 µM FeSO4/g dw), but was not statistically different than PSN (37.79 µM $FeSO_4$/g dw), the smallest activity was pronounced by PSL (21.79 µM FeSO4/g dw), but the difference was not significant compared to PSN. Free radical quenching capacity DPPH was expressed in % of quenching, but also in the form of Trolox equivalent. The assessment demonstrated that all samples are characterized by the capacity to scavenge free radicals to a similar level, with the strongest properties found for the PSL sample (66.7% and 339 µM Trolox/g dw), and PSN (64.90% and 332.25 µM Trolox/g dw), statistically different was PSP sample (55.92% and 299.72 µM Trolox/g dw). On the other hand, according tothe FCR assay reducing capacity was as follows: PSN>PSP>PSL in the range from 13.4 to 5.73 mg gallic acid/g dw.

Antimicrobial activity

All of the studied extracts demonstrated antimicrobial and fungicidal properties, and they were particularly efficient in the case of gram-negative bacteria. The results were variable, however the PSL extract was characterized by the strongest biocidal properties towards the majority of microorganisms.

PCA projection (biplot) of results for qualitative analysis of *Pinus sylvestris* L. leaf extracts in set of two first components (PC1 and PC2), responsible for approximately 58% composition deviation, presented heterogeneity of tested

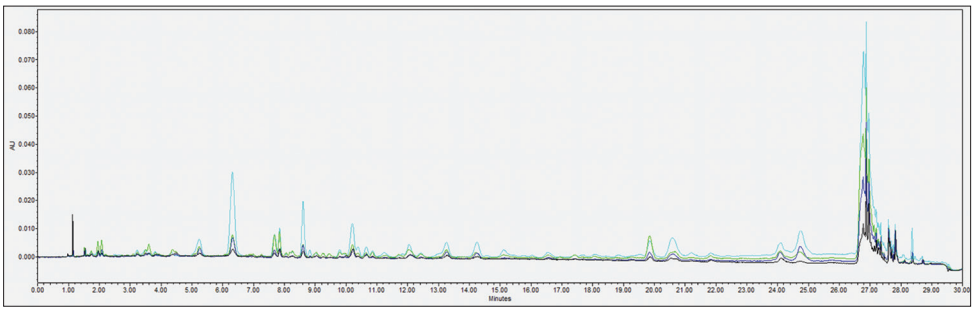

Fig 2. HPLC chromatogram of acids and standards. Legend: PSL – black line, PSP – blue line, PSN – green line, standards - light blue line

samples in terms of bioactive ingredients composition and the degree of influence of selected compounds on extracts' characteristic (Figure 3). The position of extracts on the right part of the figure indicates high deviation between samples in terms of the content of analyzed compounds and assayed antioxidative activity.

DISCUSSION

Considering the continuing growth of interest for vegetable products with health-promoting properties, it is necessary to evaluate these raw materials in order to provide consumers with functional products having the desired, efficient action (Griffiths et al., 2016). The main compounds with antioxidative nature in plants include phenolic compounds, carotenoids, chlorophylls and vitamins, and thus in vitro determination of the content of these compounds is the basic method of evaluation for the potential antioxidative properties of products (Bungau et al., 2019). Considering that determination of each component individually in complex systems such as food products would be impossible to carry out, methods such as FRA assay, FCR assay, DPPH scavenging are used, allowing a rapid, simple and sensitive determinations. Thus, these methods have been applied in the present study (Benzie and Choi, 2014).

The conducted study demonstrated that the method and conditions of the applied raw material drying have considerable impact on physical properties and antioxidant content, as well as antioxidative properties of an extract. Drying method impact on extraction yield and content of bioactive compounds is well documented regarding many raw materials as a consequence of the matrix changes during the drying process (Górnaś et al., 2014; Youssef and Mokhtar, 2014). Low efficiency characterized the air-dried shoots, whereas vacuum-dried and freeze-dried shoots were characterized by considerably higher extraction yields. This may be caused by the specificity of pine shoots containing waxes, resins and essential oils, differing from the majority of plant materials. Similar relationship was demonstrated in the study of Pham et al., where drying at 30 °C and 25 °C resulted in very low extraction efficiency, which was most likely linked to the differences in the permeability and solubility of the dried material (Pham et al., 2015). The authors noted significant differences in the color, indicating considerable impact of drying on the content of bioactive compounds, in particular of pigments (Pham et al., 2015). Spectrophotmetric testing demonstrated that pine shoots can be a source of vitamin C, which is a strong antioxidant, and fulfills a wide range of biological functions. The content of ascorbic acid differed significantly depending on the drying method. Literature includes reports on vitamin C degradation as a result of drying is frequently observed due to its susceptibility to numerous factors, i.e. light, temperature, humidity (Deng et al., 2018). In addition, vitamin C content in pine shoots, varies depending on the season of the year or the pollution level (Kalugina et al., 2018). Temperature and relative humidity are among the most important factors influencing the change of coloration and thus pigment degradation (Shahabi et al., 2014). The relationship between temperature growth and decrease of chlorophyll

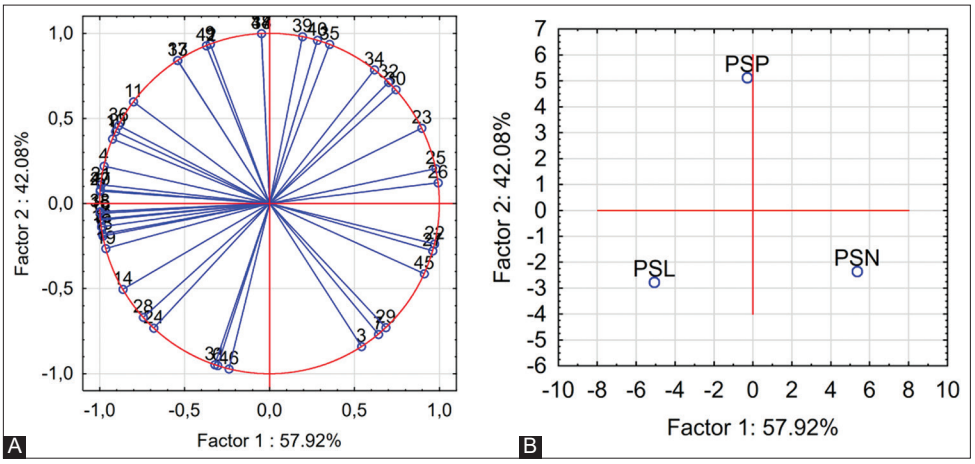

Fig 3. Principal component scatter diagram for tested samples (a) and principal components projection (b).Variables numbers as follows: 1. Gallic acid; 2. 2,5.dihydroxybenzoic acid; 3. 4.hydroxybenzoic acid; 4. caffeic acid; 5. syringic acid; 6. p.coumaric acid; 7. ferulic acid; 8. chlorogenic acid; 9. sinapic acid; 10. t.cinnamic acid; 11. vanilic acid; 12. salicylic acid; 13. Naringenin; 14. Vitexin; 15; Rutin 16. Quercitin; 17. Apigenin; 18. Kaempferol; 19. Luteolin; 20. Color L*; 21. Color a*; 22. Color b*; 23. Ascorbic acid; 24. Chlorophyll a; 25. Chlorophyll b; 26. total carotenoids 27. total flavonoids; 28. total polyphenol; 29. DPPH; 30. FRAP; 31. Klebsiella pneumoniae ATCC 31488; 32. Salmonella enteritidis ATCC 13076; 33. Pseudomonas aeruginosa ATCC 27853; 34. Acinetobacter baumannii ATCC 19606; 35. Enterococcus faecium ATCC 27270; 36. Staphylococcus aureus ATCC 25923; 37. Lactobacillus fermentum ATCC 14932; 38. Clostridium butyricum ATCC 860; 39. Listeria monocytogenes ATCC 19115; 40. Bacillus coagulans; 41. Candida utillis ATCC 9950; 42. Aspergillus sp.; 43. Fusarium sp.; 44. pH; 45. Density; 46. extraction yield.

Table 2: The content of chlorophyll and carotenoids in *Pinus sylvestris* L.

Extract	Ascorbic acid (mg/g dw)	Chlorophyll A (mg/g dw)	Chlorophyll B(mg/g dw)	Total Chlorophyll (mg/g dw)	Carotenoids (mg/g dw)	Total flavonoids (mg quercetin/g dw)
PSN	20,96^{a}±8,46	43,30^{a}±9,96	71,66^{a} ±9,04	126,11^{a}±12,49	49,34^{a}±2,07	4,44^{a}±0,34
PSP	59,12^{b}±12,25	13,43^{a}±4,44	11,51^{a}±2,24	23,05^{ab}±3,15	17,72^{b}±2,74	5,51^{b}±0,52
PSL	7,81^{a}±1,77	32,53^{b}±9,31	45,48^{a}±9,58	68,95^{b}±7,78	24,18^{c}±4,75	4,22^{a}±0,64

The mean values in the column marked with different small letters indicate the significance of differences ($p \leq 0.05$). dw - dried weight of raw material

Table 3 : HPLC analysis of phenolic compounds in *Pinus sylvestris L.*

Compound (µg/g dw)	PSN	PSP	PSL
gallic acid	134,57^{a}±2,38	174,12^{b}±5,22	184,54^{b}±8,58
2,5-dihydroxybenzoic acid	10,65^{a}±0,59	13,12^{b}±0,97	18,66^{c}±0,73
4-hydroxybenzoic acid	674,09^{a}±2,27	619,94^{b}±1,57	671,68^{a}±29,67
caffeic acid	954,89^{a}±0,60	1336,18^{b}±31,73	1432,17^{c}±66,86
syringic acid	92,11^{a}±2,07	108,76^{b}±0,55	117,21^{c}±3,63
p-coumaric acid	234,89^{a}±0,99	220,61^{a}±3,08	256,45^{b}±14,92
ferulic acid	1380,69^{a}±1,9	1222,65^{b}±4,09	1330,81^{c}±37,18
chlorogenic acid	311,71^{a}±2,15	347,65^{b}±1,88	418,68^{c}±8,57
sinapic acid	36,95^{a}±0,06	62,59^{b}±4,03	42,92^{c}±1,4
t-cinnamic acid	72,35^{a}±1,43	114,50^{b}±2,97	120,49^{b}±1,38
vanilic acid	198,55^{a}±0,65	223,36^{b}±0,42	222,14^{b}±9,85
salicylic acid	6,50^{a}±0,44	13,04^{b}±0,88	21,47^{c}±0,53
naringenin	3221,25^{a}±5,87	3456,63^{b}±5,01	3868,04^{c}±79,99
vitexin	18,92^{a}±1,36	24,13^{b}±3,83	56,97^{c}±2,28
rutin	103,16^{a}±4,21	111,90^{b}±4,33	124,80^{c}±4,55
quercetin	155,76^{a}±7,49	187,68^{b}±10,98	243,74^{c}±7,4
apigenin	10,36^{a}±0,62	12,54^{b}±0,51	11,82^{b}±0,44
kaempferol	0,26^{a}±0,06	10,15^{b}±2,69	0,30^{a}±0,02
luteolin	4,12^{a}±0,25	5,02^{b}±0,43	8,26^{c}±0,22
Total phenolic compounds	7621,76^{a}±14,43	8264,57^{b}±48,09	9151,15^{c}±118,31

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

Table 4: Radical scavenging and antioxidant activity of *Pinus sylvestris* L. with DPPH and FRAP methods

Assay	PSN	PSP	PSL
DPPH scavenging effect (%)	64,90^{a}±2,9	55,92^{b}±4,41	66,76^{a}±5,42
DPPH (µM Trolox/g dw)	332,25^{a}±10,49	299,72^{b}±15,97	339,00^{a}±19,61
FRAP (µM FeSO4/g dw)	37,79^{a}±3,64	47,25^{ab}±14,06	21,79^{ac}±4,36
FCR (mg GAE /g dw)	13,4^{a}±4,07	8,34^{b}±2,01	5,73^{c}±2,55

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

content and carotenoids in plant material in the process drying has also been demonstrated (Oliveira et al., 2015). Similarly to this study, numerous other works showed that the freeze-drying method enables high pigment concentration to be retained. Feng et al., who studied lettuce cubes, demonstrated that freeze-drying allowed retention of higher amount of chlorophylls as compared with hot air drying and microwave drying, both in vacuum conditions as well as in bed drying (Feng et al., 2012). Freeze drying was also the most favorable method in the study of green tea, where this method enabled obtaining several-fold higher content than for fresh leaves, as well as sun dried and shade dried leaves (Roshanak et al., 2016). On the other hand, Barisa et al. demonstrated minor differences between the content of chlorophyll and carotenoids between samples dried with the following methods: convection oven drying, microwave drying, air drying with and without sun exposure and food dehydrator drying. In turn, Kumar et al. demonstrated that drying leaves of *Hibiscus sabdariffa* L. at room temperature was more favorable than freeze drying and other methods (Branisa et al., 2017; Kumar et al., 2015). Lower yields of these compounds in present study in PSL sample could be also attributed to characteristic structure of Pine shoots. As the outer layer is covered by epicuticular waxex, freeze-drying of whole shoots could contribute to build-up of internal pressure and lead up to material damage, making it more susceptible to external conditions (Bhatta et al., 2020). Therefore, it can be expected that raw material type also has a decisive impact on the stability of pigments during drying with different methods. Numerous studies determined that the content of flavonoids determines the antioxidative properties of extracts (Kobus-Cisowska et al., 2019a; Kulczyński et al., 2016; Szczepaniak et

Table 5. Antibacterial activity of *Pinus sylvestris L.*

Microrganism	Growth inhibition zone [mm]		
	PSN	PSP	PSL
Gram(-) bacteria			
Klebsiella pneumoniae ATCC 31488	22±2	12±2	25±1
Salmonella enteritidis ATCC 13076	16±2	18±7	20±3
Pseudomonas aeruginosa ATCC 27853	27±3	25±2	28±5
Acinetobacter baumannii ATCC 19606	20±2	18±2	23±3
Gram(+) bacteria			
Enterococcus faecium ATCC 27270	18±2	19±2	19±9
Staphylococcus aureus ATCC 25923	19±2	19±3	21±7
Lactobacillus fermentum ATCC 14932	13±2	15±3	14±8
Clostridium butyricum ATCC 860	17±2	16±2	20±4
Listeria monocytogenes ATCC 19115	19±2	18±2	21±5
Bacillus coagulans GBI-30, 6086	19±2	20±1	22±8
Fungi			
Candida utillis ATCC 9950	6±1	7±1	9±1
Aspergillus sp.	5±1	4±1	7±5
Fusarium sp.	5±1	5±1	6±4

al., 2019). The test of total flavonoids content showed that the extract obtained from freeze-dried shoots was characterized by lower content of these compounds Based on the example of numerous plant raw materials, it was demonstrated that drying at elevated temperature may lead to increased concentration of phenolic compounds, which is caused by altered activities of various key enzymes of phenolic biosynthetic pathways, increased activity of enzymes such as polyphenol oxidase, phenoloxidase cause abiotic stress and decreased water content in cells [39]. On the other hand, the content of 19 flavonoids and phenolic acids determined via HPLC exhibited highest concentration for the PSL and lowest for the PSN sample. In line with observations of numerous authors, pine needles were characterized by high quercetin content, whereas narginenin and ferrulic acid were predominant among phenolic acids (Metsämuuronen and Sirén, 2019). All samples were characterized by high antioxidative activity in the free radical DPPH assay and in ferric reducing antioxidant power assay. The order of activity was as follows: PSL>PSP>PSN for DPPH and PSP>PSN>PSL for FRAP. The results for FCR assay were slightly different, the sample with highest reducing capacity was PSN and PSL sample had the lowest reducing activity. These differences showed between different methods may stem from the different reactivity of the tested components in extracts towards the studied reagent. For example, FC reagent is nonspecific to phenolic compounds and can be reduced by many nonphenolic compounds such as vitamin C, which concentration was lowest in PSL sample (Górnaś et al., 2016). However, in the case of numerous phenolic compounds, relationship explained via bell-shaped curves has been observed, as many compounds can be efficient antioxidants at very low concentrations but rather inefficient or pro-oxidative depending on the concentration, conditions and co-occurrence of other compounds (Eren-Guzelgun et al., 2018). Pine shoots have wide antimicrobial and fungicidal action, which has also been demonstrated herein. Other authors refer to the essential oils contained in coniferous trees as compounds with highest antimicrobial potential, however some phenolic compounds also demonstrate this effect, such as quercetin, ferrulic acid and apigenin contained in Scots pine (Nazzaro et al., 2017).

CONCLUSIONS

Pine shoots form a raw material characterized by containing numerous bioactive compounds with high antioxidative, reducing and antimicrobial properties. This raw material may be applied in functional foods due to the wide spectrum of potential health promoting action, low price and good availability. However, the conducted study provide evidence that the applied fixing methods, i.e. drying leave marked impact on the physicochemical properties of the obtained extracts and further research is needed in order to optimize the process and to evaluate the applicability of pine shoots.

Authors' contributions

Conceptualization and methodology: Joanna Kobus-Cisowska; formal analysis: Marcin Dziedziński, Daria Szymanowska-Powałowska, Kinga Stuper-Szablewska; resources: Marlena Baranowska; writing—original draft preparation: Marcin Dziedziński; supervision: Joanna Kobus-Cisowska.

Funding

The publication was co-financed within the framework of the Ministry of Science and Higher Education program as "Regional Initiative Excellence" in years 2019–2022, project number 005/RID/2018/19 and by statutory funds of the Department of Gastronomy Sciences and Functional Foods of Poznan University of Life Sciences, grant number 506.751.03.00.

Conflicts of Interest

The authors declare no conflict of interest.

REFERENCES

Ács, K., V. L. Balázs, B. Kocsis, T. Bencsik, A. Böszörményi and G. Horváth. 2018. Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. BMC Complement. Altern. Med. 18; 227.

Ajila, C. M., S. G. Bhat and U. J. S. Prasada Rao. 2007. Valuable components of raw and ripe peels from two Indian mango varieties. Food Chem. 102: 1006-1011.

Benzie, I. F. F. and S. W. Choi. 2014. Chapter one antioxidants in food: Content, measurement, significance, action, cautions, caveats, and research needs. In: J. Henry (Ed.), Advances in Food and Nutrition Research. Academic Press, Cambridge, Massachusetts, pp. 1-53.

Bhatta, S., T. S. Janezic and C. Ratti. 2020. Freeze-drying of plant-based foods. Foods. 9: 10087.

Branisa, J., K. Jomova, M. Porubska, V. Kollar, M. Simunkova and M. Valko. 2017. Effect of drying methods on the content of natural pigments and antioxidant capacity in extracts from medicinal plants: A spectroscopic study. Chem. Pap. 71: 1993-2002.

Bungau, S., M. M. Abdel-Daim, D. M. Tit, E. Ghanem, S. Sato, M. Maruyama-Inoue, S. Yamane and K. Kadonosono. 2019. Health benefits of polyphenols and carotenoids in age-related eye diseases. Oxid. Med. Cell. Longev. 2019: 9783429.

Deng, L. Z., X. H. Yang, A. S. Mujumdar, J. H. Zhao, D. Wang, Q. Zhang, J. Wang, Z. J. Gao and H. W. Xiao. 2018. Red pepper (*Capsicum annuum* L.) drying: Effects of different drying methods on drying kinetics, physicochemical properties, antioxidant capacity, and microstructure. Drying Technol. 36: 893-907.

Eren-Guzelgun, B., E. Ince and H. Gurer-Orhan. 2018. *In vitro* antioxidant/prooxidant effects of combined use of flavonoids. Nat. Prod. Res. 32: 1446-1450.

Feng, Y. F., M. Zhang, H. Jiang and J. C. Sun. 2012. Microwave-assisted spouted bed drying of lettuce cubes. Drying Technol. 30: 1482-1490.

Górnaś, P., K. Dwiecki, A. Siger, J. Tomaszewska-Gras, M. Michalak and K. Polewski. 2016. Contribution of phenolic acids isolated from green and roasted boiled-type coffee brews to total coffee antioxidant capacity. Eur. Food Res. Technol. 242: 641-653.

Górnaś, P., E. Šnē, A. Siger and D. Segliņa. 2014. Sea buckthorn (*Hippophae rhamnoides* L.) leaves as valuable source of lipophilic antioxidants: The effect of harvest time, sex, drying and extraction methods. Indust. Crops Prod. 60: 1-7.

Griffiths, K., B. B. Aggarwal, R. B. Singh, H. S. Buttar, D. Wilson and F. De Meester. 2016. Food antioxidants and their anti-inflammatory properties: A potential role in cardiovascular diseases and cancer prevention. Diseases. 4: 28.

Hoai, N. T., H. V. Duc, D. T. Thao, A. Orav and A. Raal. 2015. Selectivity of *Pinus sylvestris* extract and essential oil to estrogen-insensitive breast cancer cells *Pinus sylvestris* against cancer cells. Pharmacogn. Mag. 11: S290-S295.

Kalugina, O. V., T. A. Mikhailova and O. V. Shergina. 2018. Biochemical adaptation of scots pine (*Pinus sylvestris* L.) to technogenic pollution. Contemp. Prob. Ecol. 11: 79-88.

