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Title: Cones of coniferous taxa as a potential source of bioactive polyphenols

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Abstract: Coniferous cones are a by-product of forestry and wood logging, potentially be utilized for a variety of purposes, including the extraction of antioxidant polyphenols. In the present article we conducted a comparative analysis of the antioxidant content of 17 selected taxa, that are either common in Hungary or that have not yet been investigated in any great detail also investigating different maturation stages of the cones. Folin-Ciocâlteu total phenol content, ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to determine the antioxidant contents. A scoring system was implemented using the three assay results to evaluate and compare the overall antioxidant power of the samples. Overall best results were found for green, followed by mature and opened cones. Taxa with the highest scores were *Tsuga Canadensis, Metasequoia glyptostroboides, Chamaecyparis lawsoniana, Cryptomeria Japonica, Thuja orientalis* and *Picea abies*. High-performance liquid chromatographic/tandem mass spectrometric polyphenol profiling was completed for the most relevant samples (green cones of *T. canadensis* and *P. abies*). Results provide a basis for future bioactivity testing of these samples.

Keywords: coniferous species; cones; antioxidants; liquid chromatography; mass spectrometry

1. INTRODUCTION

Forestry and timber production wastes (e.g. leaves, wood bark, cones, etc.) can be a rich source of antioxidants [1] with potential utilization fields (e.g. production of healthcare-related products [2, 3], natural food ingredients [4, 5], natural growth bioregulators [6], silver nanoparticles [7, 8], etc.).

Cones are exclusively born by coniferous trees and shrubs. Conifers bear "seed-cones" and "pollen-cones" out of which the female seed-cones were the subject of the present study.

The major use of forest tree cones has been seed extraction for the production of forestry propagation material or alimentation purposes [9, 10]. The opened (empty) cones are usually burned [11] or can be converted to briquettes [12]. Cone extracts and essential oils of *Pinus*, *Thuja*, and *Cedrus* spp. have been used by traditional medicine [13, 14] and have been shown to possess anticancer, antimutagenic or other health promoting effects [15-19]. Latest results indicate that pine cone and pine cone extracts can be used because of their various useful properties, e.g. being a source as dietary fibre [20], or starting materials for the production of coagulants [21] and adsorbents [22, 23].

Despite these results, the literature lacks systematic research of the antioxidant composition of cones and the assessment of their role as a source of antioxidants. Moreover, sample collection times in the presented examples – more specifically, the phenophase of cone maturity – have rarely been documented.

The aim of the present research was to investigate 17 taxa including Atlas cedar (*Cedrus atlantica* Endl.), European larch (*Larix decidua* Mill.), Norway spruce (*Picea abies* H. Karst.), mountain pine (*Pinus mugo* Turra), black pine (*Pinus nigra* J.F. Arnold), Scots pine (*Pinus sylvestris* L.), Himalayan pine (*Pinus wallichiana* A. B. Jacks.), eastern hemlock (*Tsuga canadensis* (L.) Carrière), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), Lawson cypress (*Chamaecyparis lawsoniana* (A. Murray) Parl.), bald cypress (*Taxodium distichum* (L.) Rich.), northern white-cedar (*Thuja occidentalis* L.), dawn redwood (*Metasequoia glyptostroboides* Hu and W. C. Cheng), Chinese arborvitae (*Thuja orientalis* L.), Japanese cedar (*Cryptomeria japonica* (L.f.) D. Don) and China fir (*Cunninghamia lanceolate* (Lamb.) Hook).

Antioxidant properties were determined by the Folin-Ciocâlteu total polyphenol content (TPC), ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods and using a scoring system for the combined evaluation of these methods.

The polyphenol profile of the samples with the highest antioxidant potential was also investigated using high-performance liquid chromatography/multistage mass

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spectrometry in order to identify the structure of major antioxidant compounds (polyphenols).

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

Water was prepared via double distillation using conventional distillation equipment. Acetone and acetonitrile (LCMS grade) were obtained from VWR-International (Budapest, Hungary). Gallic acid, ascorbic acid, acetic acid, sodium acetate, hydrochloric acid, sodium carbonate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-S-triazine (TPTZ), iron(III)-chloride were purchased from Sigma-Aldrich (Budapest, Hungary). Folin-Ciocâlteu reagent was obtained from Merck (Darmstadt, Germany).

