CHEMICAL ENGINEERING TRANSACTIONS
VOL. 107, 2023
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.cetjournal.it
Guest Editors: Petar S. Varbanov, Bohong Wang, Petro Kapustenko

ISBN 979-12-81206-07-6; **ISSN** 2283-9216
DOI: 10.3303/CET23107112

Utilization of Forestry By-Products as a Source of Natural Antioxidants from Hungarian Forests

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In this work, the antioxidant content of the main forestry and logging by-products (bark, leaves, cones) found in Hungary was compared. The main goal of the study was to identify those wood species and by-products that have the highest antioxidant content and, thus, can be used in the future. The results contribute to sustainable forestry and waste management. The utilization of the by-products of farming (chaff, leaves, pomace, etc.) and forestry (bark, leaves, cones, etc.) is urged not only by stricter environmental protection aspects but also by increasing social responsibility. In recent decades, the research of bioactive compounds from by-products has gained special importance, especially the extraction possibilities of antioxidants: potential areas of utilization are as ingredients in food and cosmeceutical products, production of natural antioxidants and wood preservatives, and production of nanoparticles. The antioxidant capacity and total polyphenol content were measured using several methods. In the case of samples showing the best results, polyphenols were profiled by high-performance liquid chromatography and tandem mass spectrometry (HPLC-PDA-ESI-MS^n). Results could contribute to elaborate future products and utilizations based on the extractives of the investigated Hungarian forestry by-products.

1. Introduction

The research on the utilization of forestry and agricultural waste biomass (e.g. bark, leaves, cones, silage, coffee pulp, etc.) has become an important topic in the last decades all around the world, urged by environmental restrictions, lack of natural resources and increasing prices (Titus et al., 2021). The use and reuse of by-products of forestry also supports circular and local economies, which have gained increasing importance nowadays (Korhonen et al., 2018). All of this supports sustainability and sustainable forestry (Hassegawa et al., 2022). The amount of biomass is considerable, as alone from the processing of logwood, an annual amount of 300-400M m^3 bark is generated, representing an enormous potential (Pásztory et al., 2016). The fields for utilization cover energetics (Makk et al., 2017), extraction of phytoactive compounds, production of composite materials, environmental protection, and nanotechnology (Sutrisno et al., 2020). One of the possible utilization fields is the extraction of antioxidant polyphenolic compounds. These compounds can be used as natural food additives and colorants, antibacterial agents in the packaging (Díez-Pascual, 2020), natural food preservatives and wood conservation agents (Vek et al., 2020), agents for the production of nanoparticles and ingredients in healthcare products (Häsler Gunnarsdottir et al., 2023).
The present work focused on the by-products generated in Hungary to find those tree species and tissues that have the highest antioxidant capacity and polyphenol content and can be possible candidates for future applications. To the best of our knowledge, such a comprehensive research with national - and potentially international - significance has not yet been conducted. The antioxidant capacity of the samples was determined and compared using the DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), TPC (Folin- Ciocalteâu's total polyphenol assay) and ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assays. Samples with the best values were subjected to polyphenol profiling using high-performance liquid chromatography/photodiode array detection/multistage electrospray ionization mass spectrometry (HPLC-PDA-ESI-MS^n) to identify polyphenolic composition. Results will serve as the basis for future research on the

Paper Received: 15 April 2023; Revised: 04 July 2023; Accepted: 20 October 2023
Please cite this article as: Hofmann T., Albert L., Visiné-Rajczi E., 2023, Utilization of Forestry By-Products as a Source of Natural Antioxidants from Hungarian Forests, Chemical Engineering Transactions, 107, 667-672 DOI:10.3303/CET23107112

utilization of these extracts in developing packaging materials, antibacterial agents and metal nanoparticles based on domestic raw materials.