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

Kobus-Cisowska, J., D. Szymanowska, P. Maciejewska, D. Kmiecik, A. Gramza-Michałowska, B. Kulczyński and J. Cielecka-Piontek. 2019a. *In vitro* screening for acetylcholinesterase and butyrylcholinesterase inhibition and antimicrobial activity of chia seeds (*Salvia hispanica*). Electron. J. Biotechnol. 37: 1-10.

Kobus-Cisowska, J., D. Szymanowska-Powałowska, O. Szczepaniak, D. Kmiecik, M. Przeor, A. Gramza-Michałowska, J. Cielecka-Piontek, M. Smuga-Kogut and P. Szulc. 2019b. Composition and *in vitro* effects of cultivars of *Humulus lupulus* L. hops on cholinesterase activity and microbial growth. Nutrients. 11: 1377.

Kulczyński, B., J. Kobus-Cisowska, D. Kmiecik, A. Gramza-Michałowska, D. Golczak and J. Korczak. 2016. Antiradical capacity and polyphenol composition of asparagus spears varieties cultivated under different sunlight conditions. Acta Sci. Pol. Technol. Aliment. 15: 267-279.

Kumar, S. S., P. Manoj, N. P. Shetty and P. Giridhar. 2015. Effect of different drying methods on chlorophyll, ascorbic acid and antioxidant compounds retention of leaves of *Hibiscus sabdariffa* L. J. Sci. Food Agric. 95: 1812-1820.

Meda, A., C. E. Lamien, M. Romito, J. Millogo and O. G. Nacoulma. 2005. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 91: 571-577.

Metsämuuronen, S. and H. Sirén. 2019. Bioactive phenolic compounds, metabolism and properties: A review on valuable chemical compounds in Scots pine and Norway spruce. Phytochem. Rev. 18: 623-664.

Nazari, S., N. Nazarnezhad and M. Ebrahimzadeh. 2013. Evaluation of antioxidant properties and total phenolic and flavonoids content of *Eucalyptus camaldulensis* and *Pinus sylvestris* bark. Iran. J. Wood. Pap. Sci. Res. 28: 522-533.

Nazzaro, F., F. Fratianni, R. Coppola and V. D. Feo. 2017. Essential oils and antifungal activity. Pharmaceuticals. 10: 40086.

Nicolato, E., F. Boschi, P. Marzola and A. Sbarbati. 2009. Secretory response induced by essential oils on airway surface fluid: A pharmacological MRI study. J. Ethnopharmacol. 124: 630-634.

Oliveira, S. M., I. N. Ramos, T. R. S. Brandão and C. L. M. Silva. 2015. Effect of air-drying temperature on the quality and bioactive characteristics of dried Galega kale (*Brassica oleracea* L. var. *acephala*). J. Food Proc. Preserv. 39: 2485-2496.

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

Penkina, N., L. Tatar, V. Kolesnyk, T. Karbivnycha and T. Letuta. 2017. The study of beer quality with the reduced toxic effect. EUREKA Life Sci. 1: 35-43.

Pham, H. N. T., V. T. Nguyen, Q. V. Vuong, M. C. Bowyer and C. J. Scarlett. 2015. Effect of extraction solvents and drying methods on the physicochemical and antioxidant properties of *Helicteres hirsuta* Lour. Leaves. Technol. 3: 285-301.

Ramana, K. V., A. B. M. Reddy, N. V. R. Majeti and S. S. Singhal. 2018. Therapeutic potential of natural antioxidants. Oxid. Med. Cell. Longev. 2018: 9471051.

Roleira, F. M. F., E. J. Tavares-da-Silva, C. L. Varela, S. C. Costa, T. Silva, J. Garrido and F. Borges. 2015. Plant derived and dietary phenolic antioxidants: Anticancer properties. Food Chem. 183: 235-258.

Roshanak, S., M. Rahimmalek and S. A. H. Goli. 2016. Evaluation of seven different drying treatments in respect to total flavonoid, phenolic, Vitamin C content, chlorophyll, antioxidant activity and color of green tea (*Camellia sinensis* or *C. assamica*) leaves. J. Food Sci. Technol. 53: 721-729.

Semeniuc, C. A., A. Rotar, L. Stan, C. R. Pop, S. Socaci, V. Mireşan and S. Muste. 2016. Characterization of pine bud syrup and its effect on physicochemical and sensory properties of kefir. CyTA J. Food. 14: 213-218.

Shahabi, M., S. Rafiee, S. S. Mohtasebi and S. Hosseinpour. 2014. Image analysis and green tea color change kinetics during thin-layer drying. Food Sci. Technol. Int. 20: 465-476.

Sokół-Łętowska, A., J. Oszmiański and A. Wojdyło. 2007. Antioxidant

activity of the phenolic compounds of hawthorn, pine and skullcap. Food Chem. 103; 853-859.

Szczepaniak, O. M., M. Ligaj, J. Kobus-Cisowska, P. Maciejewska, M. Tichoniuk and P. Szulc. 2019. Application for novel electrochemical screening of antioxidant potential and phytochemicals in Cornus mas extracts. CyTA J. Food. 17: 781-789.

Villalobos, M. C., M. J. Serradilla, A. Martín, C. Pereira, M. López-Corrales and M. G. Córdoba. 2016. Evaluation of different drying systems as an alternative to sun drying for figs (*Ficus carica* L). Innov. Food Sci. Emerg. Technol. 36: 156-165.

Youssef, K. M. and S. M. Mokhtar. 2014. Effect of drying methods on the antioxidant capacity, color and phytochemicals of *Portulaca oleracea* L. Leaves. J. Nutr Food. Sci. 4: 322.

Journal of Elementology ISSN 1644-2296

Dziedziński M., Kobus-Cisowska J., Stuper-Szablewska K., Cielecka-Piontek J., Wilk R., Ludowicz D. 2022.
Antioxidant potential, mineral composition and inhibitory effects of conifer needle extract on hyaluronidase – prospects of application in functional food.
J. Elem., 27(4): 831-845. DOI: 10.5601/jelem.2022.27.3.2314

RECEIVED: 7 July 2022
ACCEPTED: 21 September 2022

ORIGINAL PAPER

ANTIOXIDANT POTENTIAL, MINERAL COMPOSITION AND INHIBITORY EFFECTS OF CONIFER NEEDLE EXTRACT ON HYALURONIDASE – PROSPECTS OF APPLICATION IN FUNCTIONAL FOOD*

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Abstract

Conifers have long been used as a source of compounds with therapeutic and health-promoting potential, as well as raw materials containing characteristic aromatic and flavour substances. The aim of this study was to characterise the mineral composition of the needles of selected conifers, i.e. *Picea abies* L., *Larix decidua* Mill., *Pinus sylvestris* L., *Pseudotsuga menziesii* and *Juniperus communis* L., *Abies alba* Mill., and to evaluate them as a source of bioactive compounds with antioxidant and inhibitory activity against hyaluronidase evaluated by an *in vitro* method. The highest total mineral content was found in *Abies alba* Mill., *Pseudotsuga menziesii*, while extracts obtained from *Picea abies* L. and *Larix decidua* Mill. were characterised by the highest content of phenolic compounds. Extracts from the needles of *Larix decidua* Mill. showed the highest antioxidant activity in DPPH and FRAP radical assays. The presence of tannins, terpenoids, saponins and coumarins was demonstrated in all the extracts tested by qualitative screening tests. Conifer needles, i.e. needles of *Picea abies* L., *Larix decidua* Mill, Abies alba, *Pinus sylvestris* L., *Pseudotsuga menziesii* and *Juniperus communis* L., can be a valuable and ecological source of polyphenols and minerals.

Keywords: conifers, minerals, antioxidant activity, hyaluronidase inhibition

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* Funding: This research was supported by statutory funds of the Department of Gastronomy Sciences and Functional Foods of Poznań University of Life Sciences, grant number 506.751.03.00.

INTRODUCTION

In recent years, interest in the use of unusual plants in medicine and functional foods has been growing steadily. Many plants that were valued in traditional medicine are now being sought as natural and diverse sources of compounds to complement modern pharmacological approaches as well as enrich conventional foods and produce novel foods (Przeor 2022). Reports indicate that up to 80% of the world's population use plants and plant extracts as medication and that 60% of medical products are based on compounds derived from plants (Kumari et al. 2019). Consumers are increasingly aware of recommendations for healthier and more sustainable food consumption, which translates into an increased interest in plant-based foods and the popularity of herbal supplements (Medawar et al. 2019). Conifers are known in the traditional medicine of many nations (e.g. Korea, China, Poland), are common throughout the world, and include eight families (Pinaceae, Araucariaceae, Cupressaceae, Podocarpaceae, Cephalotaxaceae, Taxaceae, Phyllocladaceae, Sciadopityaceae), 70 genera and 630 species (BharDMaj et al. 2020). New research indicates the possible use of extracts from various conifers in the treatment of many diseases, including diabetes, neurological disorders, inflammation and cancer. Phytochemicals present in conifer extracts are non-toxic at therapeutic levels, with polyphenolic compounds, terpenoids, alkaloids having significant biological activity (BharDMaj et al. 2021). These constituents can positively influence the activity of many enzymes. Meanwhile, minerals act as cofactors for antioxidant enzymes, including superoxide dismutase, catalase and peroxidase, which are essential for optimal function of the immune system (Türkan et al. 2020). On the other hand, plant secondary metabolites often have the ability to inhibit enzymes, the high activity of which can promote disease and lead to health disorders (Zengin et al. 2017). Plant extracts, including those derived from conifers, were shown to have the ability to inhibit enzymes such as cholinesterase**,** tyrosinase, α-amylase, α-glucosidase, angiotensin--converting enzyme, hyaluronidase and others (Zengin et al. 2017, Khouja et al. 2020). Hyaluronic acid is a glucose-based polymer that is found in tissues and body fluids, especially in the dermis and epidermis (Jiratchayamaethasakul et al. 2020). Hyaluronidase is an enzyme that degrades hyaluronic acid. The main direction of action of hyaluronic acid is the effect on the skin; the activity of this acid contributes to the loss of elasticity, reduced hydration and premature aging of the skin (Jiratchayamaethasakul et al. 2020). However, the degradation of hyaluronan is also associated with a wide range of physiological and pathological processes. Therefore, the inhibition of the hyaluronidase enzyme is important as an approach to treating various diseases and disorders. Hyaluronidase inhibitors may be used in anticancer therapy, antimicrobial therapy, and as components of anti-venom and anti-toxin agents (Bhatti, Karim 2021).

The aim of this study was to evaluate the needles of selected conifers, i.e. *Picea abies* L., *Larix decidua* Mill., *Pinus sylvestris* L., *Pseudotsuga menziesii, Abies alba* and *Juniperus communis* L., in terms of mineral content, presence of secondary metabolites, antioxidant activity and inhibitory activity towards hyaluronidase.

MATERIALS AND METHODS

Material

Samples of shoots were collected from six different species of coniferous trees: *Picea abies* L. (PA), *Larix decidua* Mill; (LD), *Abies alba* (AA), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC) from the arboretum in Zielonka (Poland, 17°06′33″E, 52°06′33″N), which belongs to the Forest Experimental Department of Poznan University of Life Sciences. The raw material collected was subjected to vacuum drying at 60°C under the pressure of 470 mbar for 48 h in a VO29 drier (Memmert, Germany). The dried needles were sampled from three different shoots, crushed in a Grindomix GM 200 (Retsch, Haan, Germany) for 15 s at a rate of 500 rpm at 21°C, to a particle size of 0.5-0.9 mm.

Extraction

Depending on the assay, two different methods of extraction were conducted. In the first case, 2 g of ground needles were weighed and extracted with 40 ml of ethanol in an ultrasonic bath for 1 hour. After the first extraction, the liquid above the needles was decanted by filtering it through a filter, the needles were refilled with 40 ml of ethanol and the extraction was continued under the same conditions. After the extraction was completed, the extract was filtered through a filter, pressing the liquid from the needles as much as possible, and the two collected extracts were combined to obtain the initial concentration of about 25 mg ml^{-1} for the extracts. The extracts prepared in this way were used to conduct DPPH, FRAP, hyaluronidase inhibition assay and qualitative tests. For the HPLC analysis, water extracts were obtained by mixing 2 g of ground needles with 40 ml of distilled water. The samples were shaken in a water bath for 1 h at 80°C at constant amplitude, and the liquid above the needles was decanted by filtering it through a filter. The extracts obtained were centrifuged and the supernatants were stored at -21°C for no more than two weeks before further analyses.

Qualitative phytochemical screening

Alkaloids and saponins were estimated according to the method of Amarasingham et al. (1964). In the alkaloid test, about 0.5 g of the extract and 5 ml of 1% aqueous HCl were mixed and heated (30°C). After filtration,

2-3 drops of Dragendorff's reagent were added to the filtrate. The presence of alkaloids showed a precipitated orange-red colour. Saponin test was conducted using about 0.5 g of the extract, which was dissolved with hot distilled water. The formation of a 1 cm layer of foam after 1 min of shaking was the preliminary evidence for saponins. Tests for tannins, phenolic constituents, flavonoids, steroids, terpenoids, and cardiac glycosides were conducted according to Auwal et al. (2014). The presence of phenolic acids was assessed by mixing 1 ml of the extract with 2 ml of distilled water and subsequently adding five drops of 10% $FeCl_3$ solution. The appearance of dark green or blue colour is evidence of the presence of phenolic compounds. A tannins test was performed by dissolving the extract in 10 ml of distilled water and adding 1% aqueous solution of $FeCl_3$ after filtration. Green-purple colour confirmed the presence of tannins. The presence of flavonoids was assessed using 0.5 ml of extract to which 4 ml of 1% NH_3 and then 1 ml of concentrated H_2SO_4 were added. After a few seconds, the yellow colour indicated a positive result. A steroid test was performed with acetic anhydride (2 ml) and 2 ml of H_2SO_4 were added into a test tube with 0.5 g of the crude extract. The appearance of green or blue colour confirmed the presence of steroids in the sample. Terpenoids (Salkowski's test) test used an extract (5 ml) and 2 ml of $CHCl_3$ were added into a test tube, followed by 1 ml of conc. H_2SO_4. A positive result for terpenoids was the formation of a reddish-brown coloured layer at the interface. Cardiac glycosides (Keller Killiani test) test was performed using 5 ml of the extracts and 2 ml of glacial acetic acid mixed in the test tube, with 1-2 drops of 2% $FeCl_3$ added after that. 1 ml of conc. H_2SO_4 was carefully added to this solution. Cardiac glycosides were characterised by the presence of a brown ring and a violet--green ring below. A coumarin test was performed according to the method of Djaafar and Ridha (2014). 1 ml of ethanol was added to a test tube that contained 0.1 g of crude extract and subsequently filtered. Afterwards, 1.5 ml of 10% NaOH was added into the filtrate.

Mineral composition

The mineral composition analysis was made with a Vario MACRO Cube CN analyzer (Elementar Analysensysteme GmbH, Germany) according to the method of Telichowska et al. (2021). The analysis of the elemental composition of shoots was performed using an ICP-OES iCAP 6500 Axial and Radial Vista (Thermo Scientific, Waltham, Massachusetts, USA. Prior to multielemental analysis, the samples (approx. 0.5 g of dry mass – d.m.) underwent the mineralisation process (with 5.0 ml of 69% HNO_3) in Teflon bombs using a microwave oven Milestone Start D (Milestone S.r.l., Sorisole, Italy).

Quantative determination of phenolic acids and flavonols

Phenolic compounds in the samples were analysed after alkaline and acidic hydrolysis (Stuper-Szablewska et al. 2017). The analysis was per-

formed using an Aquity H class UPLC system equipped with an 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 λ=320 nm and 280 nm, and finally expressed as mg 100 g^{-1} DM of the sample. The compounds were identified by comparing the retention time of the analysed peak with the retention time of the standard and by adding a specific amount of the standard to the samples analysed and conducting a repeated analysis. The detection level was 1 μg g^{-1}. The retention times for phenolic acids were as follows: 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, vanillic acid – 16.80 min, sinapic acid – 17.10 min, ferulic acid – 17.50 min, salicylic acid – 17.85 min., t-cynnamic acid – 19.50 min. Retention times for flavonoids were as follows: apigenin – 1.10 min, vitexin – 8.00 min, kaempferol – 11.00 min, luteoline – 16.90 min, quercetin – 17.00 min, naringenin – 17.50 min, rutin – 17 90 min.

Antioxidant capacity screening

The DPPH assay was conducted according to the method of Studzińska-Sroka et al. (2021). The study was carried out with the use of 96-well plates. 25 μl of the appropriate extract dilution and 175 μl of DPPH reagent were added to each well, making 4 replications for each of the species' needle extract dilutions. Incubation in a dark place for 30 min was allowed. After this time, the absorbance of λ = 517 nm was measured using a plate reader. The IC50 value was determined. The reference was vitamin C. FRAP assay was conducted according to a modified method by O'Sullivan et. al (2013). The study was carried out with the use of 96-well plates. 25 μL of the appropriate extract dilution and 175 μL of FRAP reagent were added to each well, making 4 replicates for each of the species' needle extract dilutions. Incubation in the dark at 37°C for 30 min was allowed. After this time, the absorbance was measured with a plate reader at a wavelength of λ = 593 nm. The reference was vitamin C.

Hyaluronidase inhibition

An assay was performed according to the methodology of Chanaj-Kaczmarek et al. (2020). The assay was carried out with the use of 96-well plates. During the analysis, the turbidance formed as a result of the precipitation of hyaluronic acid, which was not degraded by the action of hyaluronidase, was measured spectrophotometrically because its action was inhibited by the extracts tested. The greater the turbidity, the more enzyme was inhibited, the more hyaluronic acid remained in the mixture.

Statistical analysis

All measurements were performed on three plants (i.e. three biological replicates). All data were expressed as a mean±standard deviation and subjected to one-way analysis of variance (ANOVA) using the RStudio software version 1.4 (RStudio, PBC, Delaware, USA). Statistical differences were measured at $p<0.05$.

RESULTS AND DISCUSSION

In this study, the needles of the studied trees were compared for the content of 15 selected mineral components (Table 1). The highest amount of mineral components was found in sample AA (39 248 mg kg^{-1}), slightly lower in samples PM (37 042.5 mg kg^{-1}) and PA (35 529.62 mg kg^{-1}), while the lowest content of mineral components was found in JC needles (150.26 mg kg^{-1}). In terms of quantity, potassium was the dominant element, ranging from 27 653.46 in the JC sample to 19 118.44 mg kg^{-1} in the PS sample. Calcium and magnesium were also present in high concentrations, ranging from 6687.46 in the JC sample to 3427.08 mg kg^{-1} in the PA sample, respectively, and from 3750.23 in the JC sample to 2718.76 mg kg^{-1} in the PA sample. Molybdenum was present in trace amounts. A particularly high variation was observed in terms of the Si content in the samples (160.16 -33.08 mg kg^{-1}). The mineral content of tree needles is influenced by many factors, including species, variety, tree age, climate zone, soil condition, ecosystem or environmental pollution (Pietrzykowski et al. 2013). In the study by Pongrac et. al. (2019), where mineral content was examined in *Pinus sylvestris* L. from Neris Regional Park (Lithuania), the contents of Fe, B, Mg, Cu were similar and the differences occurred in the range of 10-40%, while in the case of Si, K, Mn, P, Al, the differences were much higher, e.g. 5 times higher in the potassium content (Pongrac et al. 2019). In another study on the mineral content of *Pinus sylvestris* L., where the effect of industrial pollution in the Irkutsk region (Russia) on the level of nutrients in conifers was studied, the results for P, Ca, Mg, S, Al, Mn did not exceed several dozen per cent, except for K, the content of which was studied in this publication and was several times higher, both in the case of trees subjected to low and high exposure to technogenic pollution (Mikhailova et al. 2017). Similar trends are observed in studies where trees of *Juniperus communis* L., *Picea abies* L. species were analysed, and where the concentration of elements is similar apart from few differences (Gruwez et al. 2017, Jyske et al. 2020).

