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Combined Multi-assay Evaluation of the Antioxidant Properties of Tree Bark

Esztella TÁLOS-NEBEHAJ* – Levente ALBERT – Eszter VISI-RAJCZI – Tamás HOFMANN

Institute of Chemistry, University of Sopron, Hungary

Abstract – Wood logging generates considerable amounts of bark by-product, which are a potential antioxidant source well worth extracting and using. The present work compares the antioxidant properties of the bark of the following selected Hungarian forest tree species: white poplar (*Populus alba* L.), black locust (*Robinia pseudoacacia* L.), sessile oak (*Quercus petraea* Liebl.), black poplar (*Populus nigra* L.), silver birch (*Betula pendula* Roth), European larch (*Larix decidua* Mill.), scots pine (*Pinus sylvestris* L.), wild cherry (*Prunus avium* L.), European hornbeam (*Carpinus betulus* L.) and sweet chestnut (*Castanea sativa* Mill.). Inner and outer bark were investigated separately. Total polyphenol content (TPC) was determined by the Folin-Ciocâlteu method, whereas antioxidant capacity was assayed using the ferric reducing ability of plasma (FRAP), 2.2-diphenyl-1-picrylhydrazyl (DPPH), and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) methods. The overall antioxidant power of the samples was evaluated using a scoring system that combined the FRAP, DPPH, and ABTS assay results. The TPC levels did not always follow the FRAP, DPPH, and ABTS assay values. Differing reaction mechanisms and sample compositions are possible reasons for this. The presented scoring evaluation was suitable for the assessment and comparison of complex antioxidant properties of tree bark samples. According to the scores, inner bark showed higher scores compared to outer bark for most species with the exceptions of black poplar, black locust, white poplar, sweet chestnut, and European larch. The highest overall antioxidant capacities were determined in the inner bark of wild cherry and the outer bark of sweet chestnut. The species with the overall lowest scores were black locust and black poplar.

bark / antioxidant capacity / polyphenols / combined multi-assay evaluation

Kivonat – Fakéreg antioxidáns tulajdonságainak felmérése kombinált többmódszeres kiértékeléssel. Az erdei fakitermelés során jelentős mennyiségű kéreg melléktermék keletkezik, mely nagy mennyiségben tartalmazhat kivonható és hasznosítható antioxidánsokat. A jelen cikkben kiválasztott magyarországi erdei fafajok (fehér nyár (*Populus alba* L.), akác (*Robinia pseudoacacia* L.), kocsánytalan tölgy (*Quercus petraea* Liebl.), fekete nyár (*Populus nigra* L.), közönséges nyír (*Betula pendula* Roth), európai vörösfenyő (*Larix decidua* Mill.), erdeifenyő (*Pinus sylvestris* L.), vadcseresznye (*Prunus avium* L.), közönséges gyertyán (*Carpinus betulus* L.) és a szelídgesztenye (*Castanea sativa* Mill.)) kérgének antioxidáns tulajdonságait mértük fel és hasonlítottuk össze. Külön vizsgáltuk a külső- és a belső kéreg szöveteket. Az összes polifenol tartalmat (TPC) a Folin-Ciocâlteu módszerrel, az antioxidáns kapacitást a FRAP (vas(III)-ion redukálóképessége) a DPPH (2,2-difenil-1-pikrilhidrazil-gyök közömbösítése) valamint az ABTS (2,2'-azino-bisz(3-etilbenzotiazolin-6-szulfonsav gyök kation reakciója) módszerekkel vizsgáltuk. A minták "összesített" antioxidáns hatását egy pontrendszer segítségével értékeltük, amely kombinálta a FRAP, DPPH és ABTS módszerekkel kapott