2. Materials and methods

2.1 Sample collection and processing

Bark samples were collected from trees originating from the forests of the TAEG (Tanulmányi Erdőgazdaság) Forestry Company, Sopron, Hungary. The bark was separated into outer bark and inner bark samples and investigated separately. Leaf and cone samples were collected at the Botanical Garden of the University of Sopron in Sopron, Hungary, at specified times of the vegetation period. The samples were dried and ground using a coffee grinder (cones, leaves) or rasped using an 8-grain half-round rasp (bark).

2.2 Extraction

Samples (0.2 g) were extracted with 20 mL methanol:water 80:20 (v/v) solution using ultrasonication (Elma Transsonic T570 ultrasonic bath, Elma Schmidbauer GmbH, Singen, Germany) for 3x10 min at 25-30 ºC. Extracts were centrifuged at 12,000 1/min for 20 min and stored at 4 °C until chemical analyses.

2.3 Measurement of antioxidant capacity

TPC content was determined by the Folin-Ciocalteâu-assay (Singleton and Rossi, 1965) at 760 nm using quercetin and gallic acid as standards. Results were expressed as mg quercetin/g dry weight (mg QE/g dw.) or mg gallic acid/g dry weight (mg GAE /g dw.). The DPPH antioxidant capacity was determined using the method described by Hofmann et al. (2020). Results were calculated in IC_{50} (50 % inhibition concentration) values in µg extractives/ml assay (µg/ml) units. The ABTS antioxidant assay was run at 734 nm using Trolox as the standard. Results were given as mg trolox/g dry weight (mg TE/g dw.) (Stratil et al., 2007). The FRAP antioxidant capacity was determined based on the method of Benzie and Strain (1996) at 593 nm and using ascorbic acid as standard. Results were indicated as mg ascorbic acid/g dry weight (mg AAE/g dw.).

2.4 Polyphenolic composition of selected samples

Separation of polyphenols was achieved using a Shimadzu LC-20 type high-performance liquid chromatograph coupled with a Shimadzu SPD-M20A photodiode array detector (PDA) (Shimadzu Corporation, Kyoto, Japan) and an AB Sciex 3200 QTrap triple quadrupole/linear ion trap mass spectrometer (MS) (AB Sciex, Framingham, USA). A Phenomenex Synergy Fusion-RP 80A, 250 mm × 4.6 mm, 4 µm column with (Phenomenex Inc., Torrance, USA) was used at 40 °C. The injection volume was 8 µL. Gradient elution was run using A (H_2O + 0.1% HCOOH) and B (CH_3CN + 0.1% HCOOH) solvents with 1.2 mL/min flowrate. The PDA signal (250–380 nm) was recorded to monitor the separation of peaks. A negative electrospray ionization mode was set for the MS detector by allowing 0.6 mL/min flow to enter the MS ion source using a split valve. Polyphenol structures were analyzed and identified between the 150-1300 m/z ion range. Ion source settings were as follows: spray voltage: −4500 V, source temperature: 500 °C; curtain gas, spray gas, and drying gas (N_2) pressures: 40 psi, 30 psi, and 30 psi, respectively. Chromatographic and mass spectrometric data were acquired and evaluated using the Analyst 1.6.3 software.

3. Results and discussion

3.1 Bark

The DPPH, FRAP, ABTS, and TPC results of the outer bark samples are shown in Table 1, while the respective results for the inner bark are included in Table 2. For the outer bark samples (Table 1), the best DPPH antioxidant capacity (lowest IC_{50} value) was found in sweet chestnut and sessile oak, while the lowest values were measured in black poplar. Interestingly, the wild cherry with the highest TPC (70.0 ± 2.43 mg QE/g dw.) showed only a moderate DPPH IC_{50} value (12.0 ± 0.32 µg/mL), while in the black locust bark, the low TPC (29.4 ± 3.13 mg QE/g dw.) was accompanied by a fairly high DPPH antioxidant capacity (5.1 ± 0.46 µg/mL). The highest FRAP activity was determined in sweet chestnut and larch extracts. Sessile oak showed moderate activity, while black poplar, white acacia, and Scots pine had the lowest values. In the case of the ABTS assay, similar results were found, with sweet chestnut and larch showing the highest antioxidant activity and Scots pine and hornbeam the overall lowest values. For the inner bark samples, wild cherry, sessile oak, and sweet chestnut resulted in the best DPPH activity, while black locust and black poplar proved to be the worst-performing samples (Table 2). Using the ABTS assay, wild cherry showed an exceptionally high antioxidant capacity (533.3 ± 11.2 mg TE/g dw.), which is almost double the value of the sweet chestnut (264.7 ± 13.9 mg TE/g dw.). The lowest TPC values were measured for Scots pine, black poplar, and birch. The present results obtained for bark extracts are comparable with the results of other researchers (Tanase et al., 2019). Storage