Qualitative screening in the conifer extracts tested revealed the presence of several primary and secondary metabolites or phytonutrients, which are summarised in Table 2. Tannins, terpenoids, saponins and coumarins were found in all the extracts studied. No steroids or cardiac glycosides were detected in any of the samples, whereas the Dragendorff reagent test only

Table 1

Mineral composition of conifer needles

Element (mg kg^{-1})	PM	AA	PA	JC	PS	LD
Fe	141.02±12.71^{ab}	142.69±11.26^{a}	112.44±5.42^{b}	121.20±5.27^{ab}	120.81±12.88^{ab}	129.11±12.94 ^{ab}
Mo	0.11±0.12^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.03±0.04^{a}
B	7.79±0.92^{ab}	5.28±0.83^{a}	6.97±1.43^{ab}	6.02±0.42^{ab}	10.64±0.60^{b}	5.63±0.15^{ab}
Si	111.57±10.43^{b}	160.16±4.05^{a}	123.13±10.79^{b}	72.32±18.13^{c}	111.72±14.50^{b}	33.08±5.04^{d}
Zn	46.65±10.53^{ab}	32.25±3.08^{a}	34.13±1.26^{a}	77.75±1.42^{b}	47.65±2.52^{ab}	59.20±2.64^{ab}
Cu	7.01±0.73^{a}	5.39±1.13^{ab}	5.45±0.58^{ab}	6.56±1.29^{ab}	4.64±0.23^{b}	4.96±0.38^{ab}
K	24364.32±637.92^{b}	25195.04±503.76^{b}	23398.59±703.01^{b}	27653.46±1080.31^{a}	19118.44±31.80^{c}	20289.25±761.73^{c}
Ca	3960.09±187.03^{ab}	4561.97±379.29^{b}	3427.09±124.37^{b}	6687.46±280.22^{a}	4246.55±411.26^{ab}	3896.63±408.68^{ab}
Mg	3170.92±29.18^{c}	3530.41±48.40^{b}	3180.27±71.41^{c}	3750.23±43.10^{a}	2718.76±24.47^{e}	2941.99±41.71^{d}
Ti	2.28±0.52^{ab}	1.45±0.20^{b}	1.23±0.31^{b}	2.77±0.45^{a}	1.97±0.71^{ab}	2.14±0.11^{ab}
P	2983.59±52.12^{c}	3324.95±16.52^{ab}	2989.42±44.88^{c}	3394.48±40.15^{a}	3141.56±36.12^{bc}	3222.25±156.05^{ab}
S	2167.50±65.54^{c}	2198.59±11.39^{ab}	2177.85±62.24^{c}	2382.99±20.1^{a}	2118.93±89.64^{bc}	2169.30±161.02^{ab}
V	2.36±0.23 ^{a}	2.44±0.49^{a}	1.81±0.65^{a}	2.00±0.93^{a}	1.77±0.39^{a}	2.45±0.28^{a}
Mn	50.13±10.27^{a}	56.92±3.73^{a}	59.69±1.54^{a}	61.70±5.39^{a}	48.85±1.83^{ab}	35.37±2.19^{b}
Al	27.15±1.68^{b}	30.47±3.41^{b}	11.54±1.00^{c}	48.88±8.07^{a}	30.03±2.24^{b}	23.10±3.70^{b}

The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix decidua* Mill. (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC)

Table 2

Qualitative phytochemical screening of conifer needles extracts

Sample / Compound	Screening result					
	PM	AA	PA	JC	PS	LD
Alkaloids	-	+	-	-	-	+
Tannins	+	+	+	+	+	+
Terpenoids	+	+	+	+	+	+
Steroids	-	-	-	-	-	-
Cardiac glycosides	-	-	-	-	-	-
Saponins	+	+	+	+	+	+
Coumarins	+	+	+	+	+	+

Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix decidua* Mill. (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC); (+) detected; (-) not detected

showed the presence of alkaloids in samples AA and LD. As reported by other authors, proanthocyanidins (condensed tannins) are commonly found in conifer needles, shoots and bark in the form of flavan-3-ols, which contain catechin and epicatechin (Yang et al. 2021). Triterpene glycosides (saponins) and other terpene compounds are commonly found in the tissues of many trees, especially the bark but also leaves and needles (Mroczek 2015, Wang et al. 2018). Among secondary plant metabolites, it is well documented that polyphenols, alkaloids, and triterpenoids function as repellents and toxic agents that protect trees. The bioactivity of saponin mixtures of individual saponins *in vitro* and *in vivo* include cytotoxic, immunomodulatory, hepatoprotective, antidiabetic, hypolipidemic, anti-osteoporotic, antiviral and antifungal activities. Thus, plant extracts can be considered as promising and highly available sources of biologically active triterpene saponins (Qi et al. 2020).

The analysis of the polyphenolic compound content was deepened by running HPLC assays (Table 3). Significant variation in the phenolic content was found between the studied shoots of different conifers. The predominant phenolic compounds present in the samples were caffeic acid> chlorogenic acid>ferulic acid>4-hydroxybenzoic acid. Caffeic acid was found in the highest concentration (from 522.6 in sample AA to 5775.76 μg g^{-1} DM in sample JC), while sinapic acid and 2,5-dihydroxybenzoic acid were present in the lowest concentration. Among the samples tested, sample PA (18 727.27 μg g^{-1} DM) was the richest in phenolic compounds, followed by LD (10 906.67 μg g^{-1} DM), JC (10 875.45 μg g^{-1} DM), PM (9436.36 μg g^{-1} DM) and PS (6605.45 μg g^{-1} DM). The presence and concentration of individual secondary metabolites and their accumulation is strongly dependent on various environmental factors, including light, temperature, soil fertility and salinity (Yang et al. 2018). In the analysis of the phenolic content, the results obtained are consistent

Table 3

Phenolic compounds in conifer needles water extracts

Compound ($\mu g\ g^{-1}$ DM)	PM	AA	PA	JC	PS	LD
gallic acid	62.42 ± 0.5^{b}	106.3 ± 0.70^{ab}	801.52 ± 6.28^{a}	851.52 ± 11.00^{a}	283.64 ± 1.07^{ab}	160.61 ± 2.03^{ab}
2,5-dihydroxybenzoic acid	8.79 ± 0.12^{b}	683.1 ± 4.20^{a}	104.24 ± 0.66^{ab}	23.94 ± 0.03^{ab}	22.12 ± 0.07^{ab}	139.39 ± 0.93^{ab}
4-hydroxybenzoic acid	1227.88 ± 12.02^{ab}	3602.0 ± 21.00^{a}	4075.76 ± 54.27^{ab}	24.55 ± 0.26^{b}	952.73 ± 7.89^{ab}	957.58 ± 10.06^{ab}
Caffeic acid	1461.52 ± 15.32^{ab}	522.6 ± 2.70^{a}	5409.70 ± 70.06^{ab}	5775.76 ± 48.91^{b}	2116.67 ± 13.80^{ab}	2899.70 ± 37.4^{ab}
Syringic acid	188.79 ± 2.05^{ab}	79.1 ± 0.40^{b}	346.97 ± 1.72^{ab}	60.00 ± 0.58^{a}	157.58 ± 1.05^{ab}	375.76 ± 0.44^{ab}
p-coumaric acid	65.45 ± 0.50^{a}	209.9 ± 1.20^{ab}	151.21 ± 0.90^{ab}	107.88 ± 1.54^{ab}	353.33 ± 2.27^{b}	275.76 ± 1.99^{ab}
Ferulic acid	5350.30 ± 41.48^{b}	280.2 ± 1.10^{a}	1309.09 ± 18.50^{ab}	1537.58 ± 12.60^{ab}	1924.85 ± 34.01^{ab}	4348.48 ± 45.36^{ab}
Chlorogenic acid	987.88 ± 10.28^{ab}	1460.7 ± 3.20^{ab}	4493.94 ± 50.76^{a}	2130.30 ± 27.92^{ab}	512.12 ± 4.23^{b}	581.82 ± 4.46^{ab}
Sinapic acid	11.82 ± 0.10^{b}	13.4 ± 0.10^{ab}	1287.88 ± 6.06^{a}	216.97 ± 0.98^{ab}	96.97 ± 0.56^{ab}	79.70 ± 1.02^{ab}
t-cinnamic acid	71.52 ± 0.28^{b}	141.2 ± 0.90^{ab}	746.97 ± 5.19^{ab}	146.97 ± 0.59^{ab}	185.45 ± 3.01^{ab}	1087.88 ± 2.62^{a}
Total (mg g^{-1} DM)	9.44	7.10	18.73	10.88	6.61	10.91

The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p\leq0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix decidua* Mill. (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC)

with those obtained in previous studies, where conifer shoots dried by different methods were analysed, as well as in studies where extraction was performed using ethanol, which yielded more compounds (Dziedzinski et al. 2020*a*). In another study (Sahin Yaglioglu, Eser 2017) on methanolic extracts of needles from 4 different juniper species (*J. communis, J. excelsa, J. foetidissima, J. oxycedrus subsp. oxycedrus*), the authors identified catechin as the major phenolic compound in the needle extracts at 273.36-274.85 mg g^{-1} DM and rutin at 146.57 mg g^{-1} DM, which were not detected in this study. In turn, in a study conducted on needles of common spruce, the authors used 95% ethanol for extraction and detected the same compounds as in the present study, i.e. chlorogenic acid, gallic acid, kaempferol, quercetin, but at concentrations below the limit of quantification of approx. 0.02 µmol g^{-1} DM. The dominant compound, as in the previously mentioned study, was catechin (Ganthaler et al. 2017). The result of extraction of phytochemical compounds is critically influenced by the composition of the solvent used, extraction time and temperature; in the case of phenolic compounds in plants, many authors observe that ethanol often yields the highest concentrations of polyphenols (Mohd Hazli et al. 2019).

Figure 1 summarises results related to the antioxidant capacity of conifer extracts evaluated by DPPH and FRAP methods. The lowest IC50 value for the inhibition of DPPH radical was 2.73 mg ml^{-1} for LD sample, while the highest value was 7.94 mg ml^{-1} for AA sample. The results for PM, PA, and JC samples ranged from 6.83 - 5.09 mg ml^{-1} and were not statistically significantly different. In the FRAP assay, LD extract (1.51 mg ml^{-1}) showed the

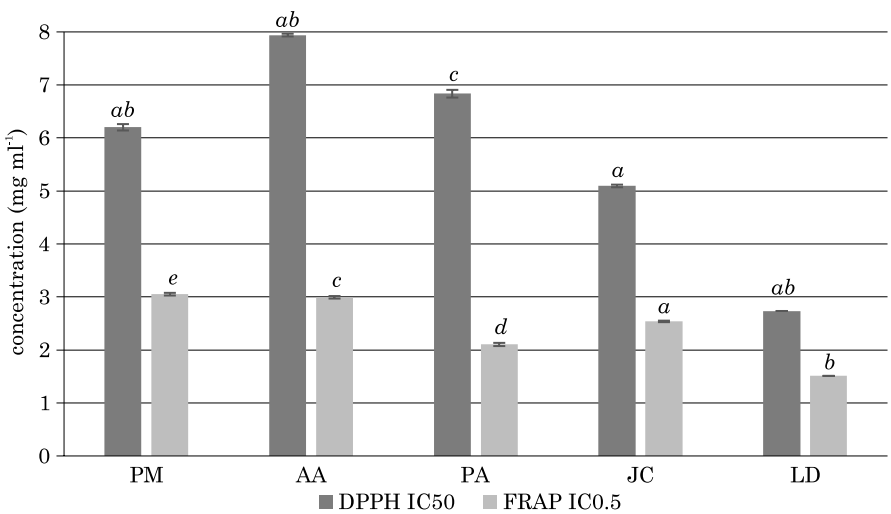

Fig. 1. Antioxidative capacity of conifer extracts measured by DPPH and FRAP assays
The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix decidua* Mill. (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC)

lowest IC0.5 value, followed by PA extract (2.1 mg ml^{-1}). PM (3.05 mg ml^{-1}) and AA (2.99 mg ml^{-1}) samples, which were not statistically significantly different from each other, showed the highest result in the study. For pine needle extracts (PS), neither IC50 nor IC0.5 values could be determined with DPPH and FRAP. DPPH and FRAP are among the most commonly used methods to estimate antioxidant activity (Tang et al. 2020). The IC_{50} values obtained from the DPPH assay are comparable to those obtained by other authors for essential oils from *Pinus sylvestris* var *mongolica* needles (14.36 mg ml^{-1}), while the results are significantly higher for both DPPH from essential oils obtained from *Pinus pinea* needles and methanol extracts from *Juniperus oxycedrus* subsp. *oxycedrus* (Chaouche et al. 2013, Halloum et al. 2019, Namshir et al. 2020). The relatively low antioxidant activity compared to some studies by other authors may be due to the difference in solvent used and extraction method. For many plant raw materials, the highest levels of phenols, flavonoids, alkaloids and terpenoids are observed in methanol extracts, which also significantly affects the measured biological activity, which was lower due to the use of ethanol in the study (Truong et al. 2019). The content of polyphenols and their antioxidant activity is often a determining factor for the functionality of plant raw materials and further of food products due to the potential of polyphenols to have protective effects in acute and chronic diseases, including obesity, neurodegenerative diseases, type 2 diabetes, and cardiovascular diseases (Cory et al. 2018).

The hyaluronidase inhibition assay compared the activity of ethanolic extracts (Figure 2). The LD sample had the highest ability to inhibit the hyalu-

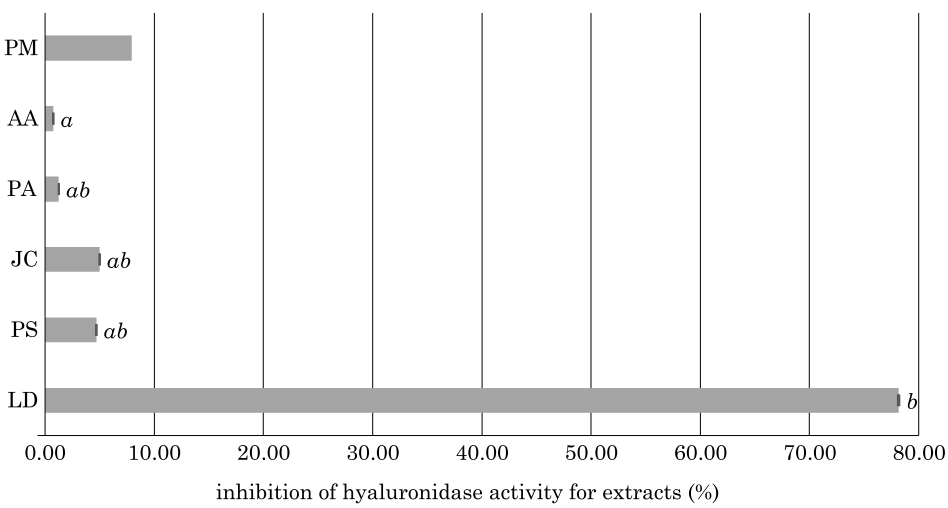

Fig. 2. Inhibition of hyaluronidase activity by conifer needle extracts
The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix decidua* Mill. (LD), *Pinus sylvestris* L. (PS), *Pseudotsuga menziesii* (PM) and *Juniperus communis* L. (JC)

ronic acid depolymerising enzyme (78.16% inhibition effect), which was statistically significantly higher compared to the other samples. The AA sample had the lowest ability to inhibit hyaluronidase (0.75% inhibition effect). The remaining samples had varying degrees of inhibition (7.95-1.26%) but the differences between them were not statistically significant. mong other things, the ability of polyphenols to inhibit dermal proteases and photoprotective activity, mostly studied using dermal fibroblasts or epidermal keratinocytes cell lines in cosmetics, as well as the ability of polyphenols to inhibit certain digestive enzymes were observed, which may be the basis for developing new and more effective anti-obesity and antidiabetic agents. (Zillich et al. 2015, Martinez-Gonzalez et al. 2017)

A study on the inhibition of hyaluronidase activity showed a relatively low inhibitory effect for most of the samples tested, except for *Larix decidua* Mill., where the inhibitory effect was 78%. In a study on essential oils of needles from different pine species (*Pinus brutia* Ten., *Pinus halepensis* Mill*., Pinus nigra* Arn., *Pinus pinea* L. and *Pinus sylvestris* L.), hyaluronidase inhibition was moderate, ranging from 10.14-30.28% (Süntar et al. 2012). Hyaluronidase is a mucopolysaccharide related to inflammation by the histamine released from mast cells. Hyaluronidase inhibitors can effectively reduce allergic and inflammatory reaction (Furusawa et al. 2011). Anti-hyaluronidase and anti-elastase properties were observed for tannin-rich plant materials, i.e. *Lythrum salicaria* L. or *Geum urbanum* L., which are characterised by a high concentration of polyphenols (Piwowarski et al. 2011).

CONCLUSIONS

Conifer needles, i.e. needles of *Picea abies* L., *Larix decidua* Mill, *Abies alba*, *Pinus sylvestris* L., *Pseudotsuga menziesii* and *Juniperus communis* L., can be a valuable and ecological source of polyphenols and minerals. The presence of tannins, terpenoids, saponins and coumarins was demonstrated in all the extracts tested by qualitative screening tests. Among phenolic compounds, a high content of 2,5-dihydroxybenzoic acid, 4-hydroxybenzoic acid and caffeic acid was found. Needles from *Abies alba* shoots had the highest antioxidant capacity in DPPH and FRAP assays. Needles may be a potential raw material that could find wide applications in pharmacology, cosmetics, as well as health-promoting and functional foods. This is a promising new source of bioactive compounds that has not yet been sufficiently developed.

REFERENCES

Amarasingham R.D., Bisset N.G., Millard A.H., Woods M.C. 1964. *A phytochemical survey of Malaya*. Part III. *Alkaloids and saponins*. Econ Bot, 18: 270-278. https://doi.org/10.1007/ /BF02908122

Auwal M.S., Saka S., Mairiga I.A., Sanda K.A., Shuaibu A., Ibrahim A. 2014. *Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa).* Vet Res Forum, 5: 95-100.

Bhardwaj K., Dhanjal D.S., Sharma A., Nepovimova E., Kalia A., Thakur S., Bhardwaj S., Chopra C., Singh R., Verma R., Kumar D., Bhardwaj P., Kuča K. 2020. *Conifer-derived metallic nanoparticles: green synthesis and biological applications.* Int J Molec Sci, 21: 9028. https://doi.org/10.3390/ijms21239028

Bhardwaj K., Silva A.S., Atanassova M., Sharma R., Nepovimova E., Musilek K., Sharma R., Alghuthaymi M.A., Dhanjal D.S., Nicoletti M., Sharma B., Upadhyay N.K., Cruz-Martins N., Bhardwaj P., Kuča K. 2021. *Conifers phytochemicals: a valuable forest with therapeutic potential.* Molecules, 26: 3005. https://doi.org/10.3390/molecules26103005

Bhatti M.Z., Karim A. 2021. *Plant natural products: a promising source of hyaluronidase enzyme inhibitors*. IntechOpen. https://doi.org/10.5772/intechopen.98814

Chanaj-Kaczmarek J., Paczkowska M., Osmałek T., Kaproń B., Plech T., Szymanowska D., Karaźniewicz-Łada M., Kobus-Cisowska J., Cielecka-Piontek J. 2020. *Hydrogel delivery system containing calendulae flos lyophilized extract with chitosan as a supporting strategy for wound healing applications.* Pharmaceutics, 12: E634. https://doi.org/10.3390/pharmaceutics12070634

Chaouche T.M., Haddouchi F., Ksouri R., Medini F., Atik-Bekara F. 2013. *In vitro evaluation of antioxidant activity of the hydro-methanolic extracts of Juniperus oxycedrus subsp. oxycedrus.* Phytothérapie, 11: 244-249. https://doi.org/10.1007/s10298-013-0779-5

Cory H., Passarelli S., Szeto J., Tamez M., Mattei J. 2018. *The role of polyphenols in human health and food systems: a mini-review.* Front Nutrit, 5: 87. https://doi.org/10.3389/fnut.2018.00087

Djaafar Z., Ridha O.M. 2014. *Phytochemical study of selected medicinal plant, solanum nigrum, the algerian desert.* Int Lett Chem, Phys Astron, 20: 25-30. https://doi.org/10.18052/www.scipress.com/ILCPA.20.25

Dziedziński M., Kobus-Cisowska J., Powałowska D.S., Szablewska K.S., Baranowska M. 2020. *Polyphenols composition, antioxidant and antimicrobial properties of Pinus sylvestris* L. *shoots extracts depending on different drying methods.* Emir J Food Agric, 229-237. https://doi.org/10.9755/ejfa.2020.v32.i3.2080

Dziedzinski M., Kobus-Cisowska J., Szymanowska D., Stuper-Szablewska K., Baranowska M. 2020. *Identification of polyphenols from coniferous shoots as natural antioxidants and antimicrobial compounds.* Molecules, 25: 3527. https://doi.org/10.3390/molecules25153527

Furusawa M., Narita Y., Iwai K., Fukunaga T., Nakagiri O. 2011. *Inhibitory effect of a hot water extract of coffee "silverskin" on hyaluronidase.* Biosci Biotech Biochem, 75: 1205-1207. https://doi.org/10.1271/bbb.110106

Ganthaler A., Stöggl W., Mayr S., Kranner I., Schüler S., Wischnitzki E., Sehr E.M., Fluch S., Trujillo-Moya C. 2017. *Association genetics of phenolic needle compounds in Norway spruce with variable susceptibility to needle bladder rust.* Plant Mol Biol, 94: 229-251. https://doi.org/10.1007/s11103-017-0589-5

Gruwez R., De Frenne P., De Schrijver A., Vangansbeke P., Verheyen K. 2017. *Climate warming and atmospheric deposition affect seed viability of common juniper (Juniperus communis) via their impact on the nutrient status of the plant.* Ecol Res, 32: 135-144. https://doi.org/10.1007/s11284-016422-3

Halloum B., Shakya A.K., Elagbar Z.A., Naik R.R. 2019. *Gc-ms analysis and biological activity of essential oil of fruits, needles and bark of pinus pinea grown wildly in jordan.* Acta Pol Pharm – Drug Research, 76: 825-831. https://doi.org/10.32383/appdr/109894