* Corresponding author: nebehaj.esztella@gmail.com; 9072 NAGYSZENTJÁNOS, Arany János u. 38, Hungary

eredményeket. A TPC értékei nem mindig követték a FRAP, ABTS illetve DPPH értékeket, feltételezhetőleg a különböző mintaösszetételek, illetve a módszerek eltérő szelektivitása miatt. A bemutatott kiértékelő módszer alkalmas volt a kéregminták antioxidáns tulajdonságainak összehasonlító elemzésére. A pontszámok alapján a legtöbb faj esetében a belső kéreg magasabb antioxidáns tartalommal rendelkezett, mint a külső kéreg, kivéve az akác, fekete- és fehér nyár, vörösfenyő és a szelídgesztenye. A legmagasabb antioxidáns tartalmat a vadcseresznye belső kérgében és a szelídgesztenye külső kérgében mértük. Az összességében legalacsonyabb pontszámokkal jellemzett fajok az akác és a feketenyár voltak.

fakéreg / antioxidáns kapacitás / polifenolok / kombinált többmódszeres kiértékelés

1 INTRODUCTION

Waste products from the food, forestry, and agricultural industries are promising raw materials because they are inexpensive and their reuse provides environmental benefits (Vázquez et al. 2012). In this regard, forest tree bark is especially significant (Molnár 2004, Pietarinen et al. 2006, Diouf et al. 2009, Ekman et al. 2013, Ghitescu et al. 2015) because it is generated during the processing of wood logs in large amounts, with an estimated annual volume of 300-400 million m^3 (Pásztory et al. 2016). Of this amount, about 0.5-0.6 million m^3 are generated in Hungary alone (Molnár 2004). *Table 1* summarizes the proportion of different woody species by occupied area and by wood logging volume in Hungary in 2018.

Table 1. The proportion of woody species by occupied area (KSH1 2019) and wood logging volume (KSH2 2019) in Hungary based on 2018 data

Species	Occupied area (1000 ha)	Wood logging (1000 m^3)
Quercus petraea and *robur* L.	389.3	1004
Quercus cerris L.	212.3	840
Fagus sylvatica L.	112.6	726
Carpinus betulus L.	97.1	259
Robinia pseudoacacia L.	454.2	1586
Other high density hardwood species	119.3	324
Hybrid poplar	197.4*	1188
Indigenous poplar		339
Salix spp.	n/a	61
Other low density hardwood species	97.3	273
Pinus sylvestris L.	110.9	n/a
Pinus nigra J.F.Arnold	59.2	1166**
Other conifers	17.8	n/a
Total	**1867.5**	**7766**

* sum of poplars (*Populus* spp.)
** sum of all coniferous species

Pásztory et al. (2016) recently reviewed potential fields for wood bark utilization. One of these fields is the extraction and use of natural antioxidants. Wood bark contains various types (enzymatic and non-enzymatic) of antioxidants. These compounds not only contribute to the protection of living tissues in trees, but have also beneficial health effects on humans as well.

Regarding tree bark antioxidant utilization, non-enzymatic antioxidants are considered more important and are better researched. The bark of *Cinchona* spp. contains the compound

quinine, which has been applied to cure malaria and was also evidenced to have antioxidant properties (Krishnaveni et al. 2015). The polyphenolic compounds present in high concentration *Acacia mangium* Willd. bark are a promising antioxidant source for cosmetic and pharmaceutical products (Rosdiana et al. 2017). The various types and mixtures of polyphenols in tree bark and bark extracts (e.g. in *Salix* spp.: salicin, catechins, procyanidins, in *Pinus pinaster* Aiton: catechins, procyanidins; and in the dragon blood tree *Draceana cinnabari* Balf. f.: flavylium compounds) were shown to have excellent-health improving and healing properties (Packer et al. 1999, Gupta et al. 2008, Sousa et al. 2008, Zaiter et al. 2016). In *Betula spp.*, the triterpenoids contribute significantly to the antioxidant (Eom et al. 2016) and to the health-related effects of the bark extracts (Hordyjewska et al. 2019).