2.2. Sample collection and extraction

Sample collection occurred at the Botanical Garden of the University of Sopron in Sopron, Hungary between July-October 2018 and 2019. Altogether three ripening stages were sampled: green cones (collected in July when cones are green, yet nearly at their full size at the final year of maturation), mature cones (collected in August/September when the cones turned brown in colour and scales began to open) and opened cones (taken in September/October, at a fully opened state having released their seeds and found on trees or to the ground). One healthy individual of each taxon was sampled at each ripening stage. Cone samples were stored at -20ºC until sample preparation. Prior to extraction, samples were thawed and ground. Ultrasonic extraction was performed using an Elma Transsonic T570 ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) as follows: 0.45 g ground sample was homogenized with 45 ml acetone:water 80:20 v/v in a 50 ml centrifuge tube and sonicated for 3 x 10 min as described by Hofmann et al. [24].

2.3. Determination of antioxidant properties

TPC determination was completed by applying the Folin-Ciocâlteu assay [25] using gallic acid as the standard at 760 nm. The results were expressed as mg equivalents of gallic acid/g dry bark units (mg GAE/g d.w.). The method described by Benzie and Strain [26] was applied for the measurement of the FRAP antioxidant capacity at 593 nm using ascorbic acid as a standard. Results were given in mg equivalents of ascorbic acid/g dry weight (mg AAE/g dw.). The slightly modified method [24] of Sharma and Bhat [27] was used for running the DPPH assay at 515 nm. Results were calculated in IC_{50} (50% inhibition concentration) values in µg extractives/ml assay (µg/ml) units.

2.4. HPLC-PDA-ESI-MS/MS analyses

Separation of the cone extracts of Norway spruce and eastern hemlock was achieved using a Shimadzu LC-20 type high-performance liquid chromatograph (HPLC) coupled with a Shimadzu SPD-M20A type diode array detector (PDA) and an AB Sciex 3200 QTrap triple quadrupole/linear ion trap mass spectrometric (MS) detector. A Phenomenex Synergy Fusion-RP 80A, 250 mm x 4.6 mm, 4µm column was used for the separation at 40ºC. The injection volume was 15 µl. The binary gradient of A (H_2O + 0.1% HCOOH) and B (CH_3CN + 0.1% HCOOH) solvents was run with 1.2 ml/min flow-rate. The PDA detector signal (250-350 nm) was recorded to monitor separation of peaks. Negative electrospray ionization (ESI) mode was used for the MS detector. Polyphenols were identified with the Information Dependent Analysis (IDA) scanning function of the mass spectrometer using a survey (Q1) scan between 150-1300 m/z and respective dependent (Q3) product ion scans between 80-1300 m/z. Chromatographic data were acquired and evaluated using the Analyst 1.6.3 software.

2.5. Statistics

In order to compare the respective antioxidant capacities of the extracts, ANOVA analysis was run using Statistica 11 (StatSoft Inc., Tulsa, USA) software with the Tukey HSD method.

3. RESULTS AND DISCUSSION

3.1. Evaluation of the TPC, FRAP and DPPH results

Table 1. includes the TPC, FRAP and DPPH data of the samples indicating statistical comparison for the 10 best results within each method. In all of the investigated taxa, the highest TPC was measured in green, followed by mature and opened cone samples. The highest TPC was determined in the green cones of eastern hemlock (157.25 ± 9.98 mg GAE/g dw.), Lawson cypress (131.68 ± 4.35 mg GAE/g dw.), Japanese cedar (131.74 ± 3.00 mg GAE/g dw.) and dawn redwood (113.60 ± 4.81 mg GAE/g dw.). For mature and opened cones highest TPC values were measured in dawn redwood (mature: 91.25 ± 3.69 mg GAE/g dw., opened: 60.16 ± 8.23 mg GAE/g dw.), Chinese arborvitae (mature: 81.22 ± 5.30 mg GAE/g dw., opened: 68.88 ± 4.91 mg GAE/g dw.), Japanese cedar (mature: 74.18 ± 2.09 mg GAE/g dw., opened: 57.41 ± 2.93 mg GAE/g dw.) and Norway spruce (mature: 64.64 ± 2.68 mg GAE/g dw., opened: 46.39 ± 3.54 mg GAE/g dw.).