Jiratchayamaethasakul C., Ding Y., Hwang O., Im S.T., Jang Y., Myung S.W., Lee J.M., Kim H.S., Ko S.C., Lee S.H. 2020. *In vitro screening of elastase, collagenase, hyaluronidase, and tyrosinase inhibitory and antioxidant activities of 22 halophyte plant extracts for novel cosmeceuticals.* Fish Aquatic Sci, 23: 6. https://doi.org/10.1186/s41240-020-00149-8

Jyske T., Järvenpää E., Kunnas S., Sarjala T., Raitanen J.E., Mäki M., Pastell H., Korpinen R., Kaseva J., Tupasela T. 2020. *Sprouts and needles of norway spruce (Picea abies (*L.*)* Karst.*) As nordic specialty-consumer acceptance, stability of nutrients, and bioactivities during storage*. Molecules, 25: 4187. https://doi.org/10.3390/molecules25184187

Khouja M., Elaissi A., Ghazghazi H., Boussaid M., Khouja M.L., Khaldi A., Messaoud C. 2020. *Variation of essential oil composition, antioxidant and anticholinesterase activities between Pinus halepensis* Mill. *plant organs*. J Essent Oil-Bear Plants, 23: 1450-1462. https://doi.org/10.1080/0972060X.2020.1868348

Kumari P., Luqman S., Meena A. 2019. *Application of the combinatorial approaches of medicinal and aromatic plants with nanotechnology and its impacts on healthcare*. DARU J Pharm Sci, 27: 475-489. https://doi.org/10.1007/s40199-019-00271-6

Martinez-Gonzalez A.I., Díaz-Sánchez Á.G., de la Rosa L.A., Vargas-Requena C.L., Bustos-Jaimes I., Alvarez-Parrilla E. 2017. *Polyphenolic compounds and digestive enzymes: in vitro non-covalent interactions*. Molecules, 22: 669. https://doi.org/10.3390/molecules22040669

Medawar E., Huhn S., Villringer A., Veronica Witte A. 2019. *The effects of plant-based diets on the body and the brain: a systematic review*. Transl Psychiatry, 9: 1-17. https://doi.org/10.1038/s41398-019-0552-0

Mikhailova T.A., Afanasieva L.V., Kalugina O.V., Shergina O.V., Taranenko E.N. 2017. *Changes in nutrition and pigment complex in pine (Pinus sylvestris L.) needles under technogenic pollution in Irkutsk region, Russia*. J For Res, 22: 386-392. https://doi.org/10.1080/13416979.2017.1386020

Mohd Hazli U.H.A., Abdul-Aziz A., Mat-Junit S., Chee C.F., Kong K.W. 2019. *Solid-liquid extraction of bioactive compounds with antioxidant potential from Alternanthera sesillis (red) and identification of the polyphenols using UHPLC-QqQ-MS/MS*. Food Res Int, 115: 241-250. https://doi.org/10.1016/j.foodres.2018.08.094

Mroczek A. 2015. *Phytochemistry and bioactivity of triterpene saponins from Amaranthaceae family*. Phytochem Rev, 14: 577-605. https://doi.org/10.1007/s11101-015-9394-4

Namshir J., Shatar A., Khandaa O., Tserennadmid R., Shiretorova V.G., Nguyen M.C. 2020. *Antimicrobial, antioxidant and cytotoxic activity on human breast cancer cells of essential oil from Pinus sylvestris. var Mongolica needle*. Mong J Chem, 21: 19-26. https://doi.org/10.5564/mjc.v21i47.1428

O'Sullivan A.M., O'Callaghan Y.C., O'Connor T.P., O'Brien N.M. 2013. *Comparison of the antioxidant activity of commercial honeys, before and after in-vitro digestion*. Pol. J. Food Nutr Sci, 63: 167-171. https://doi.org/10.2478/v10222-012-0080-6

Pietrzykowski M., Woś B., Haus N. 2013. S*cots pine needles macronutrient (N, P, K, Ca, Mg, and S) supply at different reclaimed mine soil substrates – as an indicator of the stability of developed forest ecosystems*. Environ Monit Assess, 185: 7445-7457. https://doi.org/10.1007/s10661-013-3111-9

Piwowarski J.P., Kiss A.K., Kozłowska-Wojciechowska M. 2011. *Anti-hyaluronidase and anti-elastase activity screening of tannin-rich plant materials used in traditional Polish medicine for external treatment of diseases with inflammatory background*. Journal of Ethnopharmacol, 137: 937-941. https://doi.org/10.1016/j.jep.2011.05.039

Pongrac P., Baltrenaite E., Vavpetič P., Kelemen M., Kladnik A., Budič B., Vogel-Mikuš K., Regvar M., Baltrenas P., Pelicon P. 2019. *Tissue-specific element profiles in Scots pine (Pinus sylvestris* L.*) needles*. Trees, 33: 91-101. https://doi.org/10.1007/s00468-018-1761-5

Przeor M. 2022. *Some common medicinal plants with antidiabetic activity, known and available in europe (a mini-review)*. Pharmaceuticals, 15: 65. https://doi.org/10.3390/ph15010065

Qi Y., Duan C., Ren L., Wu H. 2020. *Growth dynamics of galls and chemical defence response of Pinus thunbergii Parl. to the pine needle gall midge, Thecodiplosis japonensis Uchida & Inouye (Diptera: Cecidomyiidae)*. Sci Rep, 10: 12289. https://doi.org/10.1038/s41598-020-69231-4.

Sahin Yaglioglu A., Eser F. 2017. *Screening of some Juniperus extracts for the phenolic compounds and their antiproliferative activities*. S Afr J Bot, 113: 29-33. https://doi.org/10.1016/j.sajb.2017.07.005.

Studzińska-Sroka E., Galanty A., Gościniak A., Wieczorek M., Kłaput M., Dudek-Makuch M., Cielecka-Piontek J. 2021. *Herbal infusions as a valuable functional food*. Nutrients, 13: 4051. https://doi.org/10.3390/nu13114051

Stuper-Szablewska K., Kurasiak-Popowska D., Nawracała J., Perkowski J. 2017. *Response of non-enzymatic antioxidative mechanisms to stress caused by infection with Fusarium fungi and chemical protection in different wheat genotypes*. Chem Ecol, 33: 949-962. https://doi.org/10.1080/02757540.2017.1381689

Süntar I., Tumen I., Ustün O., Keleş H., Küpeli Akkol E. 2012. *Appraisal on the wound healing and anti-inflammatory activities of the essential oils obtained from the cones and needles of Pinus species by in vivo and in vitro experimental models*. J Ethnopharmacol, 139: 533-540. https://doi.org/10.1016/j.jep.2011.11.045

Tang J., Dunshea F.R., Suleria H.A.R. 2020. *LC-ESI-QTOF/MS characterization of phenolic compounds from medicinal plants (hops and juniper berries) and their antioxidant activity*. Foods, 9: 7. https://doi.org/10.3390/foods901000

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 Chem, 19: 1052-1061. https://doi.org/10.1515/chem-2021-0088

Truong D.-H., Nguyen D.H., Ta N.T.A., Bui A.V., Do T.H., Nguyen H.C. 2019. E*valuation of the use of different solvents for phytochemical constituents, antioxidants, and in vitro anti -inflammatory activities of Severinia buxifolia*. J Food Qual, 2019: e8178294. https://doi.org/10.1155/2019/8178294

Türkan F., Atalar M.N., Aras A., Gülçin İ., Bursal E. 2020. *ICP-MS and HPLC analyses, enzyme inhibition and antioxidant potential of Achillea schischkinii* Sosn. Bioorg Chem, 94: 103333. https://doi.org/10.1016/j.bioorg.2019.103333

Wang R., Wang K., Si C.-L., Luo Y.-Y., Liu W., Zhang X., Tian Y., Wang J. 2018. *Triterpene Saponins from Branches of Pinus massoniana*. Chem Nat Comp, 54: 717-720. https://doi.org/10.1007/s10600-018-2453-5

Yang H., Wang Z., Song W., Zhao Z., Zhao Y. 2021. *Isolation of proanthocyanidins from Pinus thunbergii needles and tyrosinase inhibition activity*. Proc Biochem, 100: 245-251. https://doi.org/10.1016/j.procbio.2020.10.003

Yang L., Wen K.-S., Ruan X., Zhao Y.-X., Wei F., Wang Q. 2018. *Response of plant secondary metabolites to environmental factors*. Molecules, 23: 762. https://doi.org/10.3390/molecules 23040762

Zengin G., Locatelli M., Stefanucci A., Macedonio G., Novellino E., Mirzaie S., Dvorácskó S., Carradori S., Brunetti L., Orlando G., Menghini L., Ferrante C., Recinella L., Chiavaroli A., Leporini L., Mollica A. 2017. *Chemical characterization, antioxidant properties, anti-inflammatory activity, and enzyme inhibition of Ipomoea batatas* L. *leaf extracts*. Int J Food Prop, 20: 1907-1919. https://doi.org/10.1080/10942912.2017.1357127

Zillich O.V., Schweiggert-Weisz U., Eisner P., Kerscher M. 2015. *Polyphenols as active ingredients for cosmetic products*. Int J Cosmetic Sci, 37: 455-464. https://doi.org/10.1111/ics.12218.

Electronic Journal of Biotechnology 63 (2023) 10–17

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Research Article

Supplementation of beer with *Pinus sylvestris* L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles

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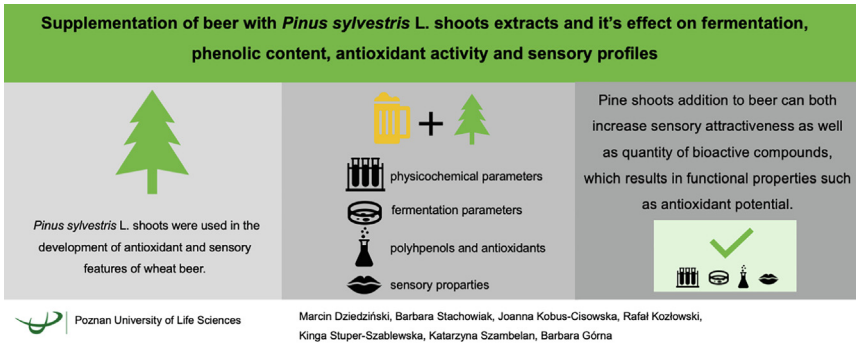
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 December 2022
Accepted 25 January 2023
Available online 29 March 2023

Keywords:
Alcohol
Antioxidant activity
Beer
Bitterness
Craft beer
Fermentation
Phenolic content
Pinus sylvestris
Sensory profiles
Shoots extracts
Wheat beer

ABSTRACT

Background: The aim of this study was to determine the role of *Pinus sylvestris* L. shoots in the development of antioxidant and sensory features of wheat beer.
Results: After storage, the alcohol content of the experimental beer was 4.04%v/v, and its bitterness was 15.83 IBU (bitterness units). Higher levels of bitterness were found compared to the control beer. Other analyzed fermentation parameters (extract, degree of fermentation) and physicochemical parameters (pH, titratable acidity, color) were similar for both types of beer. The addition of pine shoots at the brewing stage affected the profile of biologically active compounds - both polyphenolic acids and flavonols. The content of both groups of those compounds was almost 30% higher in the sample with pine shoots compared to the control sample. The sensory evaluation confirmed the high attractiveness of the beer with pine shoots. During the three-month storage period, the tested samples were microbiologically stable.bd268
Conclusions: It was concluded that pine shoots may be an attractive functional addition to flavored craft beer. It can increase both sensory attractiveness and quantity of bioactive compounds, which results in functional properties such as antioxidant potential.

Peer review under responsibility of Pontificia Universidad Católica de Valparaíso
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https://doi.org/10.1016/j.ejbt.2023.01.001

How to cite: Dziedziński M, Stachowiak B, Kobus-Cisowska J, et al. Supplementation of beer with *Pinus sylvestris* L. shoots extracts and its effect on fermentation, phenolic content, antioxidant activity and sensory profiles. Electron J Biotechnol 2023;63. https://doi.org/10.1016/j.ejbt.2023.01.001.

1. Introduction

Beer is one of the oldest and most popular alcoholic beverages in the world. In 2020, the global consumption of that beverage was 177.5 million kiloliters. The leader in beer consumption is China (360 million hectoliters), followed by the USA (241 million hectoliters), Brazil (138 million hectoliters), Russia (86 million hectoliters) and Germany (77 million hectoliters) [1]. In 2021, in the EU, the production of alcoholic beer amounted to 33.1 billion liters and of beer that contained less than 0.5% alcohol reached 1.7 billion liters [2]. The traditional raw materials for beer production are water, malt and hops, from which a hopped wort is prepared, which is then fermented with bottom- or top-fermenting yeast in the next stage. The addition of unconventional raw materials shapes the sensory qualities of beer, first of all, but it can also affect the content of bioactive compounds with functional effects, including antioxidants [3]. The source of such raw materials can be the forest environment, including pine species, individual elements of which, such as bark, cones, shoots, have been and are used in traditional medicine for various ailments, most often in the treatment of respiratory and dermatological diseases. The preparations obtained from them (resin, extracts, ointments, lotions, oils) show antimicrobial, antioxidant, anti-inflammatory and cytoprotective properties. They can also be used in the treatment of neurodegenerative disorders such as Alzheimer's or Parkinson's diseases, as well as in the treatment of wounds [4]. Pycnogenol, an extract from the bark of the French maritime pine, is the most powerful antioxidant known to modern medicine. It contains polyphenolic compounds (mainly procyanidins, organic acids and bioflavonoids). The quality of this extract is determined by the United States Pharmacopeia (USP 28) [5]. In turn, the extract from *Pinus sylvestris* aetheroleum is officially listed in the European Pharmacopoeia [6]. It has antibacterial, expectorant and analgesic properties and is used as an antiseptic for respiratory tract, urinary tract and kidney infections. It facilitates the dissolution of kidney stones [6]. The shoots of the Scots pine (*Pinus sylvestris* L.) are also a rich source of bioactive compounds. Among those, the most important are essential oil (0.4%) phenolic compounds (flavonoids, tannins, phenolic acids and their derivatives), vitamin C (lithium). Young shoots of *Pinus sylvestris* are used to treat respiratory diseases (e.g. asthma, cough and tracheitis). Pine shoot extract has been used as a folk remedy in the treatment of chronic inflammation, circulatory disorders and asthma, and now, it is an ingredient of pharmaceutical supplements [4].

Phenolic compounds are well-known antioxidants capable of reducing oxidative stress, which is the direct cause of most civilization diseases, such as cardiovascular diseases, cancer, and neurodegenerative diseases (Parkinson, Alzheimer). There are more than 50 components in pine shoot essential oils. Their concentration depends on the species, growing conditions, morphological part of the plant. The following components of essential oils are added in the largest amount: α-pinene, β-pinene, β-phellandrene, β-caryophyllene, camphene, α-terpineol, germacrene D, bornyl acetate, citronellol. They can determine the sensory properties of the final products [7]. Additionally, they are characterized by antiviral and antimicrobial properties. Pinens have the GRAS status, i.e. they can be used as food ingredients [8].

Beer that contains coniferous tree extracts, mainly from pine shoots, appear on the craft beer market; however, the impact of the extracts on the antioxidant activity of beer, polyphenol content and sensory properties of that beverage has not been analyzed so far. The aim of this study was to determine the role of *Pinus sylvestris* L. shoots in the development of antioxidant and sensory properties of wheat beer. To evaluate the effect on the addition of pine shoots on the characteristics of the beer, a test beer and a control beer were produced under laboratory conditions and stored for one month. Basic physicochemical properties, concentration of polyphenols and their profile, the microbiological condition of beer and its sensory quality were analyzed.

2. Materials and methods

2.1. Material

The test material was pine shoots (*Pinus sylvestris* L). Shoots collected in 2021 from the arboretum in Zielonka (Poland, 17°06′33″E, 52°06′33″N), a part of the Forest Experimental Department of Poznan University of Life Sciences. The material was air-dried at 20°C with 55% humidity and stored before usage. A ready-made brew-kit BA Hefeweizen (Browamator, Poland) was used to prepare the beer. The kit included a blend of ground malts in the following proportions: pale wheat Weyermann® – 58%, pilsner Weyermann® – 37%, carmel Carahell® – 5%; granulated aromatic hop Relax (Germany) – 30 g; dried top-fermenting *Saccharomyces cerevisiae* yeast (Safbrew™ WB-06) – 11.5 g.

2.2. Methods

2.2.1. Laboratory beer production

In the first stage, the infusion mashing with stirring was carried out under laboratory conditions. For that process, 15 l of top water and 4.3 kg of blend of malts (a weight ratio: 3.5:1) were used at the beginning. The temperature of mash was adjusted to 45°C and maintained for 10 min. Then, the temperature of mash was raised as follows: to 53°C for 15 min (for β-glucan denaturation); to 63°C for 30 min (for protein denaturation) and to 72°C up to the negative iodine test (for starch denaturation). Then, the mash-out temperature was raised to 76°C for 10 min (for enzyme denaturation). The ready mash was transferred to a plastic filter tank. The mash was left for about 30 min to create a filter bed from the malt spent grains of mainly the husk fraction. After this time, the proper filtration stage took place. After separating the first wort, the grains were washed with water at the temperature of 75°C until 20 l of liquid was obtained. The obtained wort was boiled for 80 min. Hops (15 g/l) were added after 15 min of boiling, and then, pine shoots (15 g/L) were added after 30 min. The control sample was prepared with the addition of hops, in two portions of 15 g, which were added in the same way as in the case of the pine shoot sample. The boiled wort was cooled down to the temperature of 25°C. The content of the extract was measured with the use of the Balling hydrometer by cooling down the wort to the temperature of 20°C.

Yeast was added to the cooled wort. Fermentation was carried out in a closed 30 l plastic fermentation vessel, at the temperature of 20°C for 10 d in a thermostat with a cooling system (ST700,

POL-EKO Aparatura, Poland). Next, the beer was poured into 500 ml glass bottles and kept refrigerated (4°C) for one month. They were analyzed at 3 stages of production - as wort (W), as beer after fermentation (FB), and beer after one month of storage (B1).

Explanations of sample acronyms used in the manuscript are provided below.

CW – control wort
EW – experimental wort, wort with pine shoots addition
CB – control beer after main fermentation
EB – experimental beer, beer with pine shoots after main fermentation
CB1 – control beer after one month of storage
EB1 - experimental beer, beer with pine shoots after one month of storage

2.2.2. Basic physico-chemical parameters

For the physico-chemical analysis, the samples of beer were degassed by manual shaking (5 min), filtrated through a layer of cotton wool and centrifuged (2000 × g for 15 min, 20°C).

The alcohol concentration by volume was determined after distillation (Super Dee Digital Distillator Gibertini, Italy) using automatic densitometer (DDM-2910, Rudolph Research Analytical, USA) by mechanical oscillator method. The extract content/the density in the samples was measured with the use of the Balling hydrometer at 20°C. The pH was determined using a pH-meter (Elmetron CP-411, Poland). To determine the titratable acidity, 25 ml of each sample was titrated with 0.1 N NaOH solution from the initial pH to 7.0. Total acidity was expressed in units of ml 1 M NaOH /100 ml beer. The color of beer was determined with the use of a spectrophotometer (Halo SB-10, Dynamica Scientific Ltd) at 430 nm wavelength.

The beer bitterness analysis was performed according to Analytica-EBC (2010) recommendation. An amount of 10 ml degassed beer was transferred to Falcon tubes (50 ml), and then, 0.5 ml of a hydrochloric acid solution (6 N HCl) and 20 ml of isoacetate were added. The tubes were shaken for 5 min. Next, 10 ml of the sample was placed into 15 ml Falcon tubes and centrifuged (3000 rpm, 5 min). For analysis of beer bitterness, the absorbance A275 of the isooctane layer was measured in quartz cuvettes at a wavelength of 275 nm (spectrophotometer Halo SB-10, Dynamica Scientific Ltd.) against pure isooctane. The value of bitterness is expressed in units of bitterness (IBU).

2.2.3. Microbial analysis

The pour plate method was used to determine the total count of microorganisms (nutrient agar – NA, BTL, Łódź, Poland; 30°C, 48 h), the total count of lactic acid bacteria (LAB) (de Man, Rogosa and Sharpe agar – MRS, Oxoid) under anaerobic conditions (30°C, 72 h), the total count of yeast (Yeast Extract Glucose Chloramphenicol – YGC Agar, BTL, Łódź, Poland; 25°C, 72 h).

2.2.4. Polyphenol content

Phenolic compounds in samples were analyzed after alkaline and acidic hydrolysis [9]. 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 (100 mm × 2.1 m m, particle size 1.7 μm) (Waters, Ireland). The elution was carried out 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 λ = 320 nm and 280 nm. The compounds were identified based on a comparison of the retention time of the analyzed peak with the retention time of the standard and by adding a specific amount of the standard to the analyzed samples and a repeated analysis. The detection level is 1 μg/g.