As seen from the previous examples, the polyphenolic compounds are the most important and abundant types of antioxidants in tree bark. Potential polyphenol uses are broad and include the production of natural food preservatives (Seeram – Heber 2007, Coté et al. 2011, Gyawali – Ibrahim 2014, Kobus-Cisowska et al. 2014), healthcare, and healthcare-related products (Packer et al. 1999, Dzialo et al. 2016, Watson et al. 2018), natural growth bioregulators (Popa et al. 2002, 2008, Vyvyan 2002), food and beverage products (Frydman et al. 2005, Sawalha et al. 2009), and silver nanoparticle production (Fahimirida et al. 2019, Ranoszek-Soliwoda et al. 2019, Rolim et al. 2019). A recent global survey predicted a boom in the polyphenol market due to increasing demand and market size. An annual growth rate of 6.1% is expected (Ameer et al. 2017).

The present study aimed to provide a comparative antioxidant properties analysis of the bark of selected tree species: white poplar (*Populus alba* L.), black locust (*Robinia pseudoacacia* L.), sessile oak (*Quercus petraea* Liebl.), black poplar (*Populus nigra* L.), silver birch (*Betula pendula* Roth), European larch (*Larix decidua* Mill.), Scots pine (*Pinus sylvestris* L.), wild cherry (*Prunus avium* L.), European hornbeam (*Carpinus betulus* L.), and sweet chestnut (*Castanea sativa* Mill.). These important Hungarian industrial wood species either potentially yield large amounts of bark by-products or have not been investigated in detail to date. Although European beech is listed in the topmost section in *Table 1*, the species was not included in the present study as detailed investigations on the antioxidant and bioactive properties of beech bark extracts have recently been completed (Hofmann et al. 2015a,b, 2017, 2019, Tănase et al. 2018, Tanase et al. 2018, 2019, Coșarcă et al. 2019).

The primary functions of tree bark include assimilate storage and translocation as well as physical and physiological protection. Tree bark has two major parts: the inner and outer bark. The inner bark plays an important role in nutrient transport and storage as well as chemical protection, while the outer bark provides protection against mechanical impacts (e.g. chewing by wild animals, fire). As a whole, bark plays a crucial role in thermal insulation and water storage as well (Molnár 2004, Wagenführ–Scholz 2008). The present study investigated the inner and outer bark composition separately to provide detailed results on the antioxidant properties of each species studied. It must be noted, however, that in industrial practice, bark is usually collected as a whole without separating inner and outer parts.

The total polyphenol content (TPC) of the bark extracts was measured using the Folin-Ciocâlteu assay, while the antioxidant capacity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and FRAP (ferric reducing ability of plasma) assays. Antioxidant capacity assays are varyingly selective to different types of compounds (Prior – Cao 1999). Therefore, the comprehensive measure of overall antioxidant properties for each sample was determined and compared by the combined evaluation of the results using a scoring system (Tálos-Nebehaj et al. 2017).

Using the scoring system, samples were ordered according to overall antioxidant power. The species/extracts with the best antioxidant parameters could be potential antioxidant sources for food, nutrition supplements, healthcare products, medical products, or other future applications.

2 MATERIALS AND METHODS

2.1 Chemicals and reagents

Double distilled water was prepared for the extractions using conventional distillation equipment. Methanol (HPLC grade) was obtained from VWR International (Budapest, Hungary). Quercetin, ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox), ABTS, potassium persulfate, DPPH, 2,4,6-tripyridyl-S-triazine (TPTZ), iron(III)-chloride, acetic acid, sodium acetate, hydrochloric acid, sulfuric acid, sodium carbonate, potassium hydrogen phosphate, and potassium dihydrogen phosphate were obtained from Sigma-Aldrich (Budapest, Hungary). Folin-Ciocâlteu reagent was purchased from Merck (Darmstadt, Germany).

