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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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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

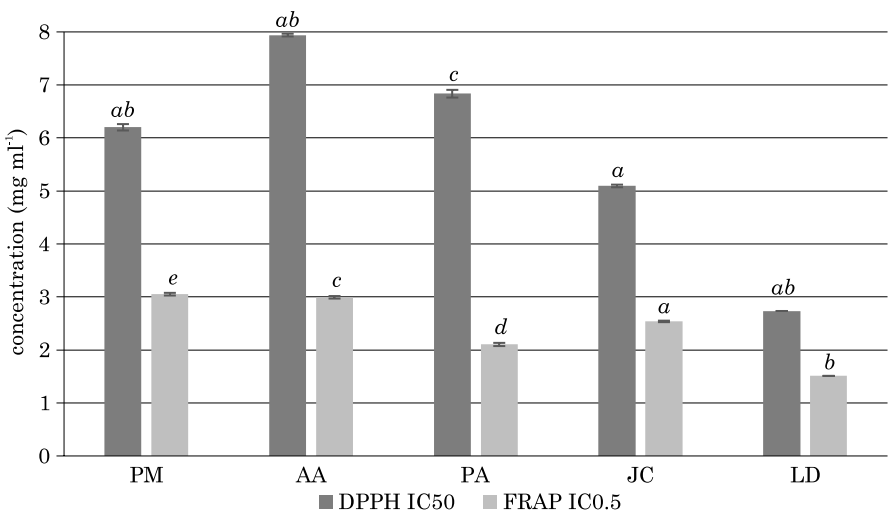

Fig. 1. Antioxidative capacity of conifer extracts measured by DPPH and FRAP assays
The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (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-

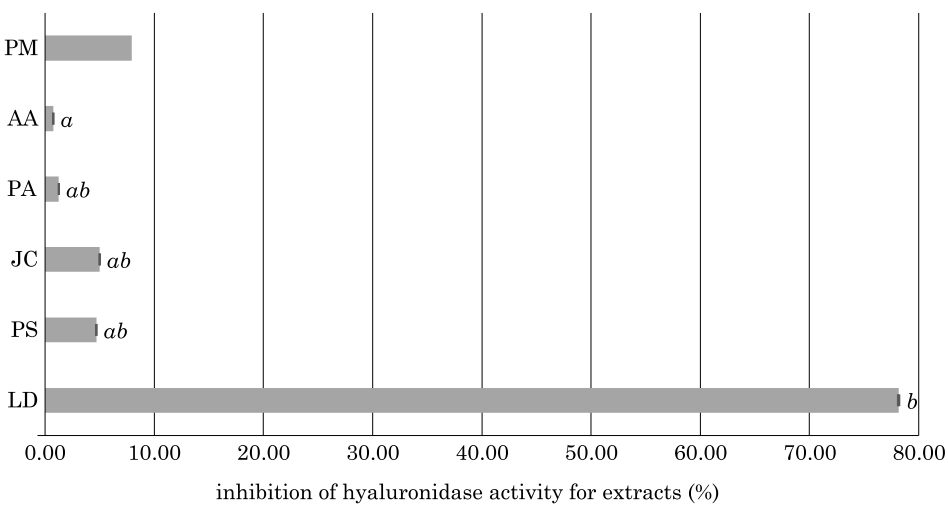

Fig. 2. Inhibition of hyaluronidase activity by conifer needle extracts
The results are mean values of three determinations±standard deviation. The values sharing the same letter in a line were not significantly different ($p \leq 0.05$). Abbreviations: *Picea abies* L. (PA), *Abies alba* (AA), *Larix 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.

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