and drying of wood bark as well as the proper choice of solvent also influence the measured antioxidant content for bark samples (Rodríguez-Seoane et al., 2021). Results also outline the different selectivity and complementing feature of the applied antioxidant assays, which was already outlined and discussed in earlier studies (see Munteanu and Apetrei (2021) for review). In fact, none of these assays is individually able to measure the total antioxidant power of all compounds in plant extracts. Therefore, the use of multiple assays to estimate the "overall" antioxidant potential of complex extracts is recommended.

Table 1: Antioxidant capacity of outer bark samples indicated as mean ± standard deviation. Within a given column, lowercase letters in the superscript indicate a significant difference. Values highlighted in bold show the best antioxidant capacity values

Species	DPPH (IC_{50}, µg/mL) p<0.02	FRAP (mg AAE/g dw.) p<0.05	ABTS (mg TE/g dw.) p<0.01	TPC (mg QE/g dw.) p<0.02
European hornbeam	6.2 ± 0.26^{cd}	30.1 ± 1.01^{d}	86.1 ± 0.81^{ab}	25.2 ± 0.63^{ab}
Black locust	5.1 ± 0.46^{c}	19.5 ± 0.86^{b}	103.3 ± 5.85^{b}	29.4 ± 3.13^{b}
European beech	11.1 ± 0.90^{e}	36.4 ± 0.67^{e}	146.6 ± 2.48^{c}	42.7 ± 3.00^{bc}
Sessile oak	**4.0 ± 0.10^{b}**	29.3 ± 0.76^{d}	86.5 ± 9.19^{ab}	71.6 ± 1.20^{d}
Wild cherry	12.0 ± 0.32^{ef}	35.9 ± 0.89^{e}	207.0 ± 7.71^{d}	70.0 ± 2.43^{d}
Sweet chestnut	**2.8 ± 0.11^{a}**	**82.8 ± 0.71^{g}**	**320.1 ± 5.73^{e}**	**89.0 ± 3.90^{e}**
Black poplar	30.2 ± 2.89^{g}	18.3 ± 0.62^{b}	154.7 ± 10.75^{c}	52.8 ± 2.83^{c}
White poplar	6.9 ± 0.60^{d}	38.1 ± 1.38^{e}	153.9 ± 2.53^{c}	49.2 ± 1.35^{c}
Birch	12.8 ± 0.06^{f}	23.4 ± 0.30^{c}	205.2 ± 17.13^{d}	57.3 ± 6.21^{c}
European larch	5.8 ± 0.16^{cd}	**51.4 ± 2.06^{f}**	**371.5 ± 18.53^{f}**	**121.0 ± 4.11^{f}**
Scots pine	11.20 ± 0.61^{e}	10.9 ± 0.62^{a}	61.7 ± 4.37^{a}	16.4 ± 3.32^{a}

Table 2: Antioxidant capacity of inner bark samples indicated as mean ± standard deviation. Within a given column, lowercase letters in the superscript indicate a significant difference. Values highlighted in bold show the best antioxidant capacity values.