2.2.5. DPPH assay

The extract's antiradical scavenging potential against DPPH radicals was analyzed. To that end, a methanolic solution of DPPH was used to evaluate the free-radical scavenging potential of the samples [10]. The degree of the solution's discoloration indicated the scavenging efficacy of the added substance. For this analysis, 1 ml of the tested solution was supplemented with 2 ml of pure methanol (Honeywell, United Kingdom), followed by 0.25 ml of 1 mM DPPH• ethanolic solution. The mixture was vortexed for ∼60 s and left for 20 min at room temperature. Absorbance was recorded at λ = 517 nm (Meterek SP 830, Taiwan). Methanol was used to prepare a reference sample and the control. To plot a calibration curve, the absorbance values were measured simultaneously for samples containing respective concentrations of Trolox (Sigma-Aldrich, Germany) as a standard (0.5, 1.0, 1.5, and 2.0 mg/ml; r2 = 0.9639). The results are expressed as % of inhibition.

2.2.6. Sensory evaluation

The sensory evaluation of beer and beer supplemented with shoots of *Pinus sylvestris* L. was carried out at sensory laboratory of Poznan University of Life Sciences. It was performed by a panel of 20 assessors (14 women and 6 men), at the age from 21 to 55, all of them were university staff members or students trained in performing sensory analysis of various alcoholic beverages (including beer). During preliminary sessions, the panelists generated 10 taste descriptors (sweet, sour, bitter, tart, fruity, yeasty, pine, malty honey, hop) and 8 aroma descriptors (citrus, malty, hoppy, yeasty, pine, caramel, foreign, fruity). The panelists were seated in separate purpose-made booths, and the environment was free of interference in terms of noise, visual stimulation and ambient odor. The samples were evaluated in duplicate and were placed in random order into standard tasting glasses filled with 50 ml of beer, and marked with a three-digit code. The beer samples were served at 12°C under white light. The panelists used an unstructured scale with boundary markings to rate the intensity of each attribute (0 = very weak, 10 = very intense), and the mean scores of attributes were submitted to quantitative descriptive analysis in order to generate the sensory profile of the two types of beer.

2.2.7. Statistical analysis

All measurements were performed using three samples (different bottles). All data were expressed as a mean ± standard deviation and subjected to one-way analysis of variance (ANOVA) using the RStudio software version 1.4 (RStudio, PBC, Delaware, USA). Statistical differences were measured at $P < 0.05$.

3. Results

3.1. Physico-chemical and microbiological parameters of beer

The results of physicochemical and microbiological tests of the wort and the produced beer are presented in Table 1. The determined base wort extract of the experimental beer (with pine shoots) and the control beer was 12% and 11.5%v/v, respectively. As a result of the wort fermentation, the actual amount of the extract in the beer decreased and was: 5.20% v/v for CB and 5.00% for SB. During storage, further, but small, consumption of the extract took place and, after a month, the parameter reached the value of 4.30% v/v for both types of beer. The content of ethanol in beer with pine shoots was higher compared to the control sample, at all controlled production stages. After one month of storage,

Table 1
Physico-chemical and microbial parameters of the prepared worts (CW, SW) and beer – young beer after main fermentation (CB, SB) and beer after a month of storage in a fridge (CB1, SB1).

Analyzed sample	Ethanol	Extract		Degree of fermentation		Acidity		Bitterness	Color	Yeast count
	% v/v	real %w/w	apparent %w/w	real %	apparent %	active pH	titratable 1 M NaOH/ 100 ml	IBU	EBC	log cfu/ml
CW	nd	11.50	nd	nd	nd	5.44 ± 0.01^{a}	0.42	17.95 ± 0.21^{a}	17.01 ± 0.19a^{bcd}	7.39
SW	nd	12.00	nd	nd	nd	5.17 ± 0.00^{b}	0.54	16.70 ± 0.43^{f}	15.00 ± 0.29^{abcd}	7.39
CB	4.27 ± 0.06^{a}	5.20 ± 0.08^{a}	4.10 ± 0.1^{bc}	54.78	64.35	4.31 ± 0.00^{c}	2.70 ± 0.02^{a}	15.39 ± 0.07^{b}	16.94 ± 0.42^{abc}	6.95
SB	4.90 ± 0.07^{de}	5.00 ± 0.11^{de}	3.70 ± 0.13 ^{fg}	58.33	69.17	4.31 ± 0.01^{d}	2.46 ± 0.03^{f}	16.39 ± 0.61 ^{g}	17.36 ± 0.09^{a}	6.74
CB1	4.81 ± 0.02^{ab}	4.30 ± 0.02^{ab}	3.10 ± 0.03 ^{cd}	62.61	73.06	4.06 ± 0.05^{e}	3.04 ± 0.01^{b}	10.37 ± 0.69^{c}	10.47 ± 0.13^{ab}	7.00
SB1	5.36 ± 0.03^{ef}	4.30 ± 0.05^{ef}	2.50 ± 0.06^{gh}	64.17	79.17	4.04 ± 0.00^{f}	3.25 ± 0.01 ^{g}	15.83 ± 0.74 ^{h}	9.77 ± 0.39^{abc}	7.30

Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (*P* value < 0.05).
* - original extract; nd – no data was collected at this point.

its concentration in the experimental beer was 5.36% v/v, while in the control beer - it was 4.81% v/v. Also, the actual attenuation, determined after fermentation and after one month of storage, was higher for the beer with the shoots and was 58.33% for SB and 64.17% for SB1. For the control sample, the extract was 54.78% for CB and 62.61% for CB1. The pH of the control wort was 5.44 and was higher than that of the wort that included pine shoots – 5.04. During primary fermentation and storage, the pH of both types of beer decreased, reaching approximately 4.0. The total acidity of the control wort was 0.41 and that of the wort with the shoots was 0.55. During fermentation and storage, an increase in the total acidity of both types of beer was noticed.

The bitterness in the control wort was 17.95 IBU, and it was higher than the bitterness in the wort with the pine shoots. Bitterness in the tested types of beer decreased during fermentation and storage; in the case of the control beer, it reached the final value of 10.37 IBU, while in the case of the experimental beer - it reached 15.83 IBU.

The color of the wort with pine shoots was lighter - at 15 EBC, while that of the control wort was 17 EBC. No changes in the wort color were observed after the turbulent fermentation process. In turn, after a month of storage, the color of both types of beer was definitely lighter. Once yeast was added to the wort, the yeast count was 7.39 log/ml. After fermentation, the count decreased in both types of beer, in the CB beer to 6.95 log/ml and in the SB to 6.74 log/ml. A significant increase in yeast count was found in the beer with pine shoots after one month of storage. In both types of tested beer, mesophilic bacteria and lactic acid bacteria were not found at any stage of the production process.

3.2. Polyphenols and antioxidant activity

The polyphenol content and their profile are included in Table 2. It was found that ferulic acid, caffeic acid, cinnamic acid and 4-hydroxybenzoic acid predominated among the phenolic acids found in the analyzed wort and beer samples. Naringenin was dominated among the flavonols. In the control wort, the dominant polyphenols were ferulic acid and naringenin. In contrast, the highest content of ferulic acid was noticed in beer with pine shoots. In the entire production process, the lowest level of the tested polyphenols was found for luteolin and kaempferol.

Table 2
Polyphenol content in the tested beer.

	Samples					
Compounds	CW	CB	CB1	SW	SB	SB1
Gallic acid	1.89 ± 0.04^{a}	1.83 ± 0.07^{b}	1.73 ± 0.04^{c}	11.53 ± 0.25^{bc}	11.63 ± 0.11^{bc}	12.34 ± 0.08^{ab}
2.5-Dihydroxybenzoic acid	0.67 ± 0.02^{ab}	0.89 ± 0.04^{ab}	1.31 ± 0.02^{a}	0.68 ± 0.05^{bc}	0.80 ± 0.1^{ce}	1.20 ± 0.1^{be}
4-hydroxybenzoic acid	0.23 ± 0.02^{a}	0.43 ± 0.02^{b}	1.60 ± 0.02^{ab}	50.13 ± 0.31^{ab}	57.25 ± 1.03^{ab}	59.27 ± 1.04^{ab}
Protocatechuic acid	1.30 ± 0.2^{a}	1.65 ± 0.06^{a}	1.50 ± 0.1^{c}	2.17 ± 0.12^{ac}	2.49 ± 0.2^{ac}	2.50 ± 0.04^{ac}
Caffeic acid	0.35 ± 0.03^{a}	0.45 ± 0.04^{b}	0.82 ± 0.03^{a}	50.37 ± 0.25^{ab}	81.63 ± 1.4^{ab}	85.60 ± 0.44^{ab}
Syringic acid	0.66 ± 0.02^{a}	0.75 ± 0.03^{b}	1.79 ± 0.06^{a}	6.30 ± 0.2^{ab}	7.72 ± 0.2^{ab}	10.30 ± 0.12^{ab}
P-coumaric acid	0.28 ± 0.01^{a}	0.38 ± 0.03^{ab}	0.36 ± 0.03^{bc}	18.37 ± 0.25^{ac}	20.67 ± 0.21^{ac}	26.37 ± 0.19^{ac}
Ferulic acid	56.67 ± 0.78^{a}	58.60 ± 0.7^{b}	48.37 ± 1.2^{bc}	195.47 ± 2.36^{abc}	107.41 ± 2.01^{ac}	139.33 ± 0.71^{abc}
Chlorogenic acid	3.50 ± 0.1^{a}	4.10 ± 0.1^{b}	4.37 ± 0.09^{ac}	52.67 ± 0.87^{ab}	26.45 ± 0.57^{bc}	29.10 ± 0.6^{ab}
Sinapinic acid	2.80 ± 0.1^{a}	1.74 ± 1.15^{b}	2.17 ± 0.04^{a}	4.60 ± 0.1^{ab}	3.37 ± 0.31^{b}	3.63 ± 0.25^{ab}
Cinnamic acid	10.60 ± 0.26^{a}	11.43 ± 0.32^{b}	13.57 ± 0.11^{ab}	35.73 ± 0.5^{ab}	6.14 ± 0.15^{ab}	7.37 ± 0.21^{bf}
Vanillic acid	2.20 ± 0.1^{a}	4.88 ± 0.09^{ab}	4.40 ± 0.16^{c}	1.57 ± 0.15^{bc}	6.41 ± 0.28^{ac}	6.80 ± 0.1^{ac}
Salicylic acid	0.13 ± 0.06^{a}	0.21 ± 0.04^{b}	0.13 ± 0.06^{c}	0.44 ± 0.04	0.88 ± 0.07^{abcd}	0.90 ± 0.05^{abcd}
Total phenolic acids	**81.28 ± 17.43**	**87.34 ± 17.96**	**82.12 ± 14.81**	**430.03 ± 59.33**	**332.85 ± 37.35**	**384.71 ± 45.68**
Naringenin	68.57 ± 0.83^{a}	65.00 ± 0.79^{b}	53.33 ± 0.93^{ac}	105.20 ± 1.35^{bc}	107.13 ± 0.57^{ab}	107.47 ± 0.74^{ab}
Vitexin	0.44 ± 0.02^{a}	0.50 ± 0.03^{b}	0.50 ± 0.05 ^{cd}	0.62 ± 0.04^{abc}	0.62 ± 0.03^{abc}	0.79 ± 0.07^{abc}
Rutin	2.70 ± 0.1^{a}	2.20 ± 0.09^{a}	2.41 ± 0.04^{b}	2.16 ± 0.03^{aef}	3.50 ± 0.1^{bde}	3.50 ± 0.21^{bdf}
Quercetin	1.57 ± 0.15^{a}	1.83 ± 0.09^{b}	1.13 ± 0.03^{bc}	3.60 ± 0.2^{abc}	5.30 ± 0.2^{abc}	5.36 ± 0.14^{abc}
Apigenin	0.10 ± 0^{a}	0.30 ± 0.03^{ab}	0.13 ± 0.04^{bc}	0.17 ± 0.06^{f}	0.28 ± 0.07^{ac}	0.36 ± 0.06^{acdf}
Kaempferol	0	0	0	0	0	0.02 ± 0.01^{a}
Luteolin	0	0	0	0	0.11 ± 0.02^{a}	0.17 ± 0.04^{b}
Catechine	1.11 ± 0.04^{a}	1.44 ± 0.04^{b}	0.86 ± 0.04^{bc}	1.20 ± 0.1 ^{cd}	1.25 ± 0.14 ^{cd}	2.12 ± 0.03^{acd}
Total flavonols	**74.49 ± 23.96**	**71.27 ± 22.68**	**58.36 ± 18.62**	**112.95 ± 36.82**	**118.19 ± 37.37**	**119.79 ± 37.42**

Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (*P* value < 0.05).

Antioxidant activity was determined by testing the ability to quench DPPH radicals (Fig. 1). It was found that the antiradical activity of the worts was 53.16% for the CW and 58.60% for the SW wort. It was shown that the anti-radical activity of beer was 53.16% in the case of the CW test and 64.74% for the SB1 sample. In all storage periods, samples containing the active components of pine shoots had a higher capacity but statistical analysis of the results showed that the differences were not statistically significant.

3.3. Sensory evaluation

The sensory profile was visualized in the form of a flavor and aroma profile (Fig. 2). For the control sample, the taste profile was characterized as intensely sweet and malty. The bitter and fruity tastes were at similar levels, while the sour and astringent tastes were of very low intensity. In the case of the sample with pine shoots, the profile was characterized as more complex, where

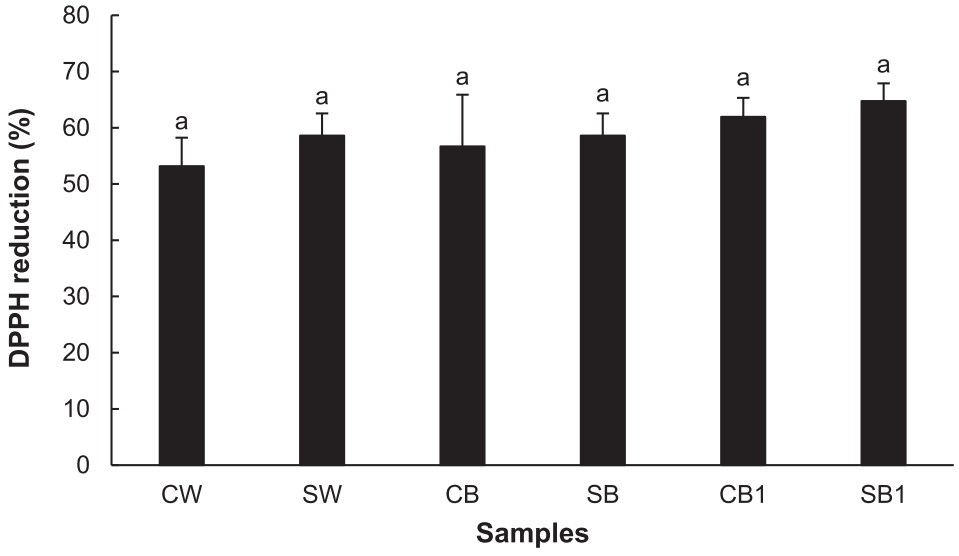

Fig. 1. DPPH scavenging effectiveness of wort and beer samples. Values are expressed as the mean (n = 3) ± standard deviation. Mean values with different the same letter (a) are not statistically different (*P* value < 0.05).

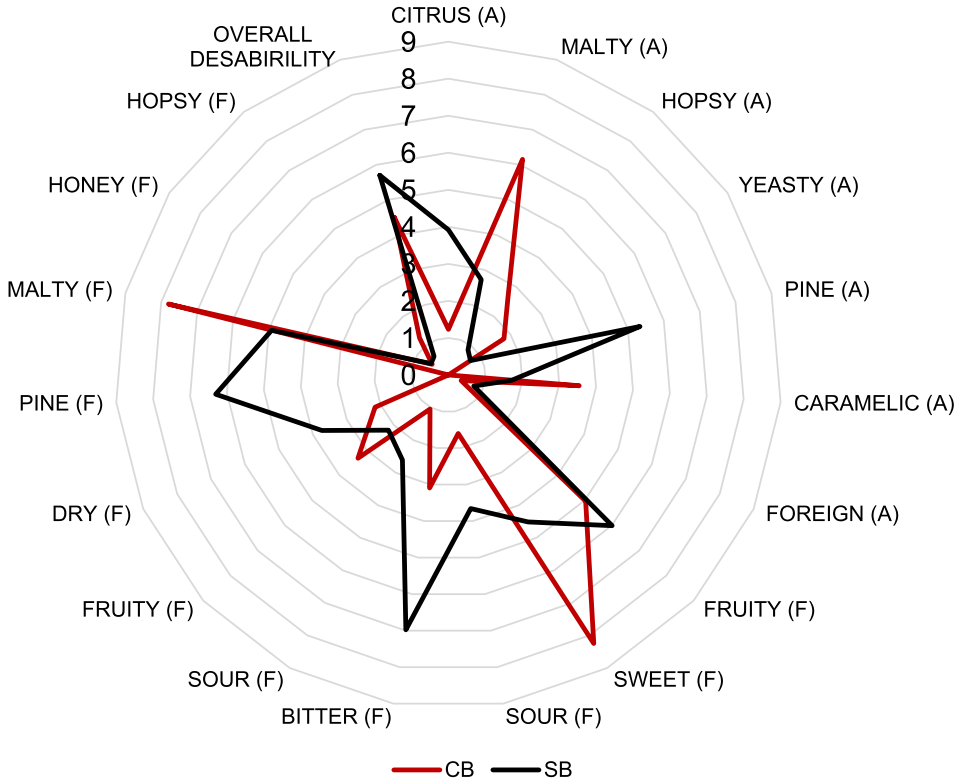

Fig. 2. Sensory profile of fresh beer samples. CB: control young beer; SB: young beer supplemented with *P. sylvestris* shoots; (F): flavour descriptor; (A): aroma descriptor.

bitter and pine flavors were also found. The level of the malt and sour tastes was determined as low.

The profile assessment of the aroma showed significant differences between the samples of the tested beer. The profile of the control sample was characterized as malty and fruity. Caramel and hop aromas were also noticeable. The level of intensity of citrus and yeasty odor descriptors was assessed as low. The experimental beer sample had an intense pine and fruit aroma. The level of intensity of citrus and malt flavor was assessed as moderate, while that of hop and yeast flavor was assessed as very low. No foreign smell was noticed in both beer samples. With regard to the assessment of overall desirability, the pine shoot sample got a higher score: 5.7, while the control sample received an average score of 4.5. However, based on statistical analysis, there were no statistically significant differences.

PCA was used to identify aroma and flavor descriptors best discriminating the two produced types of beer. The scores for each beer descriptor for the two components are presented in Fig. 3 representing the bi-plot, which globally explained 100% of the total variance. The first principal component (PC1) explained the variation across samples. Moreover, looking at the bi-plots, the differentiation of sensory profiles across samples can be noticed. There are also evident sensory variables that characterize the beer produced with the use of pine shoots, suggesting a greater complexity of the flavor profile and smaller complexity of the aroma profile, which was dominated by the pine descriptor. In the latter, a correlation could be assumed between yeasty, pine, tart and bitter flavor descriptors and pine, citrus aroma descriptors.

4. Discussion

Pine shoots are a raw material that is relatively rarely used in food production at the moment. There are attempts to use them as a food ingredient or as a raw material for the production of tinctures, as well as an additive to beer [11]. As part of the study, beer similar to the Hefe-Weizen type of that beverage, which comes from Bavaria, was produced, to which Scots pine shoots were added at the wort brewing stage. The purpose of the study was to assess the possibility to demonstrate whether the compounds in the shoots would enable the fermentation process and whether beer with new sensory properties could be obtained.