2.2 Bark material and extraction

Bark samples were collected from trees originating from the forests of the TAEG (Tanulmányi Erdőgazdaság) Forestry Company, Sopron (Hungary) during December 2015. As the specimens of each tree species were from the same plot, the climatic and other environmental effects were regarded as similar. The trees selected for sampling were mature and healthy, with a diameter at breast height of 30-50 cm. For each species, one representative tree was sampled immediately after felling. An axe was used to strip the bark from the trunk at heights between 1.5-3 meters. About 5 kg of bark material was collected from each tree. The bark samples were immediately taken to the laboratory and were dried for 2 days in the laboratory climate (18^{o}C) in the dark. The inner bark was rasped from the whole bark pieces using a half round wood rasp (8 grain); the outer bark was also separated this way. With European hornbeam, the whole bark was investigated as the bark was too thin to be precisely separated into inner and outer parts. Bark powder in the amount of 0.15 g was extracted with 15 ml methanol:water 80:20 (v/v) solution using ultrasonication (Elma Transsonic T570 ultrasonic bath, Elma Schmidbauer GmbH, Singen, Germany) for 20 min at room temperature. The extracts were filtered using 0.45 μm cellulose-acetate syringe filters and were stored at -20 °C in amber glasses until analysis.

2.3 Spectrometric assays

All measurements were conducted in triplicate (the same extract three times) by using a U-1500 type spectrophotometer (Hitachi Ltd., Tokyo, Japan).

2.3.1 Determination of the TPC

TPC determination was completed by applying the Folin-Ciocâlteu assay (Singleton – Rossi 1965) using quercetin as the standard as follows: extract solution was mixed with 2.5 ml 10-fold diluted Folin-Ciocâlteu reagent. After 1 min, 2 ml 0.7 M Na_2CO_3 solution was added and the reaction mixture was heated for 5 min in a 50 °C water bath. Reaction was stopped by cooling to room temperature in a cold water bath. Solution absorbance was measured at 760 nm. The results were expressed as mg equivalents of quercetin/g dry bark units (mg QE/g d.w.).

2.3.2 Antioxidant assays

The ABTS assay was run based on the method of Stratil et al. (2007), using Trolox as standard and 10 min reaction time at 734 nm. The results were expressed as mg equivalents of Trolox/g dry bark units (mg TE/g d.w.). The FRAP assay was performed as described by Benzie – Strain (1996), using ascorbic acid as standard. Results were expressed as mg equivalents of ascorbic acid/g dry bark units (mg AAE/g d.w.). The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts was determined using a slightly modified method of Sharma – Bhat (2009) as follows: methanol (2090 µl), methanolic DPPH solution (900 µl, 2×10^{-4} M) and 10 µl of the extract were mixed. After incubation in the dark at room temperature for 30 min, the decrease in absorbance was measured at 515 nm. Results were calculated as IC_{50} (50% inhibition concentration) and expressed as µg extractives/ml assay (µg/ml) units, representing the amount of extractives that react with 50% of the added $DPPH^{\bullet}$ radicals in the total volume of the assay (3000 µl) under the conditions used. The calculation was executed using equations 1 and 2 as follows:

$$I = \frac{100\cdot(A_{DPPH}-A)}{A_{DPPH}} \quad (1)$$

$$DPPH\ (IC_{50}) = \frac{1000\cdot E\cdot V}{3000}\cdot\frac{50}{I} \quad (2)$$

I: the rate of inhibition (%)
A_{DPPH}: the initial absorbance of the reaction mixture
A: the absorbance of reaction mixture after the reaction
E: extractive content (mg/ml)
V: the volume of plant extract in the reaction mixture (µl)
3000: the final volume of the reaction mixture (µl).

2.4. Total extractives

The extracts (5 ml) were evaporated to dryness at 70°C in a laboratory oven and the remaining solids were weighed. The total extractive content was expressed as mg extractives/ml extract unit. Results were used to calculate the DPPH IC_{50} values.

2.5 Statistics

Analysis of variance (ANOVA) was performed using Statistica 11 (StatSoft Inc., Tulsa, USA) software, using Tukey's HSD test to compare the respective chemical parameters of the extracts. Prior to ANOVA, data was tested for normal distribution and the homogeneity of variances was checked using Bartlett's *Chi*-square test.