Species	DPPH (IC_{50}, µg/mL) p<0.02	FRAP (mg AAE/g dw.) p<0.05	ABTS (mg TE/g dw.) p<0.01	TPC (mg QE/g dw.) p<0.02
European hornbeam	6.2 ± 0.26^{b}	30.1 ± 1.01^{b}	86.1 ± 0.81^{a}	25.2 ± 0.63^{b}
Black locust	11.1 ± 0.90^{f}	36.4 ± 0.67^{b}	146.6 ± 2.48^{b}	42.7 ± 3.00^{d}
European beech	13.3 ± 1.88^{f}	13.6 ± 0.12^{a}	63.7 ± 2.72^{a}	9.9 ± 0.05^{a}
Sessile oak	**4.6 ± 0.13^{a}**	44.5 ± 0.12^{c}	138.4 ± 7.91^{b}	46.2 ± 1.39^{d}
Wild cherry	**4.7 ± 0.05^{a}**	**80.1 ± 3.98^{f}**	**533.3 ± 11.20^{g}**	**139.0 ± 4.00^{h}**
Sweet chestnut	**4.8 ± 0.17^{a}**	**70.9 ± 3.47^{e}**	264.7 ± 13.91^{d}	61.4 ± 1.73^{e}
Black poplar	44.0 ± 2.41^{g}	17.6 ± 0.27^{a}	94.7 ± 4.48^{a}	36.3 ± 0.51^{c}
White poplar	8.8 ± 0.27^{e}	34.6 ± 0.40^{b}	143.2 ± 4.31^{b}	44.1 ± 1.71^{d}
Birch	6.6 ± 0.22^{bc}	32.9 ± 2.23^{b}	**300.4 ± 10.53^{e}**	76.6 ± 0.54^{f}
European larch	6.7 ± 0.04^{c}	**62.3 ± 3.58^{d}**	**345.6 ± 9.28^{f}**	**106.9 ± 0.70^{g}**
Scots pine	7.2 ± 0.09^{d}	42.4 ± 2.66^{c}	219.0 ± 13.98^{c}	76.2 ± 3.15^{f}

3.2 Leaves

The leaf samples were collected and compared during the month of July since the antioxidant content can vary significantly during the growing season (Tálos-Nebehaj et al., 2017). Based on the results of Table 3, it was verified again that the methods used to determine the individual antioxidant capacity resulted in different "orders", which can be explained by the different selectivity and reaction mechanisms of the methods. Overall, the leaves of the European hornbeam showed the best values. The antioxidant capacity of Turkey oak leaves was also very high, despite the fact that the value obtained for the total polyphenol content can be considered average (65.9 ± 1.54 mg QE/g dw.). The DPPH value of Norway maple leaves is also remarkable (7.32 ± 0.44 µg/mL). Compared with the bark samples, it can be concluded that the leaves have a slightly lower antioxidant content. Results are comparable with the values of other studies performed on the antioxidant content of the leaves of forest trees (Pirvu et al., 2013).

Table 3: Antioxidant capacity of leaf samples collected in July, indicated as mean ± standard deviation. Within a given column, lowercase letters in the superscript indicate a significant difference. Values highlighted in bold show the best antioxidant capacity values.