As none of the antioxidant capacity assays is individually able to measure the total antioxidant power of all compounds in plant extracts, multiple assays are used to estimate the "overall" antioxidant potential of complex extracts [28]. The present study used the FRAP and the DPPH methods to provide further results on the antioxidant capacity of the samples.

In general, green cone samples showed the best FRAP results. The only reverse tendency was observed with dawn redwood and Chinese arborvitae, where mature cones (D.r.: 147.00 ± 6.83 mg AAE/g dw., C.a: 93.12 ± 4.84 mg AAE/g dw.) had superior FRAP values compared to green cone results (D.r.: 129.16 ± 3.01 mg AAE/g dw., C.a: 78.49 ± 1.55 mg AAE/g dw.) showing excellent FRAP. The highest FRAP was found in the green cones and opened cones of previous two taxa and for the green cones of eastern hemlock (100.11 ± 0.40 mg AAE/g dw.). According to Lesjak et al. [9, 29], the FRAP of *Juniperus* spp. cones varies between 3.61 ± 0.03 mg AAE/g dw. (*Juniperus macrocarpa* Sibth. et Sm.) to 35.26 ± 1.12 mg AAE/g dw. (*Juniperus sibirica* Burgsdorf.), indicating that there can be big differences between related taxa just as with eastern (100.11 ± 0.40 mg AAE/g dw.) and western hemlock (59.11 ± 1.73 mg AAE/g dw.) cones evaluated the present study.

The DPPH radical scavenging activity was determined using the IC_{50} value (50% inhibition concentration), with low IC_{50} indicating high antioxidant power. The DPPH results also showed the general decreasing tendency of the order green > mature > opened cones within a taxon. The best results were obtained for the mature (4.42 ± 0.07 μg/ml) and green (6.22 ± 0.42 μg/ml) cone samples of dawn redwood, as well as for green cones of Lawson cypress (7.23 ± 0.41 μg/ml) and eastern hemlock (7.83 ± 0.29 μg/ml). The excellent DPPH activity [30, 31] and bioactivity [30, 32, 33] of dawn redwood cone extracts has already been reported previously.

Analyzing the TPC, FRAP and DPPH data it is apparent that all of the three assays indicated different orders for the best results, which was explained with the different compositions of the extracts as well as with the different working principle of the assays [34, 35].

In order to obtain a comprehensive measure of the overall antioxidant power of the samples and to consider the different selectivity of methods, the summarized evaluation of results of the three different methods was carried out.

Table 1. TPC[1], FRAP[2] and DPPH[3] antioxidant capacity of the cones (mean ± standard deviation). Different superscript letters indicate significant differences at $p < 0.05$ (TPC, FRAP, DPPH) between the samples with the 10 best values withing a method.