Unconventional raw materials and additives can affect not only sensory qualities or functional properties but they can also change the basic physicochemical parameters of beer or determine the course of the fermentation process. Reports on the antioxidant and antimicrobial properties of pine shoots may be important con-

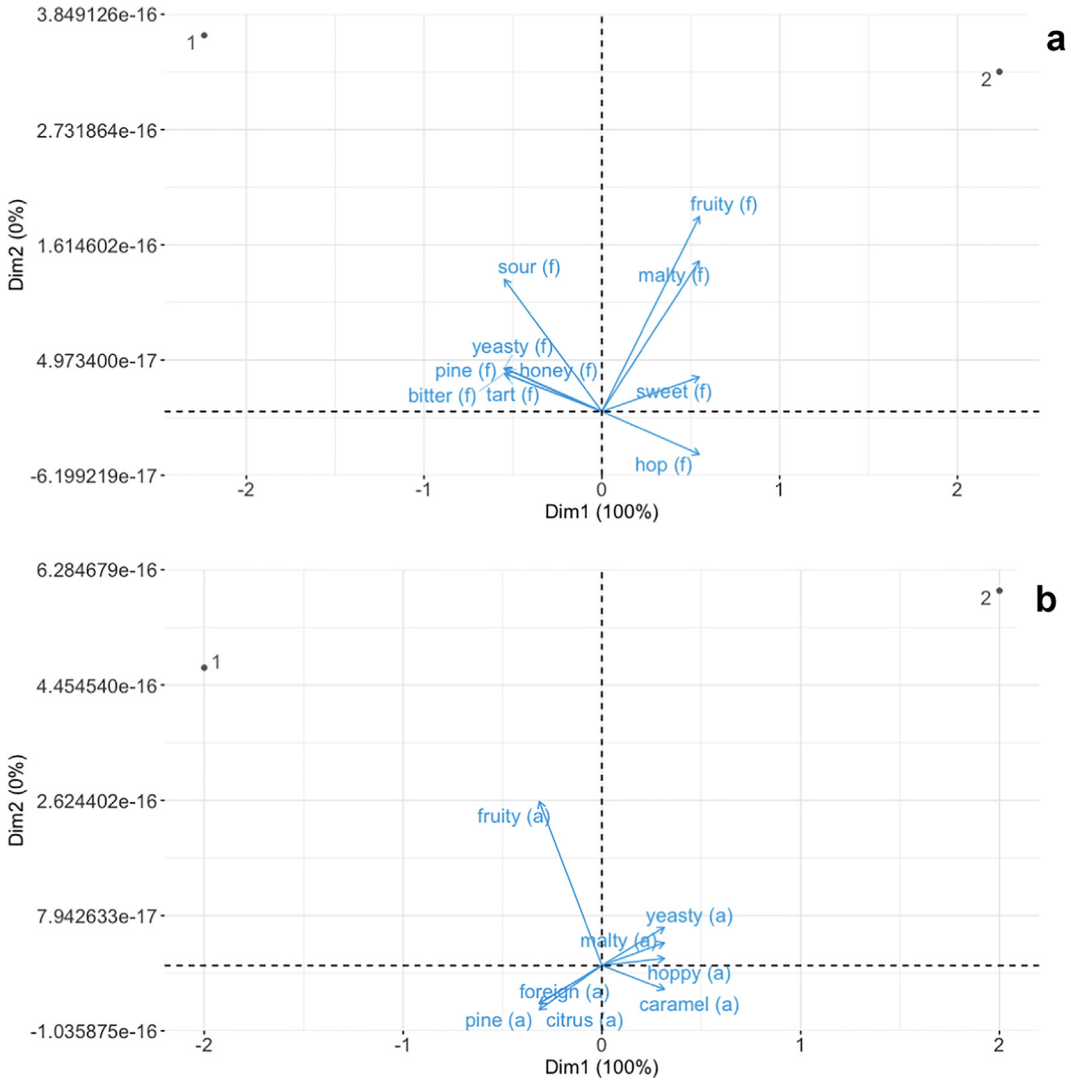

Fig. 3. Principal component analysis bi-plot of flavor (a) and aroma (b) descriptors of the control beer (1) and beer supplemented with pine shoots (2). PC1 vs PC2 accounts for 100% of the explained variation.

sidering the beer production process, during which high yeast activity is necessary [12]. Due to the potential contamination in the beer production process, microbiological quality control at every stage is an important task. The amount of brewing yeast and the total number of mesophilic microorganisms or lactic acid bacteria (LAB) is analyzed, due to their similar nutritional and environmental requirements. Their growth can cause the most undesirable consequences, such as delaying or disrupting the wort fermentation process or introducing undesirable sensory changes in beer. LAB spoil beer through acidification, haze formation, and/or diacetyl production, which gives the beer an intense aroma of artificial butter. Many strains can also produce exopolysaccharides (EPSs) in beer [13]. The presence of undesirable microorganisms, i.e. lactic acid bacteria and mesophilic bacteria, was not confirmed in the beer produced as part of the study. The only microorganisms present in the beer were yeasts. A well-known fact is that failure to adjust the inoculum to the conditions of the fermentation process prolongs the process of cell adaptation and delays proliferation and the fermentation process. Subsequently, the alcohol present in beer, as well as other changes to the parameters of the fermentation medium (e.g. lowering the pH, loss of substrates in the wort) slow down yeast metabolism and contribute to the deactivation of the weakest cells [14]. In our experiment, the largest amount of yeast in the beer wort was noticed before alcoholic fermentation started and after its completion, the number decreased in both types of beer due to the above-described regularities.

Adding pine shoots during brewing made it possible to obtain a slightly richer extract of the basic wort compared to the control wort, a higher alcohol content and actual attenuation rate in the beer after the fermentation process and after a storage period of one month. Pine shoots contain a number of soluble components that are found in the extract. Among them, there are approximately 5.15 g/100 g of soluble sugars, including glucose, fructose and sucrose. The metabolic processes of yeast are related not only to the production of ethanol but also to the production of organic acids, as a result of which the pH of beer changes (compared to the pH of the wort) [14]. Such a correlation can also be noticed in the research conducted for this thesis - the pH of the wort reached a higher value than that of the beer. Usually, the pH of beer wort varies between 5.3 and 5.5 [15]. It should also be noted that a lower value was achieved by the wort with pine shoots, due to the effect of the shoots on the pH values. In turn, the pH of wheat beer after fermentation usually reaches a value of approximately 4.3, which is also comparable to the results obtained in this study as it oscillated around 4 and 4.3 pH [16]. Both the control and test samples showed similar values of total acidity from the beginning of the performance of the tests - immediately after fermentation - until the end of the fermentation process and the completion of the tests on the samples after storage. Changes in total acidity during fermentation should be considered normal as the processes involving yeast cause an increase in total acidity, in contrast to pH - as described earlier in the case of changes in pH [17].

Beer, in studies on bitterness in beer and standards, is not classified based on that value [18]. However, in the case of light beer, the IBU level of less than 40 is considered as standard, therefore both samples - the control sample and the sample with pine shoots - should be considered valid and as meeting the standards for the level of bitterness in beer [18]. The observed discrepancy in bitterness values between wort and fresh beer may be due to the concentration of polyphenols and may be the result of the processes occurring during fermentation. The study conducted by Lazzari et al. showed a negative correlation between total flavonoid content and IBU [19]. In contrast, Kishimoto et al. noticed a decrease in IBU values during fermentation as a result of the disappearance of AA alpha-acids, e.g, as the pH decreases during fermentation, the hydrophobic components become insoluble in beer and interact with the cell walls of yeast [20].

The polyphenols and terpenes in the plant material are responsible for the typical bitter aftertaste. Therefore, their addition to food can significantly affect the sensory qualities and also the nutritional and health-promoting value [21]. With regard to the phenolic acids, chlorogenic and caffeic acids are responsible for the tangy and bitter taste [22]. In studies of polyphenol content in beer, the concentration of that compound ranged between 40 and 600 mg/l, depending on the adopted methodology and test material [23]. When comparing the values from the aforementioned study to the results obtained for the control sample in this research, it should be noted that the results are similar, as the results range from 125 to 160 mg/l, depending on the beer storage period and the fermentation process. The test sample that contained pine shoots had a significantly higher polyphenol content, of 450–600 mg/l.

The characteristics that determine the sensory quality of beer include the following: aroma, flavor, palatability, saturation, bitterness, clarity, color and the amount of the frothy foam. The most important feature is the palatability of beer, which depends on the amount of perceptible positive and negative features in taste and aroma [24]. Due to the complexity of the human sense of taste, it is difficult to determine at what level the above-mentioned quality characteristics should be present for the final effect - the high consumer acceptance - to be the best possible. The taste of beer is influenced by many factors, including the quality of malt, the strain and quality of yeast, the conditions under which the individual technological processes are performed, and the storage conditions of the finished product [25]. Factors that negatively affect the quality are as follows: too high or too low fermentation temperature, osmotic pressure, inadequate oxygenation of the wort, deficiency of nutrients in the wort, inappropriate pH, toxic agents (e.g. too high concentration of ethanol due to the inappropriate adaptation of the yeast strain to the style or disinfectants being the residue after disinfection of technical installations), and the water content in freeze-dried yeast [26]. The study showed that the control sample was a higher clarity beer and more brown compared to the sample with pine shoots, which was most likely due to the phenolic compounds in the shoots. Studies have shown that the concentration of those compounds and their transformation in beer can significantly affect its color [27]. The observed changes of color and a lighter color after storage may result from the instability of color compounds that show relative instability and are susceptible to several factors such as storage temperature, pH, oxygen, light, chemical structure, concentration and the presence of enzymes, proteins and metallic ions, as reported in studies [27]. Both samples were free of foreign flavor and smell, which is crucial in assessing the quality of beer [18]. The current development of the craft beer market indicates that consumers are looking for new flavors and aromas of beer more and more often [28]. Based on consumer preference studies, assessment of beer characteristics varies depending on whether or not the consumers have previously tried craft beer; generally, that type of beer is perceived as better quality than commercial beer due to the type of raw materials used in brewing [29].

5. Conclusions

The active compounds in pine shoots can be used as ingredients of functional beer, as they affect the composition of polyphenols and flavor. The research has confirmed that replacing half of the amount of hops indicated in the recipe with pine shoots enables fermentation and obtaining beer of good quality. Beer with pine shoots has a slightly acidic character, tested both on the pH and

total acidity scale. It is a relatively low-bitterness beer. A broad spectrum of biologically active compounds was present in the produced beer. It was shown that replacing hops with pine shoots did not reduce antioxidant properties. At the same time, it was found that the new beer was characterized by a high content of antioxidant compounds - polyphenols, among which ferulic acid, caffeic acid and naringenin predominated. The content of those compounds was statistically significantly higher in beer with pine shoots. Adding Scots pine shoots (*Pinus sylvestris* L.) at the brewing stage of wheat beer changed the sensory characteristics and did not impair the microbiological quality during storage, nor did it reduce physico-chemical quality parameters compared to the control sample.

Ethical approval

All participants in this study gave informed consent to Poznań University of Life Sciences.

Author contributions

- Study conception and design: M Dziedziński, J Kobus-Cisowska, B Stachowiak.
- Data collection: M Dziedziński; R Kozłowski; K Szambelan; B Górna; K Stuper-Szablewska.
- Analysis and interpretation of results: M Dziedziński.
- Draft manuscript preparation: M Dziedziński.
- Revision of the results and approval of the final version of the manuscript: J Kobus-Cisowska; B Stachowiak.

Financial support

This research was supported by statutory funds of the Department of Gastronomy Sciences and Functional Foods of Poznań University of Life Sciences, grant number 506.751.03.00.

Conflict of interest

The authors report no potential conflict of interest.

References

[1] Kirin Holdings Company. Global Beer Consumption by Country in 2020. 2022.
[2] Eurostat. Beer production increased in 2021. Available at Internet: https://ec.europa.eu/eurostat/web/products-eurostat-news/-/ddn-20220830-1 [cited October 12, 2022].
[3] Habschied K, Živković A, Krstanović V, et al. Functional beer—A review on possibilities. Beverages 2020;6(3):51. https://doi.org/10.3390/beverages6030051.
[4] Papp N, Purger D, Czigle S, et al. The importance of pine species in the ethnomedicine of Transylvania (Romania). Plants 2022;11(18):2331. https://doi.org/10.3390/plants11182331. PMid: 36145732.
[5] Torras MAC, Faura CA, Schönlau F, et al. Antimicrobial activity of Pycnogenol®. Phytother Res 2005;19(7):647–8. https://doi.org/10.1002/ptr.1662. PMid: 16161029.
[6] Allenspach M, Valder C, Flamm D, et al. Verification of chromatographic profile of primary essential oil of *Pinus sylvestris* L. combined with chemometric analysis. Molecules 2020;25(13):2973. https://doi.org/10.3390/molecules25132973. PMid: 32605289.
[7] Hajdari A, Mustafa B, Nebija D, et al. Essential oil composition of *Pinus peuce* Griseb. needles and twigs from two national parks of Kosovo. Sci World J 2016;2016:e5393079. https://doi.org/10.1155/2016/5393079. PMid: 27579344.
[8] Dziedziński M, Kobus-Cisowska J, Stachowiak B. *Pinus* species as prospective reserves of bioactive compounds with potential use in functional food—Current state of knowledge. Plants 2021;10(7):1306. https://doi.org/10.3390/plants10071306. PMid: 34203162.
[9] Stuper-Szablewska K, Kurasiak-Popowska D, Nawracała J, et al. Response of non-enzymatic antioxidative mechanisms to stress caused by infection with *Fusarium* fungi and chemical protection in different wheat genotypes. Chem Ecol 2017;33(10):949–62. https://doi.org/10.1080/02757540.2017.1381689.
[10] Kulczyński B, Kobus-Cisowska J, Kmiecik D, et al. Antiradical capacity and polyphenol composition of asparagus spears varieties cultivated under different sunlight conditions. Acta Sci Pol Technol Aliment 2016;15(3):267–79. https://doi.org/10.17306/J.AFS.2016.3.26. PMid: 28071026.
[11] Bradley WG, Holm KN, Tanaka A. An orally active immune adjuvant prepared from cones of *Pinus sylvestris*, enhances the proliferative phase of a primary T cell response. BMC Complement Altern Med 2014;14:163. https://doi.org/10.1186/1472-6882-14-163. PMid: 24884568.
[12] Leandro LF, Cardoso MJO, Silva SDC, et al. Antibacterial activity of *Pinus elliottii* and its major compound, dehydroabietic acid, against multidrug-resistant strains. J Med Microbiol 2014;63(12):1649–53. https://doi.org/10.1099/jmm.0.081711-0. PMid: 25261060.
[13] Vriesekoop F, Krahl M, Hucker B, et al. 125th Anniversary Review: Bacteria in brewing: The good, the bad and the ugly. J Inst Brew 2012;118(4):335–45. https://doi.org/10.1002/jib.49.
[14] Walker GM, Stewart GG. *Saccharomyces cerevisiae* in the production of fermented beverages. Beverages 2016;2(4):30. https://doi.org/10.3390/beverages2040030.
[15] Grassi S, Amigo JM, Lyndgaard CB, et al. Beer fermentation: Monitoring of process parameters by FT-NIR and multivariate data analysis. Food Chem 2014;155:279–86. https://doi.org/10.1016/j.foodchem.2014.01.060. PMid: 24594186.
[16] Mascia I, Fadda C, Dostálek P, et al. Preliminary characterization of an Italian craft durum wheat beer. J Inst Brew 2014;120(4):495–9. https://doi.org/10.1002/jib.176.
[17] Popescu V, Soceanu A, Dobrinas S, et al. A study of beer bitterness loss during the various stages of the Romanian beer production process. J Inst Brew 2013;119(3):111–5. https://doi.org/10.1002/jib.82.
[18] Mosher M, Trantham K. Beer styles. In: Mosher M, Trantham K, editors. Brewing science: A multidisciplinary approach. Cham: Springer International Publishing; 2017. p. 35–61. https://doi.org/10.1007/978-3-319-46394-0_2.
[19] Lazzari A, Barbosa HD, Machado Filho ER, et al. Effect on bioactive compounds and antioxidant activity in the brewing process for beers using Rubim and Mastruz as hop replacements. J Am Soc Brew Chem 2022. https://doi.org/10.1080/03610470.2022.2053638.
[20] Kishimoto T, Teramoto S, Fujita A, et al. Evaluation of components contributing to the International Bitterness Unit of wort and beer. J Am Soc Brew Chem 2022;80(1):53–61. https://doi.org/10.1080/03610470.2021.1878684.
[21] Canivenc-Lavier M-C, Neiers F, Briand L. Plant polyphenols, chemoreception, taste receptors and taste management. Curr Opin Clin Nutr Metab Care 2019;22(6):472–8. https://doi.org/10.1097/MCO.0000000000000595. PMid: 31490201.
[22] Soares S, Silva MS, García-Estevez I, et al. Human bitter taste receptors are activated by different classes of polyphenols. J Agric Food Chem 2018;66(33):8814–23. https://doi.org/10.1021/acs.jafc.8b03569. PMid: 30056706.
[23] Cetó X, Céspedes F, del Valle M. Assessment of individual polyphenol content in beer by Means of a Voltammetric BioElectronic Tongue. Electroanalysis 2013;25(1):68–76. https://doi.org/10.1002/elan.201200299.
[24] Parker DK. Beer: production, sensory characteristics and sensory analysis. In: Piggott J, editor. Alcoholic Beverages. Woodhead Publishing; 2012. p. 133–58. https://doi.org/10.1533/9780857095176.2.133.
[25] Mosher M, Trantham K. Quality assurance and quality control. In: Mosher M, Trantham K, editors. Brewing Science: A Multidisciplinary Approach. Cham: Springer International Publishing; 2017. p. 359–77. https://doi.org/10.1007/978-3-319-46394-0_12.
[26] Annemüller G, Manger H-J, Lietz P. Zur geschichtlichen Entwicklung der Züchtung von reinen Bierhefen. In: Die Hefe in der Brauerei: Hefemanagement, Kulturhefe-Hefereinzucht, Hefepropagation im Bierherstellungsprozess. Brauerei: Verlag der VLB-Versuchs-u. Lehranstalt f; 2008. p. 25–59.
[27] Martínez A, Vegara S, Herranz-López M, et al. Kinetic changes of polyphenols, anthocyanins and antioxidant capacity in forced aged hibiscus ale beer. J Inst Brew 2017;123(1):58–65. https://doi.org/10.1002/jib.387.
[28] Medoro C, Cianciabella M, Camilli F, et al. Sensory profile of Italian craft beers, beer taster expert versus sensory methods: A comparative study. Food Nutr Sci 2016;7(6):454–65. https://doi.org/10.4236/fns.2016.76047.
[29] Aquilani B, Laureti T, Poponi S, et al. Beer choice and consumption determinants when craft beers are tasted: An exploratory study of consumer preferences. Food Qual Prefer 2015;41:214–24. https://doi.org/10.1016/j.foodqual.2014.12.005.

Research Article

Marcin Dziedziński*, Barbara Stachowiak, Joanna Kobus-Cisowska, Miguel A. Faria, Isabel M. P. L. V. O. Ferreira

Antioxidant, sensory, and functional properties of low-alcoholic IPA beer with *Pinus sylvestris* L. shoots addition fermented using unconventional yeast

https://doi.org/10.1515/chem-2022-0360
received March 27, 2023; accepted June 23, 2023

Abstract: The study investigated the potential use of pine (*Pinus sylvestris* L.) shoots and standard and unconventional yeast strains for the production of low-alcohol IPA beer. For this purpose, control worts without added shoots, and worts enriched with pine shoots at 10 g/L were prepared. Worts were inoculated with yeast: *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii, Pichia kluyveri* NEER™, and *Saccharomyces cerevisiae* Safale US-05. The final beer was tested for yeast cell number, basic physicochemical properties, effects on cell line and antioxidant properties. Pine shoots and the tested yeast strains were shown to be suitable for developing low-alcohol beers with potential functional effects. Pine shoots have no negative impact on the beer-making process and may increase the antioxidant potential of beer. Beers supplemented with pine shoots were shown to increase the ability to quench DPPH free radicals *in vitro*, while all low-alcohol beers tested were found to have the ability to reduce nitrite production by lipopolysaccharide-induced RAW264.7. Depending on the yeast used, pine shoots affect the flavour and aroma profile differently, possibly masking foreign aftertastes and odours resulting from the use of unconventional yeast.

Keywords: *Pinus sylvestris* L., low-alcoholic beer, *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, *Pichia kluyveri* NEER™, *Saccharomyces cerevisiae* Safale US-05

1 Introduction

Beer is one of the most widely consumed alcohols in the world. It is a fermented alcoholic beverage produced with water, malted barley, and *Saccharomyces cerevisiae* yeast [1]. The most popular lager beer's distinctive features are its light, clear colour, carbonation, presence of foam, and ethanol content of 4.5–5.5% [1]. Since the beginning of the twenty-first century, however, there has been a clear diversification in the beer market and changes in consumer preferences [2]. Currently, the Beer Judge Certification Program distinguishes more than 80 different styles of beers, which vary significantly in terms of physicochemical and sensory parameters [3]. In addition to the differentiation among styles, there are also new assortment groups, classifying beers by alcohol by volume (ABV), i.e. alcohol-free (≤0.5% ABV) and low-alcohol (≤3.5% ABV) beers [4]. Beer contains B vitamins, minerals, polyphenols, fibre, and prebiotics. However, excessive alcohol consumption has negative health and social consequences, which is why the range of products with reduced ethanol content is developing rapidly [5]. Low-alcohol beer can be seen as a functional beverage, i.e. a beverage in which herbal ingredients, amino acids, vitamins, minerals, and ingredients derived from vegetables or fruit are found that enhance the nutritional value of this group of beverages [6]. The use of herbs and additives in beer production is a well-known practice and is aimed at enhancing flavour and aroma [6].

