Coniferous cones as a forestry waste biomass - a source of antioxidants

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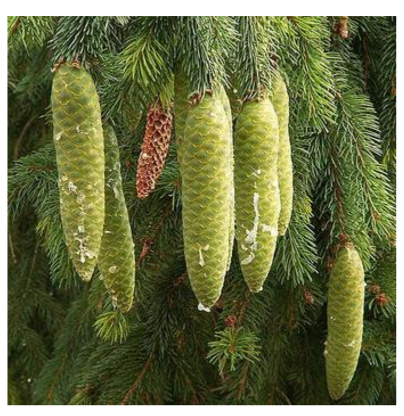
Introduction

The cones of conifers are a waste biomass, potentially be utilized for a variety of purposes, including the extraction of bioactive materials, particularly antioxidant polyphenols. In the present work we conducted a comparative analysis of the antioxidant content of selected taxa that are either common in Hungary or that have not yet been investigated in any great detail *(Cedrus atlantica, Larix decidua, Picea abies, Pinus mugo, Pinus nigra, Pinus sylvestris, Pinus wallichiana, Tsuga Canadensis, Tsuga heterophylla, Pseudotsuga menziesii, Chamaecyparis lawsoniana, Taxodium distichum, Thuja occidentalis, Metasequoia glyptostroboides, Thuja orientalis, Cryptomeria Japonica, Cunninghamia lanceolata*). A comparison of different maturation stages (green, mature, and opened cones) was carried out. Folin-Ciocâlteu total phenol content, ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays were used to assess the antioxidant contents. Total antioxidant power was determined by a scoring system that combined the three assay results. For each taxon the overall best results were found for green cones, 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 profiling of the polyphenols was completed for selected samples. Results provide a basis for future bioactivity testing of these samples.

Samples and extraction

- Samples originated from the Botanical Garden of the University of Sopron, Sopron, Hungary
- 17 taxa were investigated; green, mature and opened cones



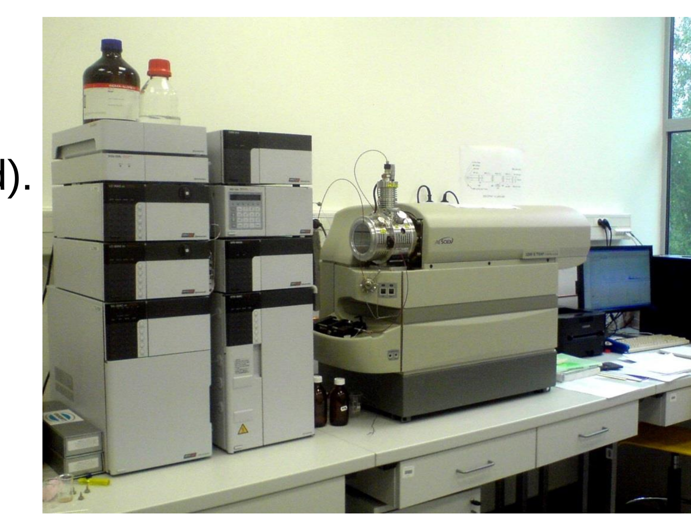
- Samples were ground, then extracted (0.45 g + 45 ml 4:1 acetone:water mixture for 30 minutes by sonication).

Antioxidant capacity measurement

Folin-Ciocalteu total polyphenol content (TPC)
Ferric Reducing Antioxidant Power (FRAP)
2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Chromatographic (HPLC-PDA-ESI-MS/MS) measurements

HPLC: Shimadzu LC-20 liquid chromatograph
MS: AB Sciex 3200 QTRAP® LC/MS/MS system
Stationary phase: Phenomenex Synergy Fusion-RP 80A, 250 mm x 4.6 mm, 4µm , 40 ºC
Mobile phase: A (water + 0.1% formic acid), B (acetonitrile + 0.1% formic acid).
Gradient elution (2%B → 100%B, 106 min.), 1.2 ml/min.
Sample injection: 15 µl
Detection:
1. MS/MS detection (negative electrospray ionization, 80-1300 m/z; MS/MS experiments) for identification
2. PDA detection (250-380 nm) for monitoring the separation of peaks



Antioxidant capacity of the samples

	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

- Highest antioxdant capacity was determined in green cones, followed by mature and opened cones.
- 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.
- 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 using a scoring system.