	TPC (mg GAE/g dw.)			**FRAP (mg AAE/g dw.)**			**DPPH (IC_{50}) (µg extractives/ml)**		
	Green	Mature	Opened	Green	Mature	Opened	Green	Mature	Opened
Atlas cedar	88.41 ± 1.68	14.96 ± 2.24	7.46 ± 0.26	62.08 ± 3.13^{a}	4.48 ± 0.11	3.37 ± 0.10	21.44 ± 2.94	88.82 ± 12.86	56.92 ± 15.87
European larch	83.44 ± 4.27	25.98 ± 0.94	17.60 ±2.15	55.96 ± 0.93	14.18 ±0.83	4.09 ± 0.17	9.07 ± 1.39	12.53 ± 0.38	28.21 ± 6.84
Norway spruce	105.58±7.92^{ab}	64.64 ± 2.68	46.39 ± 3.54	72.02 ± 8.76^{ab}	50.19 ± 2.08	28.35 ± 3.37	10.75 ± 0.32	9.38 ± 1.14	8.57 ± 0.17^{ab}
Mountain pine	95.76 ± 9.48^{a}	22.33 ± 3.31	15.96 ± 1.10	60.06 ± 2.77	9.34 ± 0.07	7.25 ± 0.19	7.87 ± 0.31^{abc}	27.83 ± 3.73	18.86 ± 0.14
Black pine	89.22 ± 4.79	19.70 ± 3.36	7.08 ±0.34	58.21 ± 2.34	9.55 ± 0.52	4.50 ± 0.17	15.33 ± 1.39	45.90 ±2.69	62.32 ± 1.90
Scots pine	46.30 ± 1.81	18.99 ± 1.44	13.19 ± 1.53	33.42 ± 3.12	9.41 ± 0.32	7.26 ± 0.14	72.40 ± 21.26	29.32 ±1.10	22.88 ± 0.54
Himalayan pine	62.52 ± 5.09	17.76 ± 1.35	8.18 ±0.97	38.84 ± 0.69	8.33 ± 0.56	3.85 ± 0.21	25.72 ± 3.50	54.76 ± 14.54	72.58 ± 7.23
Eastern hemlock	157.25 ±9.98^{d}	56.13 ± 4.07	10.57 ± 1.69	100.11 ± 0.40^{e}	46.57 ± 1.02	5.94 ± 0.25	7.83 ± 0.29^{abc}	11.37 ± 0.67	17.74 ± 1.01
Western hemlock	89.16 ± 5.51	30.77 ± 2.22	10.01 ± 1.77	59.11 ± 1.73	31.03 ± 1.55	4.53 ± 0.09	11.16 ± 1.37	15.52 ± 0.84	40.44 ± 17.94
Douglas fir	48.67 ± 0.90	17.24 ± 0.89	11.16 ± 0.66	23.36 ± 0.17	7.51 ± 0.28	3.61 ± 0.14	11.95 ± 0.79	14.40 ± 1.24	10.18 ± 0.79
Lawson cypress	131.68 ±4.35^{c}	20.61 ± 2.27	16.21 ± 2.11	89.42 ±6.82^{cde}	9.18 ± 0.12	8.36 ± 0.13	7.23 ± 0.41^{bc}	22.46 ± 1.72	30.50 ± 6.72
Bald cypress	70.99 ± 4.49	52.20 ± 1.86	29.53 ± 3.96	57.34 ± 1.28	49.69 ± 5.07	42.42 ± 3.29	8.45 ± 0.74^{ab}	13.17 ± 2.13	13.42 ± 0.60
Northern white-cedar	93.71 ± 5.47^{a}	39.96 ± 2.59	31.38 ± 2.57	76.46 ±3.44^{abc}	49.81 ± 0.11	18.54 ± 0.83	9.93 ± 0.62	9.21 ± 0.30	8.13 ± 0.55^{ab}
Dawn redwood	113.60 ±4.81^{b}	91.25 ± 3.69^{a}	60.16 ± 8.23	129.16 ± 3.01^{f}	147.00 ±6.83^{g}	61.43 ± 3.51	6.22 ± 0.42^{c}	4.42 ± 0.07^{d}	7.15 ± 0.87^{bc}
Chinese arborvitae	106.67±2.76^{ab}	81.22 ± 5.30	68.88 ± 4.91	78.49 ±1.55^{bcd}	93.12 ± 4.84^{de}	31.60 ± 2.02	9.56 ± 0.50	15.76 ± 0.45	17.27 ± 7.71
Japanese cedar	131.74 ±3.00^{c}	74.18 ± 2.09	57.41 ± 2.93	60.87 ± 5.21	41.04 ± 2.08	24.16 ± 0.86	10.13 ± 0.76	10.55 ± 1.40	17.51 ± 0.56
China fir	92.24 ± 1.57^{a}	36.36 ± 2.29	35.94 ± 1.33	67.99 ± 8.88^{ab}	37.20 ± 2.68	20.65 ± 1.44	9.03 ± 1.19^{a}	13.79 ± 0.46	11.14 ± 0.45

[1]: Total polyphenol content, [2]: Ferric reducing antioxidant power, [3]: 2,2-diphenyl-1-picrylhydrazyl

3.2. Combined evaluation of the TPC, FRAP and DPPH results

Combined evaluation of the TPC, FRAP and DPPH results was achieved using a scoring system further developed from the method of Hofmann et al. [24]. This method, presented here for the first time has several advantages over the previous evaluation method: it is combining results in a simpler way, it is appendable, thus it can be extended with the results of previous investigations. In this method the overall antioxidant efficiency of the sample was estimated as a score calculated using the following formula: $Score = TPC \bullet FRAP / DPPH\ IC_{50}$.

The scores of the samples are included in Table 2.

Table 2. Scores of each sample representing the combined antioxidant values.