3 RESULTS AND DISCUSSION

3.1 Total amounts of polyphenolic compounds

Polyphenolic compounds are a major type of antioxidants, which are also found in large amounts in woody tissues (Popa et al. 2002, Molnár 2004, Vázquez et al., 2008, Wagenführ – Scholz 2008, Sathya – Siddhuraju 2012). First, the total amount of polyphenols was determined to see their contribution to tree bark tissue composition. TPC results are summarized in *Table 2*.

Table 2. The total polyphenol content (TPC) of inner and outer bark extracts

Species	TPC (mg QE/g d.w.)	
	Outer bark	Inner bark
European hornbeam*	25.2 ± 0.63^{ab}	25.2 ± 0.63^{b}
Black locust	29.4 ± 3.13^{b}	9.9 ± 0.05^{a}
Sessile oak	71.6 ± 1.20^{d}	46.2 ± 1.39^{d}
Wild cherry	70.0 ± 2.43^{d}	**139.0 ± 4.00^{h}**
Sweet chestnut	**89.0 ± 3.90^{e}**	61.4 ± 1.73^{e}
Black poplar	52.8 ± 2.83^{c}	36.3 ± 0.51^{c}
White poplar	49.2 ± 1.35^{c}	44.1 ± 1.71^{d}
Silver birch	57.3 ± 6.21^{c}	76.6 ± 0.54^{f}
European larch	**121.0 ± 4.11^{f}**	**106.9 ± 0.70^{g}**
Scots pine	16.4 ± 3.32^{a}	76.2 ± 3.15^{f}

Results were indicated in average ± standard deviation. Within a given column, the different superscript letters indicate a significant difference at $p<0.02$. Bold values indicate the highest TPC values.

* Whole bark was investigated as it could not be separated into inner and outer parts. The results for the inner and outer bark correspond here to the same (whole bark) sample.

In the distribution of polyphenolic compounds between inner and outer bark tissues, inner bark did not always possess a higher TPC than outer bark. Outer bark was found to be richer in polyphenolic compounds in black locust, sessile oak, sweet chestnut, black poplar, and European larch.

Outer bark sample comparisons revealed larch and sweet chestnut had the highest TPC. Earlier results, determined from the same extracts (Tálos-Nebehaj et al. 2018), indicated a high flavan-3-ol content in the outer bark tissues of European larch (20.0 mg (+)-catechin/g d.w.), and high flavonoid content (4.81 mg quercetin/g d.w.) was found in sweet chestnut. These compounds, together with other unmeasured compounds (e.g. hydrolizable tannins in chestnut bark), may account for the high TPC in the mentioned tissues. The overall lowest TPC was determined in Scots pine, E. hornbeam, black poplar, and birch.

Wild cherry and European larch had the highest TPC levels in the inner bark while European hornbeam, and black locust had the lowest. The high TPC was accompanied by very high flavan-3-ol levels in both cherry (61.8 mg (+)-catechin/g d.w.) and larch (32.0 mg (+)-catechin/g d.w.) as determined by Tálos-Nebehaj et al. (2018) from the same extracts.

Comparing the inner bark of European beech (*Fagus sylvatica* L.), the TPC of the extracts gained with ultrasonic extraction and with methanol:water 80:20 v/v was 42.66 mg QE/g d.w., which is between the values of white poplar and sessile oak (Hofmann et al. 2015a,b).

Polyphenolic compounds are not the only compounds that influence the antioxidant power of bark tissues, especially in the inner bark where other types of reducing compounds can also be present in large amounts (e.g. sugars, organic acids, enzymes, etc.). These can influence the antioxidant power of the extracts significantly (Prior et al. 2005, Everette et al. 2010). This makes the application of other assays necessary, which are in turn also selective to various other types of compounds due to their specific working mechanism and reaction principle.