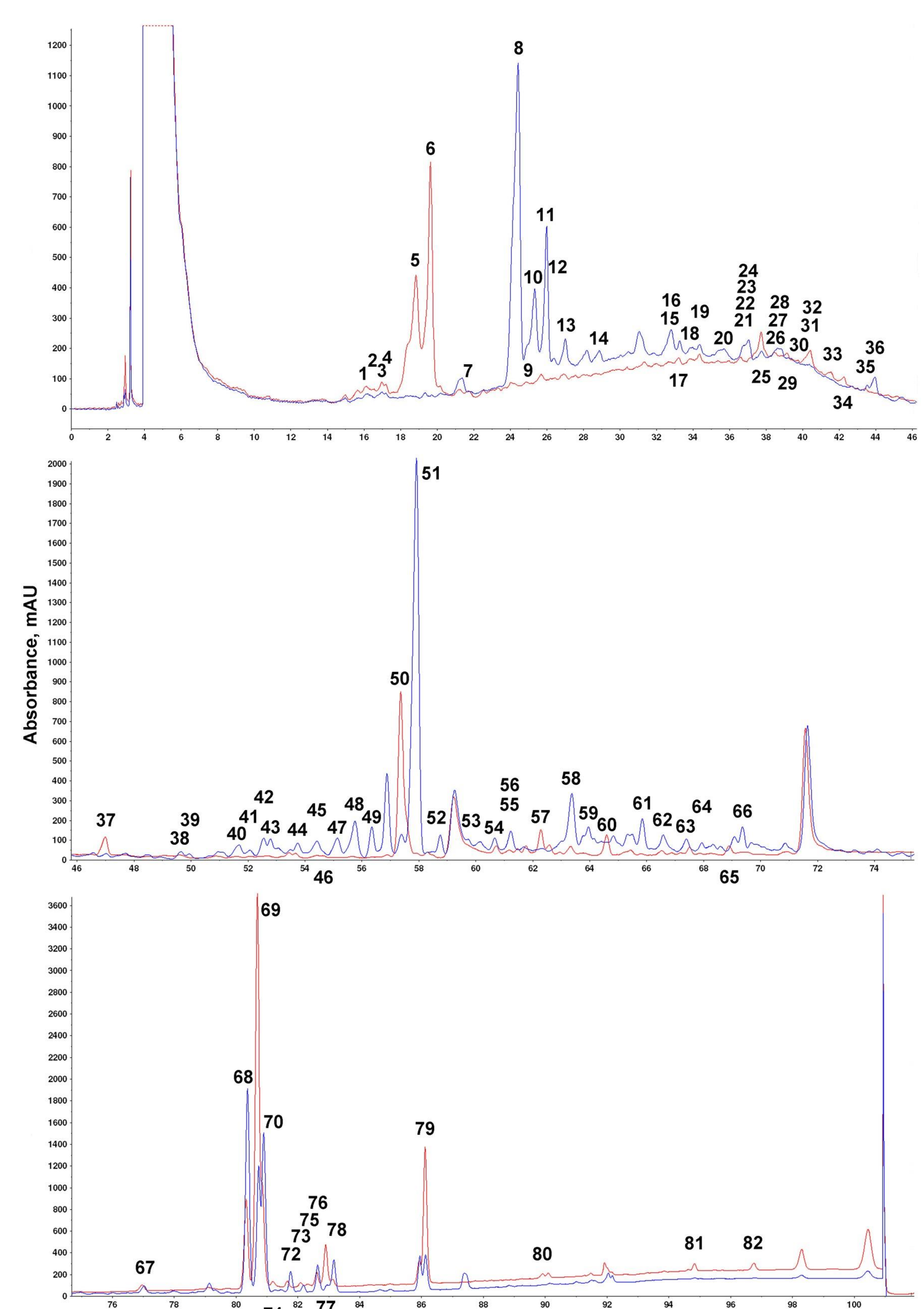
$$\text{Score} = \text{TPC} \cdot \text{FRAP} / \text{DPPH IC}_{50}$$

	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

- Highest scores (best overall antioxidant power) 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) and Norway spruce (707.5)
- Out of these taxa only Norway spruce and eastern hemlock have not yet been investigated for their polyphenolic composition and bioactivity.

polyphenol profiling by HPLC-PDA-ESI-MS/MS
(lots of hard work..)