	Score		
	Green	Mature	Opened
Atlas cedar	256.0	0.8	0.4
European larch	515.0	29.4	2.6
Norway spruce	707.5	345.8	153.4
Mountain pine	730.4	7.5	6.1
Black pine	338.8	4.1	0.5
Scots pine	21.4	6.1	4.2
Himalayan pine	94.4	2.7	0.4
Eastern hemlock	2009.0	229.8	3.5
Lawson cypress	1629.5	8.4	4.4
Bald cypress	481.6	196.9	93.3
Northern white-cedar	721.7	216.2	71.5
Dawn redwood	2358.7	3033.6	516.7
Chinese arborvitae	875.8	479.9	126.0
Japanese cedar	791.3	288.7	79.2
China fir	694.6	98.1	66.6
Western hemlock	472.4	61.5	1.1
Douglas fir	95.1	9.0	4.0

The highest scores, thus best overall antioxidant power, were determined in the green cones of eastern hemlock (2009.0), dawn redwood (2358.7), Lawson cypress (1629.5), Japanese cedar (791.3), Chinese arborvitae (875.8), mountain pine (730.4), northern white-cedar (721.7) and Norway spruce (707.5) and for the mature cones of dawn redwood (3033.6). Interestingly eastern hemlock had much higher overall antioxidant power compared to related western hemlock for green, mature, and opened cone samples. Out of these taxa, the bioactivity, antioxidant activity, or uses of their cone extracts have already been reported in the literature for Lawson cypress [36, 37], dawn redwood [17, 30-33], Japanese cedar [38] and Chinese arborvitae [39].

However, to the best of our knowledge there is no data in the scientific literature on the polyphenolic composition and bioactivity of Norway spruce and eastern hemlock cone extracts. Norway spruce is one of the most dominant coniferous tree species in Europe, possessing significant ecological, industrial, and economic importance [40, 41], while eastern hemlock is also an ecologically important foundation species in forests of eastern North America [42]. Information on molecular composition will provide a basis for the future research on the role these compounds play in possible bioactivity effects. In the following the detailed identification of cone extractives (mostly polyphenolic compounds) of the green cone tissues of Norway spruce and eastern hemlock will be discussed.

3.3. Results of the HPLC-DPA-ESI-MS/MS analyses

Figure **1** depicts the HPLC chromatograms and Table 3 includes the major compounds found in the extracts of Norway spruce and eastern hemlock green cones. Altogether 83 compounds have been tentatively identified and described by tandem mass spectrometric fragmentation (MS/MS) data. Both taxa included low amounts of (+)-catechin (**3**), (−)-epicatechin (**7**), and procyanidin B dimers (**1**, **2**, **4**). A large number of coumaric acid derivatives and flavonoid glycosides were found, yet not all of the compounds were found in both samples. Quercetin-*O*-hexosides (**18**, **19**) and taxifolin-*O*-hexosides (**12**, **13**) were detected in both taxa; however, the pentose derivative of quercetin (**21**) was only indicated in eastern hemlock. Interestingly, isorhamnetin-*O*-hexosides (**27**, **28**) were found in Norway spruce exclusively. The most abundant class of flavonoid compounds were the kaempferol derivatives (mostly glycosides) with a total count of 11 compounds. Out of these, only kaempferol-*O*-hexoside (**25**) was detected in the green cones of both taxa. The *O*-rutinoside (**24, 37**), *O*-pentoside (**29**, **30**, **31**), *O*-rhamnoside (**33**), acetyl-hexoside (**34**), coumaric acid conjugates (**50**, **58**) and an unknown derivative (**46**) of kaempferol were exclusively detected in eastern hemlock. The presence of acylated kaempferol conjugate (**34**) is especially interesting as these types of compounds were shown to have excellent antioxidant properties and to contribute significantly to the antibacterial effects of plant extracts [43], which highlights the importance in finding matrices with high content of acylated flavonols [44]. The presence of coumaric acid as part of the compounds was evidenced by the simultaneous occurence of the 163, 145, and 119 *m/z* ions in the MS/MS spectra of the compounds corresponding to the $[M\text{-}H]^-$, $[M\text{-}H_2O\text{-}H]^-$ and $[M\text{-}CO_2\text{-}H]^-$ fragment ions (M: coumaric acid). The derivatives **47**, **48**, **49**, **60**, and **67** were only indicated in Norway spruce,