Species	DPPH (IC50, µg/mL) p<0.05	FRAP (mg AAE/g dw.) p<0.03	ABTS (mg TE/g dw.) p<0.05	TPC (mg QE/g dw.) p<0.02
European beech	13.4 ± 0.63^{cd}	36.4 ± 0.53^{b}	132 ± 11.6^{bc}	48.1 ± 1.28^{bc}
European hornbeam	**5.51 ± 0.85^{a}**	**84.0 ± 2.67^{h}**	**281 ± 4.57^{f}**	**106.0 ± 5.57^{h}**
Sweet chestnut	10.5 ± 2.16^{bc}	62.8 ± 2.57^{f}	**199 ± 5.00^{e}**	62.5 ± 1.59^{de}
Black locust	10.2 ± 0.70^{bc}	40.6 ± 2.63^{bcd}	112 ± 1.84^{a}	43.2 ± 0.21^{ab}
Norway maple	**7.32 ± 0.44^{ab}**	50.1 ± 1.82^{e}	187 ± 2.96^{e}	**80.2 ± 1.47^{g}**
Downy oak	8.06 ± 0.38^{ab}	**67.0 ± 2.12^{fg}**	143 ± 2.47^{cd}	63.8 ± 3.31^{e}
Turkey oak	**7.21 ± 0.47^{ab}**	**69.2 ± 2.28^{g}**	**190 ± 4.05^{e}**	65.9 ± 1.54^{ef}
Pedunculate oak	10.4 ± 0.59^{bc}	43.1 ± 2.93^{cd}	126 ± 1.05^{b}	48.3 ± 4.82^{bc}
Sessile oak	7.73 ± 0.67^{ab}	64.2 ± 2.52^{fg}	155 ± 3.18^{d}	59.2 ± 4.06^{de}
Poplar	26.6 ± 1.58^{e}	38.6 ± 1.27^{bc}	126 ± 1.48^{b}	**73.7 ± 3.05^{fg}**
Scots pine	38.7 ± 2.19^{f}	20.0 ± 0.33^{a}	141 ± 3.23^{c}	37.5 ± 3.9^{a}
Black pine	14.8 ± 0.47^{d}	45.7 ± 1.80^{de}	134 ± 2.06^{bc}	53.8 ± 1.56^{cd}

Table 4: Antioxidant capacity of the cones (mean ± standard deviation). Different superscript letters indicate significant differences at p< 0.05 between the samples with the 10 best values

Species	TPC (mg GAE/g dw)			FRAP (mg AAE/g dw.)			DPPH IC_{50} (µg/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
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

3.3 Cones

The results obtained for the cone samples are summarized in Table 4. The composition of the cones was examined in the different phenophases of cone maturation (green, mature, opened cones). Green cones were collected in May, mature cones in July, and open cones in August/September. In all of the investigated taxa, the highest TPC was measured in green cone samples, followed by mature and opened cones. The overall highest TPC, determined for eastern hemlock green cones (157.25 ± 9.98 mg GAE/g dw.) was surprisingly higher than that of the related taxon, western hemlock (89.16 ± 5.51 GAE/g dw). Regarding FRAP results, green cone samples showed the best results in general. The only opposite tendency was observed with dawn redwood and Chinese arborvitae. Overall, the best FRAP was determined for these two species, for their green cones (d.r.: 129.16 ± 3.01 mg AAE/g dw., C.a: 78.49 ± 1.55 mg AAE/g dw.) and for their mature cones (d.r.: 147.0 ± 6.83 mg AAE/g dw., C.a: 93.12 ± 4.84 mg AAE/g dw.) and for the green cones of eastern hemlock (100.11 ± 0.40 mg AAE/g dw.). The DPPH results also showed the general decreasing tendency of the order green > mature > opened cones within a given taxon. The best results were obtained for the mature (4.42 ± 0.07 µg/mL) and green (6.22 ± 0.42 µg/ml) cones of dawn redwood and for green cones of Lawson cypress (7.23 ± 0.41 µg/mL) and eastern hemlock (7.83 ± 0.29 µg/mL). The TPC, FRAP, and DPPH data makes it apparent that all of three assays indicated different orders for the best results, which was attributed to the different compositions of the extracts as well as to the different working principle and selectivity of the assays (Müller et al., 2011).
Based on the results, it can be concluded that among the tested tissues, bark samples had the highest antioxidant content in general. The three samples with the highest values were wild cherry, sweet chestnut, and larch bark. By the investigation of the polyphenols in these samples, the potentially phytoactive substances can be identified, which may have beneficial physiological effects and can provide the basis for the future applicability of these bark extracts. The polyphenols of the bark of the three species were separated and identified using HPLC-PDA-ESI-MS^n technique. The chromatogram of the bark extracts is shown in Figure 1. A total of 123 polyphenolic compounds were identified by name and characterized by mass spectra. The identified compounds are summarized in the work of (Agarwal et al., 2021). Chromatography/mass spectrometry results on the polyphenolic composition of bark extracts also contribute to the determination of the structure of unidentified compounds and the clarification of the role of extract substances in determining the bioactivity of bark extracts.