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Nowadays, many plant raw materials are used at various stages of the production of this beverage to provide health-promoting effects. Xu et al. used okra (*Abelmoschus esculentus* L.) pulp as an additive for wheat beer [7]. The study revealed that incorporating okra resulted in heightened cloudiness, improved foam texture, and enhanced stability in the beers. Furthermore, the beers exhibited elevated levels of terpenes, specifically styrene, which contribute to the characteristic flavour of hazy wheat beer [7]. Ducruet et al. added dried goji berries (*Lycium chinense*) to ale-type beers and assessed the effect on the composition and sensory properties [8]. They found that independently adding 50 g/L of goji berries at the wort boiling stage increased the antioxidant potential of the beer [8]. Besides the addition of plant-based raw materials, unconventional yeast strains have also been increasingly used. Such yeasts include a wide range of strains of *Saccharomyces cerevisiae*, wild strains of *Saccharomyces eubayanus*, in addition to a wide array of various species comprising *Mrakia gelida*, *Torulaspora delbrueckii*, and *Lachancea thermotolerans* [9]. Compared to conventional brewer's yeasts, such strains offer a number of functional advantages, including the possibility of obtaining aromatic low-alcohol beers, reduced caloricity, reduced acidity, the possibility of improving existing beer styles, creating new styles, or faithfully recreating traditional or ancient beer styles [9]. Previous studies have shown that the consumption of alcohol-free beers has a positive effect on human health through the supplementation of biologically active polyphenols and phenolic acids and an increase in the diversity of the intestinal microflora in beneficial bacteria, whereas the presence of alcohol in standard beers impairs this effect [10]. In the present study, the use of cell line research, which is an unusual method for beer analysis, has allowed a preliminary evaluation of the functional effect of hitherto unexplored low-alcohol beers and the possibility of their positive protective effect on the human digestive system [11].

The aim of this study was to investigate the possibility of using unconventional yeast i.e. *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii*, and *Pichia kluyveri* NEER™ pine shoots (*Pinus sylvestris* L.) to obtain low-alcohol IPA beer with *Pinus sylvestris* shoots addition. The study was carried out to assess the impact of these factors on the brewing process, basic physicochemical properties of the final product, antioxidant activity, and cell lines cultures. It is also aimed to evaluate the effect of pine shoots on the flavour and aroma profile of the beer.

2 Materials and methods

2.1 Material

The experimental samples comprised of air-dried pine shoots (*Pinus sylvestris* L.) harvested in 2021 from the arboretum located in Zielonka, Poland (17°06′33″E, 52°06′33″N). These shoots were maintained at a temperature of 20°C with a relative humidity of 55%. Material also comprised hopped malt extract Coopers Brew A IPA (Coopers Brewery Limited, Australia) and four different strains of yeast: *Saccharomyces cerevisiae* Safale US-05 (Lesaffre, France), *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01 (Lesaffre, France), *Saccharomyces cerevisiae* var. *boulardii* (BART Sp. z o.o., Poland), and *Pichia kluyveri* NEER™ (Chr. Hansen Poland Sp. z o.o, Poland).

2.2 Methods

2.2.1 Beer production

In the first stage, hopped malt extract was mixed with an adequate quantity of water and transferred to 1 L glass flasks. Half of the worts were pitched with *Pinus sylvestris* L. shoots in a concentration of 10 g/L (S) and the remaining half were without shoots as controls (C). Worts were pasteurised and cooled to the temperature of 25°C. Prepared worts were inoculated with 10×10^6 yeast cells per millilitre and fermented for 10 days at 20°C and bottled. Then, they were fermented in brown glass bottles for 14 days. After fermentation, the beer was stored at 4°C for 14 days.

2.2.2 Basic physicochemical parameters

To conduct the physicochemical analysis, the beer samples were subjected to degassing through manual agitation for a duration of 5 min. Subsequently, the degassed samples were filtered using a layer of cotton wool and then centrifuged at a speed of 448 RCF for 15 min at a temperature of 20°C (Thermo Fisher Scientific, United Kingdom).

The alcohol content by volume was determined following a distillation process using the Super Dee Digital Distillator (Gibertini, Italy). An automatic densitometer (DDM-2910, Rudolph Research Analytical, USA) employing the mechanical oscillator method was utilised for this

analysis. The extract content in the samples was measured at 20°C using the Balling hydrometer. The measurement of pH was conducted utilizing a CP-411 pH-meter (Elmetron, Poland) based on the PN-A-79093-4:2000 standard.

To assess the bitterness of the beer, the recommended protocol outlined in Analytica-EBC was followed [12]. Initially, 10 mL of degassed beer sample was moved into 50 mL Falcon tubes, to which 0.5 mL of a 6 N hydrochloric acid solution and 20 mL of isoacetate were added. The mixture was vigorously shaken for a duration of 5 min. Subsequently, 10 mL of the resulting sample was transferred into 15 mL Falcon tubes and subjected to centrifugation (Thermo Fisher Scientific, United Kingdom) at a speed of 1,008 RCF for 5 min. For the bitterness analysis, the absorbance of the isooctane layer was measured against pure isooctane utilizing a Halo SB-10 spectrophotometer (Dynamica Scientific Ltd) at a wavelength of 275 nm. The level of bitterness was quantified using international bitterness units (IBU).

2.2.3 Microbiological analysis

To ascertain the overall yeast count, the pour plate technique was employed [13]. All the plates were incubated at 25°C for 72 h. Results showing 30–300 colony-forming units (CFU) were used to analysis.

2.2.4 Polyphenol content

The determination of the total polyphenolic index (TPI) involved the utilisation of Folin–Ciocalteu reagent (FCR), following a procedure outlined by Singleton and Rossi [23]. The measurement involved assessing the colour change of the radical, transitioning from light blue to dark blue, after a 30 min incubation period at 760 nm using a Shimadzu UVmini-1240 UV-Vis spectrophotometer (Kyoto, Japan). The quantification of total polyphenols (TP) involved the creation of a calibration curve using gallic acid (3–20 mg L^{-1}, $R^2 = 0.9961$). Finally, the TPI was calculated and reported as milligrams of gallic acid equivalent (GAE) per litre of beer.

2.2.5 Free radical scavenging activity

The assessment of antioxidant activity was conducted using a DPPH radical, following a modified method described by Brand-Williams et al. [24]. A stock solution was prepared by dissolving 24 mg of DPPH in 100 mL of methanol. The DPPH stock solution was then filtered using methanol, resulting in a usable mixture with an absorbance of approximately 0.973 at 517 nm. In a test tube, 3 mL of the prepared DPPH solution was combined with 100 µL of beer, while another tube contained a solution of 3 mL of DPPH in 100 µL of methanol as a control. Subsequently, the tubes were placed in complete darkness for a duration of 30 min. The absorbance was measured at 517 nm. To calculate the percentage of antioxidant activity or RSA, the following formula was applied [19]:

$$\%\text{of free radical scavenging activity} = [(\text{Ac} - \text{As}) \div \text{Ac}] \times 100,$$

where Ac is the absorbance of the control reaction; and As is the absorbance of the testing specimen.

2.2.6 Effect on induced nitric oxide (NO) production in RAW264.7 cell line

The RAW264.7 macrophage-like cell line was seeded at a density of 35,000 cells per well in 96-well plates and incubated for 24 h to evaluate the effect of beer samples on induced NO production. After the incubation period, the cell culture medium was removed, and the cells were then pre-treated with either beer samples or the control medium alone. After a 2 h interval, the cells were stimulated with 1 mg mL^{-1} of lipopolysaccharide (LPS) for 22 h [14]. To measure NO production, the level of nitrites, a stable end-product of NO metabolism, was determined [15]. Nitrites present in the cell culture medium were detected by combining 75 mL of the medium with an equal volume of Griess reagent, consisting of 1% sulphanilamide and 0.1% *N*-(1-naphthyl)ethylenediamine in 2% H_3PO_4. The plate was then incubated for 10 min in the dark at room temperature, and the absorbance was measured at 560 nm using a microplate reader (BioTek, Winooski, VT, USA). The absorbance values were standardised in relation to the non-pretreated cells that were stimulated with LPS (positive control), serving as the reference value of 100%. The outcomes are presented as relative percentages of the positive control, reflecting the extent of the response compared to the cells that were fully stimulated.

2.2.7 Measurement of cytotoxic potential of beer samples and beer digest on cancerous Caco-2/HT-29 intestinal epithelial cells

To assess the potential cytotoxic effects of beer and *in vitro*-digested beer, an MTT assay was conducted on a Caco2/HT-29 co-culture. To simulate gastrointestinal conditions, an harmonised static protocol, as described by Minekus et al.

was employed for *in vitro* gastrointestinal digestion [16]. Prior to the cell assays, the *in vitro*-digested beer samples were thawed at room temperature and diluted 12 times in a culture medium [17]. Prior to incubation for 24 h, the Caco2/HT-29 co-culture was subjected to pre-treatment with beer, *in vitro*-digested beer, or the complete medium as the control. After the incubation period, the medium was removed, and the cells were exposed to a 0.5 mg mL^{-1} stock solution of MTT for 3 h. Afterward, dimethyl sulfoxide was introduced to facilitate the dissolution of the formazan crystals. Absorbance values were measured at 570 nm using a microplate reader (Biotek, Winooski, VT, USA). The results are expressed as a percentage of the absorbance value of the control group (medium only), with the control group set to 100%. This allows for comparison and determination of the relative impact of the beer and *in vitro*-digested beer on cell viability.

2.2.8 Sensory evaluation

The sensory evaluation of the beer samples took place at the sensory laboratory of Poznań University of Life Sciences, facilitated by a professional panel consisting of 20 judges (14 women and 6 men). These judges, aged between 21 and 55, possessed extensive training in the sensory analysis of beer. To establish a comprehensive understanding of the beer's characteristics, the panel conducted preliminary sessions where they identified 8 aroma descriptors (citrus, fruity, malty, caramel, hoppy, piney, yeasty, and foreign) as well as 10 taste descriptors (hoppy, malty, yeasty, honey-like, piney, fruity, tart, bitter, sour, and sweet). During the sensory evaluation, the judges were comfortably situated in individual specialised booths, ensuring an environment devoid of disturbances such as noise, visual distractions, and extraneous odours. The beer samples, each marked with a unique three-digit code, were presented in standard tasting glasses containing 50 mL of beer. The samples were randomised to prevent any order bias. The beer was served at a temperature of 12°C, under white lighting conditions. To assess the sensory attributes, the judges employed an unstructured scale with clearly defined boundaries, allowing them to rate the intensity of each attribute on a scale from 0 (very weak) to 10 (very intense). The mean scores of these attributes were collected and subjected to quantitative descriptive analysis, enabling the generation of a sensory profile for both types of beers.

2.2.9 Statistical analysis

A total of three samples from distinct bottles were utilised for all measurements. The data obtained were presented as the mean value ± standard deviation and subjected to statistical analysis using one-way analysis of variance through the implementation of RStudio software version 1.4 (RStudio, PBC, Delaware, USA). Statistical significance was determined at a threshold of $p < 0.05$, indicating the presence of notable differences.

3 Results

The physicochemical parameters of the beer varied depending on the yeast strain used and the addition of pine shoots (Table 1). The ethanol content (% v/v) of the beer ranged from 0.98 ± 0.02 in the SC sample to 2.26 ± 0.11 in the LC sample. Beers without shoots had a slightly higher ethanol content than these supplemented, although the differences between the

Table 1: Physicochemical and microbial parameters of the prepared beer

Analysed sample	Ethanol (% v/v)	Extract		Acidity (PH)	Bitterness (IBU)	Yeast count (log CFU/mL)
		Real (% w/w)	Apparent (% w/w)			
CC	2.16 ± 0.03^{ab}	3.00 ± 0.00^{ab}	2.10 ± 0.00^{ab}	4.26 ± 0.08^{c}	28.99 ± 0.48^{ab}	1.60 × 10^{3}
CP	2.06 ± 0.01^{ab}	3.10 ± 0.00^{ab}	2.47 ± 0.29^{ab}	4.23 ± 0.06^{bc}	27.79 ± 0.41^{ab}	2.42 × 10^{6}
SC	0.98 ± 0.02^{b}	3.17 ± 0.29^{ab}	2.87 ± 0.06^{ab}	3.97 ± 0.03^{bc}	27.76 ± 0.07^{ab}	5.60 × 10^{6}
SP	1.56 ± 0.02^{ab}	3.77 ± 0.06^{b}	3.00 ± 0.00^{b}	4.27 ± 0.03d	28.73 ± 0.12^{ab}	7.20 × 10^{5}
LC	2.26 ± 0.11^{a}	2.67 ± 0.06^{a}	1.97 ± 0.06^{a}	4.37 ± 0.02^{b}	24.95 ± 0.33^{a}	1.12 × 10^{6}
LP	2.18 ± 0.02^{a}	2.80 ± 0.00^{a}	2.10 ± 0.00^{ab}	4.74 ± 0.01^{a}	30.19 ± 0.14^{b}	5.00 × 10^{6}
NC	1.49 ± 0.01^{ab}	3.57 ± 0.06^{ab}	2.80 ± 0.00^{ab}	4.18 ± 0.03^{c}	28.62 ± 0.67^{ab}	1.80 × 10^{6}
NP	1.34 ± 0.03^{ab}	3.47 ± 0.06^{ab}	2.70 ± 0.17^{ab}	4.22 ± 0.09^{c}	29.95 ± 0.29^{b}	1.98 × 10^{6}

Values are expressed as the mean value (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05).

sample beers were not statistically significant. There was less variation between beers for real and apparent extracts. The acidity of the beers described in terms of pH ranged between 3.97 ± 0.03 for the SC sample and 4.37 ± 0.02 for the LC sample. The beers with added shoots had higher pH. Bitterness as described in terms of IBU was not widely varied, except in the LC beer where IBU was 24.95 ± 0.33. Due to the lack of a pasteurisation process, all beer samples contained yeast. The CC beer had the lowest log CFU mL^{-1} content (1.60×10^3), while the SC beer had the highest (5.60×10^6).

3.1 Physicochemical and microbiological parameters of beer

3.1.1 Polyphenol content and free radical scavenging activity (RSA)

The analysed beers contained polyphenols as measured with the FC method and the ability to quench DPPH free radicals (Table 2). The content of TP ranged from 222.42 ± 12.5 mg GAE L^{-1} for the CP beer to 294.72 ± 27.05 mg GAE L^{-1} for the SC beer, while free RSA (% RSA) ranged from 31.72 ± 6.52 for the CP beer to 52.91 ± 1.40 for the CC beer, but the differences were not statistically significant. No correlation was observed between polyphenol content and % RSA. For free radical quenching, beers supplemented with pine shoots showed greater efficacy, while for polyphenol content, such a relationship did not exist for all beers and most differences between samples were not statistically significant.

Table 2: Polyphenol content and free RSA of beer samples

Analysed sample	TPI mg GAE L^{-1}	DPPH % RSA
CP	222.42 ± 12.51^{b}	31.72 ± 6.52^{ab}
CC	222.58 ± 37.65^{b}	52.91 ± 1.40^{a}
SC	294.72 ± 27.05^{a}	44.98 ± 2.76^{ab}
SP	277.85 ± 15.06^{ab}	46.56 ± 3.32^{ab}
LC	221.39 ± 11.16^{b}	52.09 ± 5.08^{ab}
LP	274.59 ± 21.48^{ab}	79.67 ± 1.53^{c}
NC	270.08 ± 29.84^{ab}	39.23 ± 2.66^{ab}
NP	244.80 ± 2.51^{ab}	42.98 ± 6.28^{ab}

Values are expressed as the mean value (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) within the same column are statistically different (p value < 0.05). Abbreviations: GAE – gallic acid equivalent; DPPH – 2.2-diphenyl-1-picrylhydrazyl; RSA – radical scavenging activity.

3.2 Cell line assays

3.2.1 Effect on induced NO production in RAW264.7 cell line

The pre-treatment of LPS-stimulated RAW264.7 cells with beer samples reduced the induced NO production (Figure 1). The lowest % NO compared to the control was shown for NP, which meant that this sample had the highest protective effect. There was a significant difference in NP activity compared to the LP sample, which showed the lowest protective effect, but no statistically significant differences were observed between the other samples.

Figure 2 presents the results for the effect of beer samples and *in vitro*-digested beer on the viability of the Caco2/HT-29 co-culture. Depending on the sample, cell viability ranged from 64.54% ± 6.16 for the LP beer sample to 100.28% ± 0.18 for the NC *in vitro*-digested beer sample. In most samples, *in vitro*-digested samples resulted in greater cell viability.

3.3 Sensory properties

Sensory testing of taste (Figure 3) and aroma (Figure 4) showed variations in profiles depending on the addition of pine shoots and the yeast strain used. The flavour defined as piney was only noticeable in beers supplemented with pine shoots. Apart from this descriptor, no particular correlations were noted in the taste profiles. The CC sample had the highest bitterness and hop flavour sensation, while the LC beer was an outlier in terms of flavour described as foreign, malty and yeasty.

In terms of aroma, higher variation was observed than in the taste profile (Figure 4).

As in the flavour profile, the pine aroma was only perceived in the pine-supplemented beers. In terms of aroma, the beers with added shoots also had an aroma described as citrus. A foreign aroma was perceived in the NP, SC, and NC beers.

4 Discussion

The study indicated the potential for the use of unconventional yeast strains and pine shoots in the production of low-alcohol functional beer. This study is of particular relevance as far as the needs of today's consumers are concerned, as they often seek to reduce alcohol in beer and are

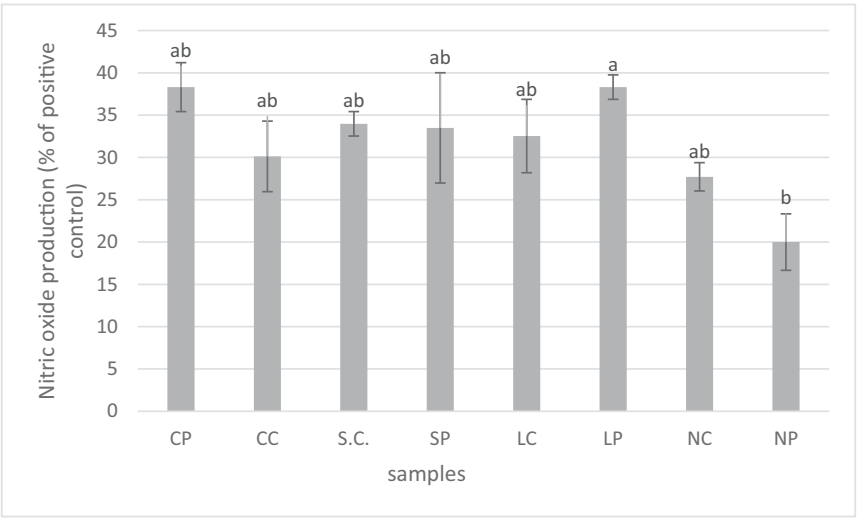

Figure 1: Effect of beer samples on nitrite production by LPS-induced RAW264.7. Values are expressed as the mean value (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) are statistically different (p value < 0.05).