while compounds **50**, **55**, **58**, **61**, and **66** were found exclusively in eastern hemlock while compound **51** in both taxa. Besides of coumaric acid only one compound of ferulic acid (**52**) was evidenced and exclusively in Norway spruce sample. Chlorogenic acid isomers (**5**, **6**) were characteristic to eastern hemlock only. Norway spruce was found to include significant amounts of piceatannol-*O*-hexosides (**10**, **11**) possibly the isomers of astringin, while their aglyone (piceatannol) was indicated only in traces (**15**, **16**). Other compounds were left unidentified only with MS/MS data for future structural identification. According to Table 3 and comparing peak heights in Figure **1**, the most abundant compounds in Norway spruce green cones were piceatannol-*O*-hexosides (**10**, **11**), coumaric acid derivative (**51**) as well as unidentified compounds **8**, **69**, **70**, **71**, whereas in eastern hemlock they were chlorogenic acid isomers **5**, **6**, kaempferol-coumaric acid derivative (**50)**, and unidentified compounds **69**, **70**, **71**, and **80**.

Figure 1. The PDA (250-350 nm) chromatogram of the green cone extracts of Norway spruce (solid red line) and eastern hemlock (dashed green line).

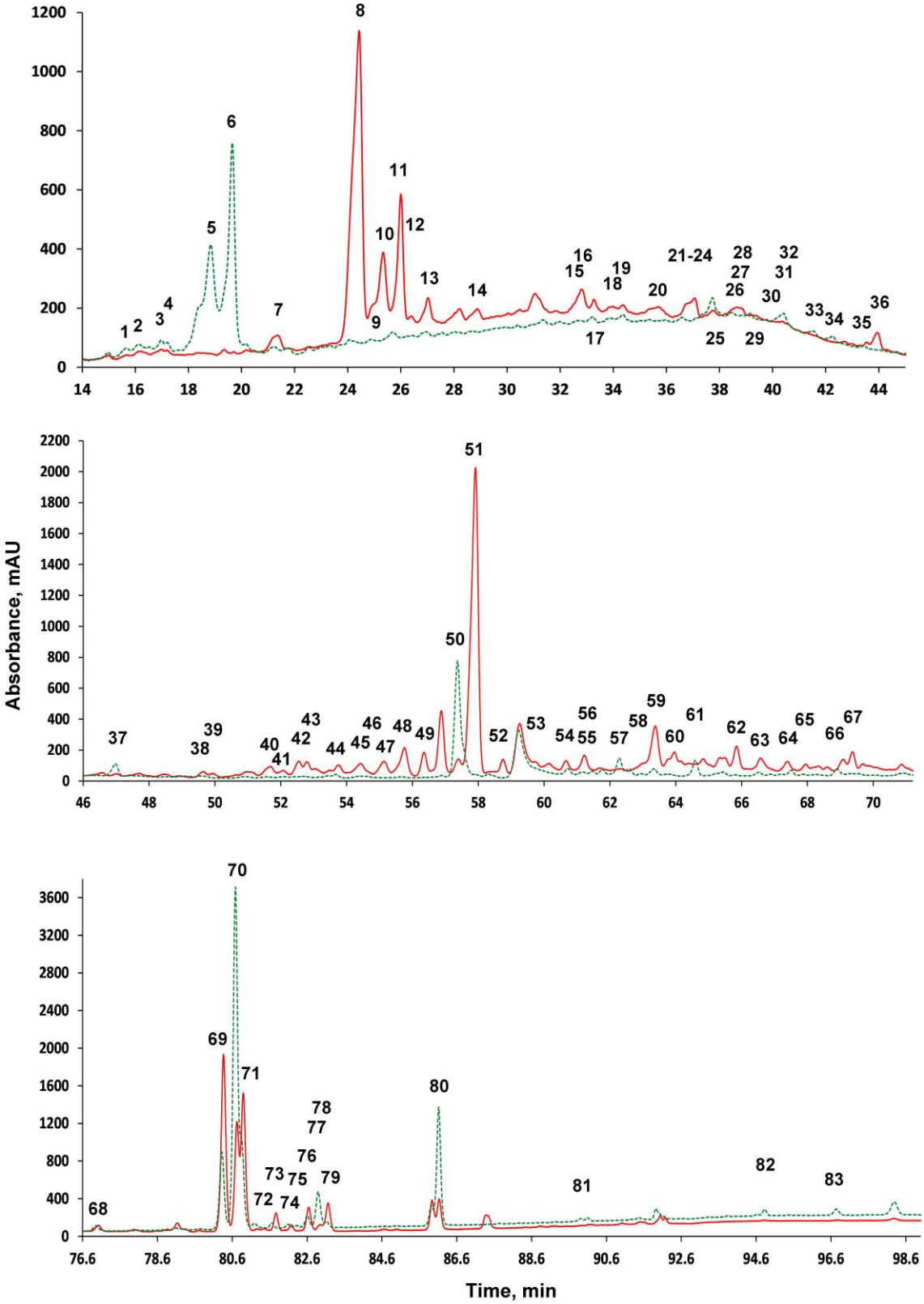

Table 3. Tentative chromatographic/mass spectrometric identification of the polyphenols in the green cones of Norway spruce (S) and eastern hemlock (H).

Peak	t_r (min)	Compound	S	H	$[M-H]^-$ *m/z*	MS/MS *m/z*
1	15.8	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
2	16.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
3	17.0	(+)-Catechin	x	x	289	245, 203, 125, 123, 109
4	17.2	Procyanidin B dimer	x	x	577	425, 407, 289, 245, 125
5	18.9	Chlorogenic acid isomer		x	353	191, 179, 161, 135
6	19.7	Chlorogenic acid isomer		x	353	191, 179, 161, 135
7	21.7	(−)-Epicatechin	x	x	289	245, 203, 125, 123, 109
8	24.0	Unidentified	x		no ion	no negative ions
9	25.0	Unidentified	x		no ion	no negative ions
10	25.3	Piceatannol-*O*-hexoside (astringin)	x		405	243, 225, 201
11	26.0	Piceatannol-*O*-hexoside (astringin)	x		405	243, 225, 201