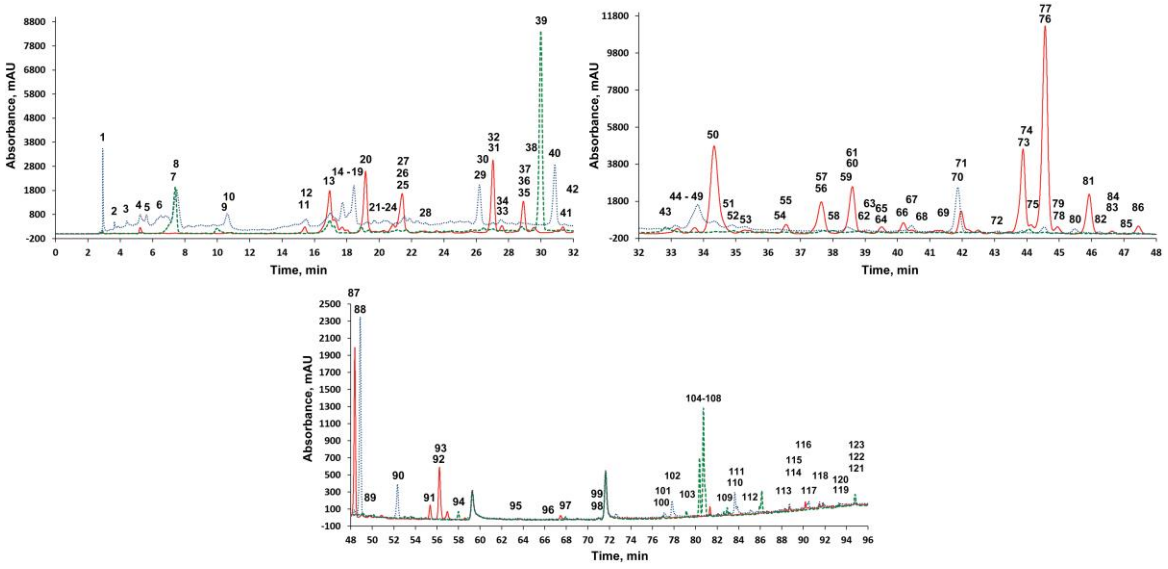

Figure 1: The HPLC-DPA (250-380 nm) chromatogram of the whole bark extracts of wild cherry (red), European larch (green) and sweet chestnut (blue) (Agarwal et al., 2021)

4. Conclusions

Sustainable forestry also requires the processing and valorization of logging and forestry by-products. In the present study, the antioxidant content of the bark, leaf, and cone tissues of major Hungarian forest tree species was investigated and compared. All three resources contained high amounts of antioxidant polyphenols, with the overall highest antioxidant capacity (FRAP) values found in the inner bark of wild cherry (80.1 ± 3.98 mg AAE/g dw.), sweet chestnut (70.9 ± 3.47 mg AAE/g dw.), European larch (62.3 ± 3.58 mg AAE/g dw), in the leaves of European hornbeam (84.0 ± 2.67 mg AAE/g dw.), downy oak (67.0 ± 2.12 mg AAE/g dw.), turkey oak (69.2 ± 2.28 mg AAE/g dw.) and in the green cones of Eastern hemlock (100.11 ± 0.40 mg AAE/g dw.) Lawson

cypress (89.42 ± 6.82 mg AAE/g dw.) and Norway spruce (72.02 ± 8.76 mg AAE/g dw.), as well as in the mature cones of dawn redwood (147.00 ± 6.83 mg AAE/g dw.). According to the results maturity and phenophase of the tissues have a significant effect on the antioxidant content, which influences the collection of basic material for future tests and applications. In the future, the effects of sample storage, drying, and use of "green" extraction solvents (e.g. overpressured water) should also be investigated for obtaining higher extraction yields, and applications using the extractives should be developed.

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