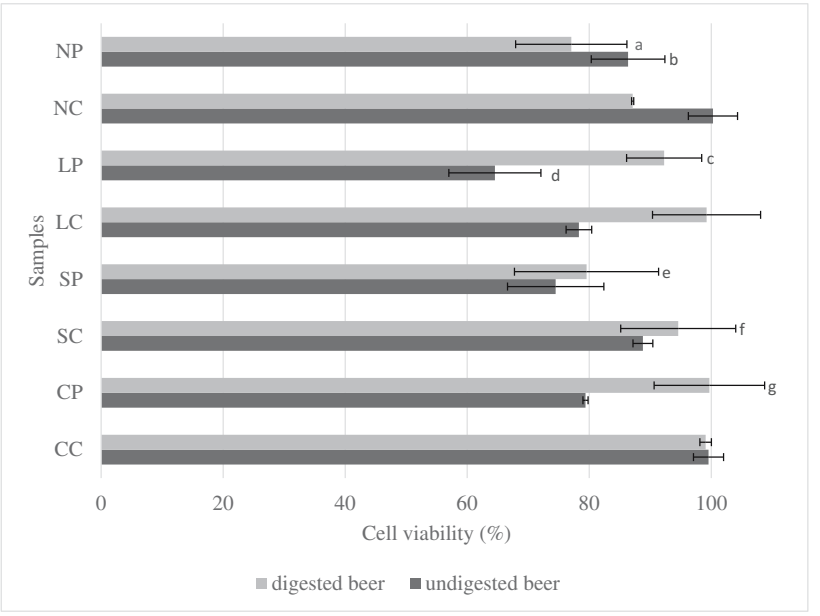

Figure 2: Effect of *in vitro* digested beer and undigested beer samples against induced cytotoxicity of Caco2/HT-29 coculture. Values are expressed as the mean value (n = 3) ± standard deviation. Mean values with different letters (a, b, c, etc.) are statistically different, those without any letter do not differ statistically (p value < 0.05).

looking for new original functional products [6]. The yeast used in the study, such as *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. *boulardii, Pichia kluyveri* NEER™, and *Saccharomyces cerevisiae* Safale US-05, had good technological and sensory properties. The *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01 yeast has not been described in detail in the literature so far. In one of the few studies on the use of this yeast, Simões et al. indicated that this yeast showed high potential for the production of lager beer, and beers made with it had high sensory acceptability and contained volatile compounds in desirable concentrations [18]. In this study, it was confirmed that IPA beers fermented with unconventional yeasts with pine shoots of *Pinus sylvestris* addition showed typical physicochemical characteristics and balanced taste and aroma. The *Saccharomyces cerevisiae* var. *boulardii* and *Pichia kluyveri* NEER™ are considered as useful in the production of low-alcohol beer, while

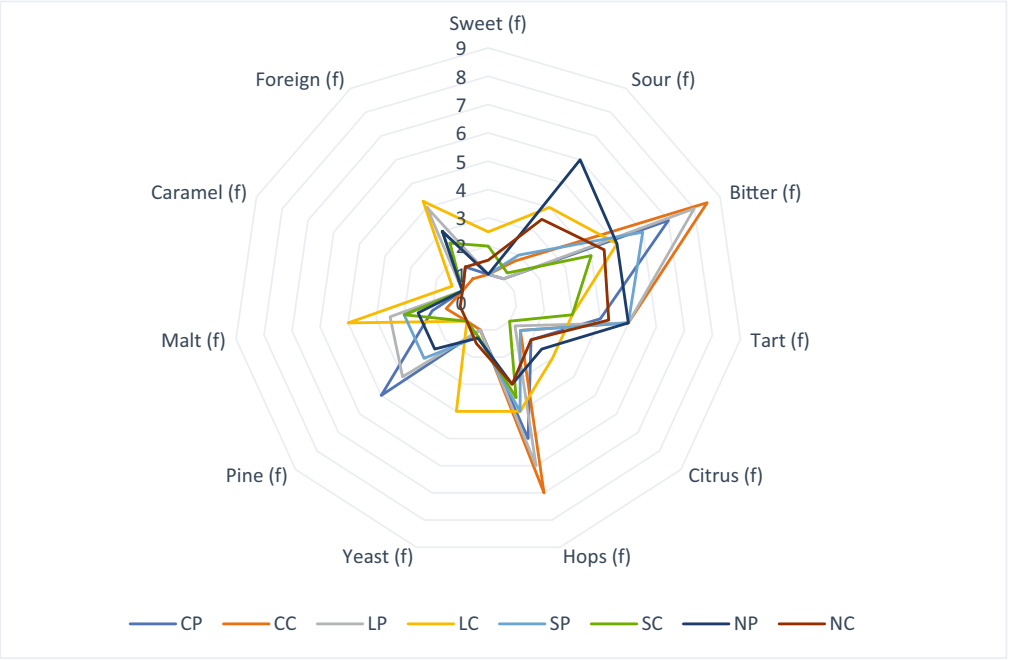

Figure 3: Flavour profile of tested beer samples. Intensities of the specific attributes are according to the compiled flavour classes, where 0 is not perceivable, 10 is very strong, and (f) stands for flavour.

in the current study, it was showed that this yeast modified the aroma of control beers and supplemented beers [19]. This may be due to the higher alcohol content in the control beers and consequently a lower content of residual sugars. Moreover, in the beers with the *Pinus sylvestris shoots* addition, the taste was found as more bitter, which

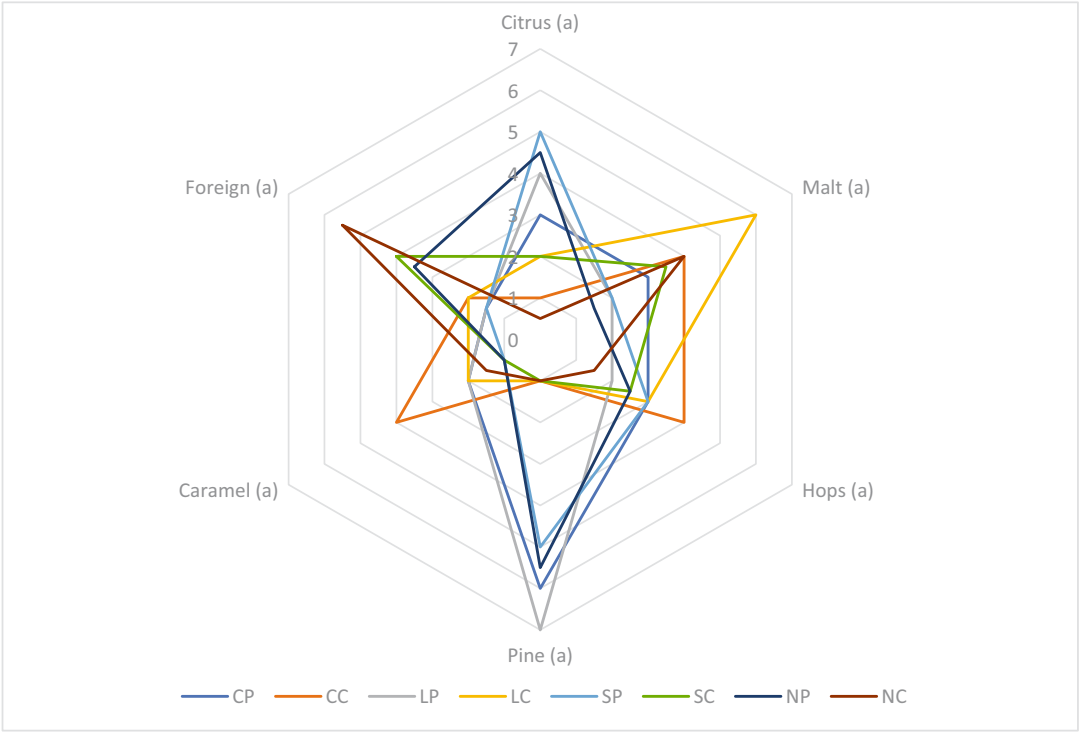

Figure 4: Aroma profile of tested beer samples. Intensities of the specific attributes are according to the compiled aroma classes, where 0 is not perceivable, 10 is very strong, and (a) stands for aroma.

may be due to the presence of compounds such as alkaloids and tannins. The addition of shoots to the wort could influence the yeast fermentation activity [19]. The technological challenge with *S. boulardii* is that these microorganisms can convert fermentable wort sugars into ethanol even at 2°C, which makes it very difficult to produce commercial low-alcohol beers containing live probiotic cultures and fermentable sugars [20]. It can be observed in the current study that ethanol content in beers fermented with S. *boulardii* is in the range of 0.98–1.56% v/v.

Functional beers are often enriched with the use of plant-based raw materials and their extracts. The literature indicates the use of, inter alia: *Coriandrum sativum, Brassica nigra, Artemisia vulgaris, Juniperus communis, Melissa officinalis, Mentha spicata, Origanum vulgare, Pimpinella anisum, Rosmarinus officinalis, Thymus serpyllum* [21]. Commercial beers are available on the market that have been produced using pine shoots, e.g. "Miłosław Sosnowe APA" (Fortuna Brewery, Poland), Forest IPA (Nepomucen Brewery, Poland), and Pine Shoot NEIPA (Austmann Bryggeri, Norway), but so far these have only been beers with standard or high alcohol content (4.8–7.8% ABV). No information was found in the literature on the possible use of pine shoots, while a few studies have used needle extract [22,23]. In the study, the authors noted that the addition of pine needle (*Pinus sylvestris* L.) aqueous extract increased the antioxidant capacity of the beer and could serve as a partial substitute for hops due to similar levels of bitterness and positive effects on sensory properties [22,23]. In the current study, pine shoots showed effects on IBU, and most tested beers supplemented with pine shoots were perceived as more bitter; however, in general, for IPA beers, the addition of pine shoots showed a positive effect on organoleptic properties. Perhaps this is since IPA-style beers are characterised by a higher bitterness expected by the consumer. Parts of other coniferous trees are also used in beer production. In a study by Balík et al., knots, aqueous extracts, or alcoholic extracts of spruce knots (*Picea abies*) were added to the wort at various stages of boiling, and then the content of lignans was measured in pilsner beers [24]. It was shown that the highest content of lignans was found in the sample where the sawdust was in the wort for 65–75 min of boiling, while the lowest was observed when alcohol extract was added, confirming the validity of using shoots as opposed to extracts in the current study [24]. In another study, juniper berries (*Juniperus communis* L.) were added to the wort at different concentrations, i.e. 0.24, 0.48, and 0.72 g L^{-1} [25]. In contrast to pine shoots, juniper berries negatively affected yeast activity and reduced fermentation, while the enriched beers showed higher polyphenol content and oxygen radical absorbance capacity measured *in vitro* in contrast to the control sample [25]. Compared to supplementation with pine shoots, beers made with juniper berries had a higher polyphenol content and a greater free RSA [25]. When it comes to cell line research, beer is not a common object of study. This may be related to the fact that it is only in recent years that an increased interest in low-alcohol and alcohol-free beer has been noted [26]. According to Kokole et al. alcohol-free beer accounted for 3.8% of total beer volume in 2019 [26]. However, as the literature indicates, studies using cell lines may be unreliable at low ethanol concentrations, i.e. below 2.5% [27]. All of the beers in the study had ethanol concentrations ranging from 0.98 ± 0.02 to 2.26 ± 0.11, which is comparable to the alcohol content of functional drinks such as kefir or kombucha [28,29]. In the current study, it was decided that intestinal epithelial cell lines and macrophage lines would be used that can tolerate the ethanol concentrations present in the investigated beers as they can be a good indicator in the study of potential cytotoxic and anti-inflammatory effects [27,30]. A study by Di Domenico et al. analysed the effects of brewing fractions from the mashing, filtration, and boiling process with the addition of hops (solution after hopping) of "La Meridionale" beer from Birrificio Bari (Italy), with the addition of Gargano's IGP orange, coriander, and borage on D-dSC stem cells and Caco-2 intestinal epithelial lines [31]. The findings of the investigation demonstrate that at low concentrations, beer fractions elicit a notable enhancement in cell proliferation. Conversely, when administered at higher doses, these fractions exhibit an inhibitory effect on cell proliferation [31]. The authors conclude that this effect may be due to the higher sugar content of the beer fractions as they are not yet fermented, which may explain the effect seen in the present study, given that low-alcohol beers are richer in sugar. In current study, *in vitro* digested beer in most cases showed higher cell viability. One possible explanation for the observed results is the good tolerance yeast on low pH of stomach and the presence of nutrient compounds in beer, which could have become more available and easier to assimilate after digestion process. Beer is known to be rich in various compounds with potential health benefits, including amino acids, prebiotics, minerals, and B vitamins, which could have contributed to the increased cell viability observed [32]. The results also suggest a potential variability in the effects of different beer samples on cell viability, this may be due to differences in the composition of the beer, which might include variation in the quantity and types of nutrient compounds present, their availability, or their interactions with other beer components [33]. In an experiment where oxidative stress was induced with H_2O_2, the results indicate that the fractions counteract the oxidative effects in D-dSC and Caco-2 cells, which is analogous to the results obtained in the current study on nitrite production

by LPS-induced RAW264.7, where a 60–80% reduction in NO production was observed.

5 Conclusion

The presented study showed that pine shoots at a concentration of 10 g L^{-1} of wort did not adversely affect the activity of the yeasts, namely, *Saccharomyces cerevisiae* Safale US-05, *Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01, *Saccharomyces cerevisiae* var. boulardii, and *Pichia kluyveri* NEER™, and the use of these yeasts and pine shoots makes it possible to obtain low-alcohol beer. Pine shoots do not adversely affect the physicochemical parameters of beer, while they can positively affect the oxygen radical absorbance capacity, as observed in the case of the study on antiradical activity with DPPH, and in the study on nitrite production by LPS-induced RAW264.7. Depending on the yeast strain used, they can affect the perceived taste and aroma in different ways, for example by masking the foreign aroma of a low-alcohol beer made with *Pichia kluyveri* NEER™ yeast.

Abbreviations

CC	control beer/*Saccharomyces cerevisiae* Safale US-05
CP	beer with pine shoots/*Saccharomyces cerevisiae* Safale US-05
LC	control beer/*Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01
LP	beer with pine shoots/*Saccharomyces cerevisiae* var. *chevalieri* SafBrew™ LA-01
SC	control beer/*Saccharomyces cerevisiae* var. *boulardii*
SP	beer with pine shoots/*Saccharomyces cerevisiae* var. *boulardii*
NC	control beer/*Pichia kluyveri* NEER™
NP	beer with pine shoots/*Pichia kluyveri* NEER™

Acknowledgements: The authors thank Susana Pinho and Helena Ramos from University of Porto and Katarzyna Szambelan and Barbara Górna from Poznan University of Life Sciences for advice and assistance in laboratory assays.

Funding information: This research was supported by statutory funds of the Department of Gastronomy Sciences and Functional Foods of Poznan University of Life Sciences, Grant number 506.751.03.00.) and a development and implementation project financed by The Agency for Restructuring and Modernisation of Agriculture (ARMA), M16 program, Cooperation, "Development of product and process innovation related to the technology of producing organic beer containing probiotic microorganisms, preserved using the refermentation process, carried out with the participation of probiotic microorganisms and the production of a food additive based on spent grain, which is a by-product in the beer production process, for the preparation of bread enriching mixes," Consortium, Biała Wrona (PROW 00053.DDD.6509.00062.2019.15, 2021–2023).

Author contributions: Study conception and design: Marcin Dziedziński, Joanna Kobus-Cisowska, Barbara Stachowiak, Miguel A. Faria, and Isabel M. P. L. V. O. Ferreira; data collection: Marcin Dziedziński and Barbara Stachowiak; analysis and interpretation of results: Marcin Dziedziński and Miguel A. Faria; draft manuscript preparation: Marcin Dziedziński; revision of the results and approved the final version of the manuscript: Joanna Kobus-Cisowska.

Conflict of interest: Authors state no conflict of interest

Ethical approval: All participants in this study gave informed consent to the University.

Data availability statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

References

[1] Wannenmacher J, Gastl M, Becker T. Phenolic substances in beer: Structural diversity, reactive potential and relevance for brewing process and beer quality. Compr Rev Food Sci Food Saf. 2018;17(4):953–88.
[2] Betancur MI, Motoki K, Spence C, Velasco C. Factors influencing the choice of beer: A review. Food Res Int. 2020 Nov 1;137:109367.
[3] Mittag R. Geographic appellations of beer. In: Patterson M, Hoalst-Pullen N, editors. The geography of beer: Regions, environment, and societies. Dordrecht; Springer Netherlands; 2014 [cited 2023 Mar 14]. p. 67–74. doi: 10.1007/978-94-007-7787-3_7.
[4] Bellut K, Arendt, EK. Chance and challenge: Non-saccharomyces yeasts in nonalcoholic and low alcohol beer brewing – A review. J Am Soc Brew Chem. 2019 Apr 3;77(2):77–91.
[5] Sohrabvandi S, Mortazavian AM, Rezaei K. Health-related aspects of beer: A review. Int J Food Prop. 2012 Mar 1;15(2):350–73.
[6] Habschied K, Živković A, Krstanović V, Mastanjević K. Functional beer—A review on possibilities. Beverages. 2020 Sep;6(3):51.

[7] Xu K, Guo M, Du J, Zhang Z. Cloudy wheat beer enriched with okra [*Abelmoschus esculentus* (L.) Moench]: Effects on volatile compound and sensorial attributes. Int J Food Prop. 2018 Jan 1;21(1):289–300.
[8] Ducruet J, Rébénaque P, Diserens S, Kosińska-Cagnazzo A, Héritier I, Andlauer W. Amber ale beer enriched with goji berries – The effect on bioactive compound content and sensorial properties. Food Chem. 2017 Jul 1;226:109–18.
[9] Cubillos FA, Gibson B, Grijalva-Vallejos N, Krogerus K, Nikulin J. Bioprospecting for brewers: Exploiting natural diversity for naturally diverse beers. Yeast. 2019;36(6):383–98.
[10] Hernández-Quiroz F, Nirmalkar K, Villalobos-Flores LE, Murugesan S, Cruz-Narváez Y, Rico-Arzate E, et al. Influence of moderate beer consumption on human gut microbiota and its impact on fasting glucose and β-cell function. Alcohol. 2020 Jun 1;85:77–94.
[11] Heinlein A, Metzger M, Walles H, Buettner A. Transport of hop aroma compounds across Caco-2 monolayers. Food & Funct. 2014;5(11):2719–30.
[12] Hagen W, Schwarz H. Analytica-EBC — precision values of wort analyses. J Inst Brew. 2000;106(3):139–46.
[13] Schisler DO. Comparison of revised yeast counting methods. J Am Soc Brew Chem. 1986 Apr 1;44(2):81–5.
[14] Pereira RB, Pinto DCGA, Pereira DM, Gomes NGM, Silva AMS, Andrade PB, et al. UHPLC-MS/MS profiling of Aplysia depilans and assessment of its potential therapeutic use: Interference on iNOS expression in LPS-stimulated RAW 264.7 macrophages and caspase-mediated pro-apoptotic effect on SH-SY5Y cells. J Funct Foods. 2017 Oct 1;37:164–75.
[15] Calvello R, Aresta A, Trapani A, Zambonin C, Cianciulli A, Salvatore R, et al. Bovine and soybean milk bioactive compounds: Effects on inflammatory response of human intestinal Caco-2 cells. Food Chem. 2016 Nov 1;210:276–85.
[16] Minekus M, Alminger M, Alvito P, Ballance S, Bohn T, Bourlieu C, et al. A standardised static in vitro digestion method suitable for food - an international consensus. Food Funct. 2014 Jun;5(6):1113–24.
[17] Rafiee Tari N, Arranz E, Corredig M. Effect of protein composition of a model dairy matrix containing various levels of beta-casein on the structure and anti-inflammatory activity of in vitro digestates. Food Funct. 2019 Apr 17;10(4):1870–9.
[18] Simões J, Coelho E, Magalhães P, Brandão T, Rodrigues P, Teixeira JA, et al. Exploiting non-conventional yeasts for low-alcohol beer production. Microorganisms. 2023 Feb;11(2):316.
[19] Methner Y, Hutzler M, Zarnkow M, Prowald A, Endres F, Jacob F. Investigation of non-saccharomyces yeast strains for their suitability for the production of non-alcoholic beers with novel flavor profiles. J Am Soc Brew Chem. 2022 Oct 2;80(4):341–55.
[20] Senkarcinova B, Graça Dias IA, Nespor J, Branyik T. Probiotic alcohol-free beer made with Saccharomyces cerevisiae var. boulardii. LWT. 2019 Feb 1;100:362–7.
[21] Djordjević S, Popović D, Despotović S, Veljović M, Atanacković M, Cvejić J, et al. Extracts of medicinal plants as functional beer additives. Chem Ind Chem Eng Q. 2016;22(3):301–8.
[22] Penkina N, Tatar L, Kolesnyk V, Karbivnycha T, Letuta T. The study of beer quality with the reduced toxic effect. Eureka: Life Sci. 2017 Mar 23;1:35–43.
[23] Penkina N, Tatar L, Kolesnyk V, Karbivnycha T, Letuta T. Research into quality of beer with the addition of pine needles extract. East-Eur J Enterp Technol. 2017;2(10):40–8.
[24] Balík J, Híc P, Tříska J, Vrchotová N, Smetana P, Smutek L, et al. Beer and beer-based beverage contain lignans. J Food Sci Technol. 2021 Feb 1;58(2):581–5.
[25] Veljovic M, Despotovic S, Djordjevic R, Pecic S, Kalusevic A, Leskosek-Cukalovic I, et al. Sensory and antioxidant properties of beer with *Juniperus communis* L. In: Full Proceedings of 11th International Congress on Engineering and Food; 2011.
[26] Kokole D, Jané Llopis E, Anderson P. Non-alcoholic beer in the European Union and UK: Availability and apparent consumption. Drug Alcohol Rev. 2022;41(3):550–60.
[27] Nguyen ST, Nguyen HTL, Truong KD. Comparative cytotoxic effects of methanol, ethanol and DMSO on human cancer cell lines. Biomed Res Ther. 2020 Jul 29;7(7):3855–9.
[28] de Miranda JF, Ruiz LF, Silva CB, Uekane TM, Silva KA, Gonzalez AGM, et al. Kombucha: A review of substrates, regulations, composition, and biological properties. J Food Sci. 2022;87(2):503–27.
[29] Tzavaras D, Papadelli M, Ntaikou I. From milk kefir to water kefir: Assessment of fermentation processes, microbial changes and evaluation of the produced beverages. Fermentation. 2022 Mar;8(3):135.
[30] Moro C, Palacios I, Lozano M, D'Arrigo M, Guillamón E, Villares A, et al. Anti-inflammatory activity of methanolic extracts from edible mushrooms in LPS activated RAW 264.7 macrophages. Food Chem. 2012 Jan;130(2):350–5.
[31] Di Domenico M, Feola A, Ambrosio P, Pinto F, Galasso G, Zarrelli A, et al. Antioxidant effect of beer polyphenols and their bioavailability in dental-derived stem cells (D-dSCs) and human intestinal epithelial lines (Caco-2) cells. Stem Cell Int. 2020 Oct 10;2020:e8835813.
[32] Cueva C, Moreno-Arribas MV, Martín-Alvarez PJ, Bills G, Vicente MF, Basilio A, et al. Antimicrobial activity of phenolic acids against commensal, probiotic and pathogenic bacteria. Res Microbiol. 2010 Jun;161(5):372–82.
[33] McClements DJ, Decker EA, Park Y, Weiss J. Structural design principles for delivery of bioactive components in nutraceuticals and functional foods. Crit Rev Food Sci Nutr. 2009 Jun 16;49(6):577–606.