Polyphenol profiling by HPLC-PDA-ESI-MS/MS



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	Unidentified	x		405	243, 225, 201
11	26.0	Unidentified	x		405	243, 225, 201
12	26.3	Unidentified	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
13	27.1	Unidentified	x	x	465	447, 437, 303, 285, 259, 217, 179, 125
14	29.0	Unidentified	x		285	241, 217, 199
15	32.6	Unidentified	x		243	225, 201, 175, 174
16	32.8	Unidentified	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	Unidentified	x		359	341, 311, 297, 282, 195, 163, 145
24	37.2	Kaempferol-O-rutinoside		x	593	447, 285, 284, 255, 227
25	37.7	Kaemperfol-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	Kaemperfol-O-pentoside		x	417	285, 284, 255, 227
30	39.8	Kaemperfol-O-pentoside		x	417	285, 284, 255, 227
31	40.4	Kaemperfol-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-rhamn.-hex.-rhamn. [1]	x	x	739	593, 453, 285, 284, 255, 229
51	58.0	Coumaric acid derivative	x	x	505	491, 477, 342, 327, 312, 177, 163, 119
52	58.8	Unidentified	x		535	520, 491, 341, 326, 193, 179, 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	63.4	Unidentified	x	x	385	317, 299, 253
59	64.0	Coumaric acid derivative	x		667	521, 403, 323, 163, 145, 119
60	64.6	Coumaric acid derivative		x	653	638, 507, 489, 353, 329, 177, 163, 145, 119
61	66.0	Unidentified	x		383	355, 315, 297
62	66.6	Unidentified	x		383	315, 299, 269
63	67.4	Unidentified	x		471	425, 403, 353, 325, 285
64	68.0	Unidentified	x	x	381	313, 269
65	68.9	Coumaric acid derivative		x	651	487, 472, 341, 326, 266, 163, 145, 119
66	69.4	Coumaric acid derivative	x		649	441, 426, 411, 321, 291, 253, 163, 145, 119
67	77.0	Unidentified	x	x	429	381, 299, 265
68	80.4	Unidentified	x	x	687	657, 301
69	80.7	Unidentified	x	x	397	301
70	80.9	Unidentified	x	x	431	401, 383, 301
71	81.2	Unidentified		x	469	425, 410, 384, 367, 339, 285
72	81.7	Unidentified	x		455	409, 391, 387, 355, 287
73	82.1	Unidentified		x	957	467, 423, 381
74	82.2	Unidentified	x		455	409, 391, 387, 355, 287
75	82.4	Unidentified		x	935	467, 424, 382, 265
76	82.6	Unidentified	x	x	721	417, 335, 317
77	82.9	Unidentified		x	467	449, 423, 408, 382, 338
78	83.1	Unidentified	x	x	633	333, 317, 315, 299
79	86.1	Unidentified		x	635	591, 333, 317, 301, 271
80	89.9	Unidentified		x	769	725, 467, 301
81	94.8	Unidentified		x	501	486
82	96.7	Unidentified		x	529	514

PDA (250-380 nm) chromatogram of spruce (blue) and eastern hemlock (red) green cone extracts and the list of identified compounds (S: Norway spruce, H: eastern hemlock)

- Altogether 82 compounds have been tentatively identified for the first time from Norway spruce and eastern hemlock green cones
- Kaempferol-, quercetin- and 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.
- Results contribute to the valorization of cones and cone extracts in the future.

standard way of cone utilization

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