12	26.3	Taxifolin-*O*-hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
13	27.1	Taxifolin-*O*-hexoside	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
14	29.0	Unidentified	x		285	241, 217, 199
15	32.6	Piceatannol	x		243	225, 201, 175, 174
16	32.8	Piceatannol	x		243	225, 201, 175, 174
17	33.3	Unidentified	x		257	241, 211,
18	33.9	Quercetin-*O*-hexoside	x	x	463	301, 300, 271, 255, 179
19	34.4	Quercetin-*O*-hexoside	x	x	463	301, 300, 271, 255, 179
20	35.4	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
21	36.6	Quercetin-*O*-pentoside		x	433	301, 300, 271, 255, 243, 179
22	36.8	Unidentified	x		373	358, 313, 305
23	37.0	Coumaric acid derivative	x		359	341, 311, 297, 282, 195, 163, 145, 119
24	37.2	Kaempferol-*O*-rutinoside		x	593	447, 285, 284, 255, 227
25	37.7	Kaempferol-*O*-hexoside	x	x	447	285, 284, 255, 227
26	38.2	Unidentified-*O*-hexoside		x	431	268, 269
27	38.6	Isorhamnetin-*O*-hexoside	x		477	315, 314, 300, 299, 271
28	38.9	Isorhamnetin-*O*-hexoside	x		477	315, 314, 300, 299, 271
29	39.2	Kaempferol-*O*-pentoside		x	417	285, 284, 255, 227
30	39.8	Kaempferol-*O*-pentoside		x	417	285, 284, 255, 227
31	40.4	Kaempferol-*O*-pentoside		x	417	285, 284, 255, 227
32	40.5	Unidentified-*O*-hexoside	x	x	447	315, 285, 217, 199
33	41.6	Kaempferol-*O*-rhamnoside		x	431	285, 284, 255, 277
34	42.2	Kaempferol-acetyl-hexoside		x	489	429, 285, 284, 255, 227
35	43.6	Unidentified	x	x	351	333, 315, 275, 251
36	43.9	Unidentified	x		291	245, 175
37	47.0	Kaempferol-*O*-rutinoside	x	x	593	447, 285, 284, 255, 227
38	49.8	Unidentified	x	x	351	333, 315, 275, 251
39	50.0	Unidentified	x		367	349, 321, 247
40	51.7	Unidentified	x		377	331
41	52.0	Unidentified	x		331	313, 273, 241, 185
42	52.6	Unidentified	x		349	331, 287, 251, 244, 207, 189, 163
43	52.8	Unidentified	x		405	375, 337, 327, 275
44	53.7	Unidentified	x		401	333, 315, 257
45	54.4	Unidentified	x		521	179, 162, 146, 135
46	54.7	Kaempferol derivative		x	635	285, 284
47	55.1	Coumaric acid derivative	x		445	427, 397, 349, 277, 251, 163, 145, 119
48	55.8	Coumaric acid derivative	x		475	457, 427, 281, 163, 145, 119

49	56.4	Coumaric acid derivative	x		505	487, 457, 311, 163, 145, 119
50	57.4	Kaempferol-coumaric acid derivative		x	739	593, 453, 285, 284, 255, 227, 163, 145, 119
51	58.0	Coumaric acid derivative	x	x	505	491, 477, 342, 327, 312, 177, 163, 119
52	58.8	Ferulic acid derivative	x		535	520, 491, 341, 207, 193, 179, 163, 149, 134
53	59.7	Unidentified	x	x	445	417, 399, 315
54	60.7	Unidentified	x	x	401	333, 315, 289, 245
55	61.1	Coumaric acid derivative		x	549	489, 353, 311, 163, 145, 119
56	61.2	Unidentified	x		349	331, 289, 245
57	62.1	Unidentified	x	x	399	367, 331, 299
58	62.7	Kaempferol-coumaric acid derivative		x	723	577, 559, 437, 285, 284, 255, 227, 163, 145, 119
59	63.4	Unidentified	x	x	385	317, 299, 253
60	64.0	Coumaric acid derivative	x		667	521, 403, 323, 163, 145, 119
61	64.6	Coumaric acid derivative		x	653	638, 489, 353, 329, 177, 163, 145, 119
62	66.0	Unidentified	x		383	355, 315, 297
63	66.6	Unidentified	x		383	315, 299, 269
64	67.4	Unidentified	x		471	425, 403, 353, 325, 285
65	68.0	Unidentified	x	x	381	313, 269
66	68.9	Coumaric acid derivative		x	651	487, 472, 341, 326, 266, 163, 145, 119
67	69.4	Coumaric acid derivative	x		649	441, 411, 321, 291, 253, 163, 145, 119
68	77.0	Unidentified	x	x	429	381, 299, 265
69	80.4	Unidentified	x	x	687	657, 301
70	80.7	Unidentified	x	x	397	301
71	80.9	Unidentified	x	x	431	401, 383, 301
72	81.2	Unidentified		x	469	425, 410, 384, 367, 339, 285
73	81.7	Unidentified	x		455	409, 391, 387, 355, 287
74	82.1	Unidentified		x	957	467, 423, 381
75	82.2	Unidentified	x		455	409, 391, 387, 355, 287
76	82.4	Unidentified		x	935	467, 424, 382, 265
77	82.6	Unidentified	x	x	721	417, 335, 317
78	82.9	Unidentified		x	467	449, 423, 408, 382, 338
79	83.1	Unidentified	x	x	633	333, 317, 315, 299
80	86.1	Unidentified		x	635	591, 333, 317, 301, 271
81	89.9	Unidentified		x	769	725, 467, 301
82	94.8	Unidentified		x	501	486
83	96.7	Unidentified		x	529	514

4. CONCLUSIONS

The present study compared and evaluated the antioxidant capacity of the cone extracts of 17 selected coniferous taxa. The overall antioxidant power was determined by a scoring system that combined the results of the three antioxidant assays of the study. The overall best antioxidant properties were determined for green cones, followed by mature and opened cones. The highest scores were determined for *Tsuga canadensis, Metasequoia glyptostroboides, Chamaecyparis lawsoniana, Cryptomeria japonica, Thuja orientalis* and *Picea abies*. The profiling of the green cone polyphenols of *Picea abies* and *Tsuga canadensis* was carried out and overall 83 compounds have been tentatively identified for the first time, including piceatannol-, kaempferol-, quercetin-, isorhamnetin-*O*-glycosides, coumaric acid derivatives, chlorogenic acids, and flavan-3-ol compounds. Presented chromatographic/mass spectrometric data on the polyphenolic composition of the cone extracts contributes to the determination of the structure of unidentified compounds and to the research on the role of extractives in determining the bioactivity of cone extracts.

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