3.2 Antioxidant capacity

The DPPH, FRAP, and ABTS antioxidant capacity results of the outer bark samples are detailed in *Table 3*, while the respective results for inner bark are included in *Table 4*.

Table 3. The DPPH, FRAP, and ABTS antioxidant capacities of the outer bark extracts

Species	Outer bark		
	DPPH (IC_{50}, μg/ml) *p < 0.02*	FRAP (mg AAE/ g d.w.) *p < 0.05*	ABTS (mg TE/g d.w.) *p < 0.01*
European hornbeam*	6.2 ± 0.26^{cd}	30.1 ± 1.01^{d}	86.1 ± 0.81^{ab}
Black locust	5.1 ± 0.46^{c}	19.5 ± 0.86^{b}	103.3 ± 5.85^{b}
Sessile oak	**4.0 ± 0.10^{b}**	29.3 ± 0.76^{d}	86.5 ± 9.19^{ab}
Wild cherry	12.0 ± 0.32^{ef}	35.9 ± 0.89^{e}	207.7 ± 7.71^{d}
Sweet chestnut	**2.8 ± 0.11^{a}**	**82.8 ± 0.71^{g}**	**320.1 ± 5.73^{e}**
Black poplar	30.2 ± 2.89^{g}	18.3 ± 0.62^{b}	154.7 ± 10.75^{c}
White poplar	6.9 ± 0.60^{d}	38.1 ± 1.38^{e}	153.9 ± 2.53^{c}
Silver birch	12.8 ± 0.06^{f}	23.4 ± 0.30^{c}	205.2 ± 17.13^{d}
European larch	5.8 ± 0.16^{cd}	**51.4 ± 2.06^{f}**	**371.5 ± 18.53^{f}**
Scots pine	11.2 ± 0.61^{e}	10.9 ± 0.62^{a}	61.7 ± 4.37^{a}

Results are expressed as average ± standard deviation. Within a given column, the different superscript letters indicate a significant difference at the given significance level. Bold numbers highlight the best antioxidant values within a method.

* Whole bark was investigated as it could not be separated into inner and outer parts. The results for the inner and outer bark correspond here to the same (whole bark) sample.

Comparing the *outer bark samples*, the best DPPH antioxidant activities (lowest IC_{50} value) were measured for sweet chestnut and sessile oak, while black poplar obtained the poorest result. The difference between white poplar (6.88 ± 0.60 μg/ml) and black polar (30.2 ± 2.89 μg/ml) was remarkable and requires further evaluation and interpretation. It was also noteworthy that wild cherry, showing one of the highest TPC (70.0 ± 2.43 mg QE/g d.w.), was characterized with only moderate DPPH IC_{50} (12.0 ± 0.32 μg/ml), while in the case of black locust, the low TPC (29.4 ± 3.13 mg Q/g d.w.) was accompanied by a fairly good DPPH antioxidant power (5.11 ± 0.46 μg/ml). The findings in these samples indicate two possibilities: it is not the polyphenolic compounds alone that determine the DPPH activity, or the DPPH reducing power of the polyphenols present in these samples is quite variable. The highest FRAP antioxidant power was present in sweet chestnut and larch; sessile oak showed only moderate FRAP activity; while the lowest activity was measured in black poplar, black locust, and Scots pine. When the DPPH and FRAP assays results were compared, a slightly different order was found, which was explained by the different selectivity and reaction mechanism of the two assays. The ABTS assay results were similar to the FRAP assay results, indicating similar selectivity: highest ABTS power was found for larch and sweet chestnut, while pine and hornbeam extracts demonstrated the lowest ABTS activity.

In *inner bark extracts*, cherry, sessile oak and sweet chestnut showed the best DPPH activity, while black locust and black polar produced the poorest results. Interestingly, the sessile oak sample showed one of the best DPPH radical scavenging activities (4.56 ± 0.13 μg/ml) even though it had only a medium TPC (46.2 ± 1.39 mg QE/g d.w.). Cherry, sweet chestnut, and larch inner bark extracts exhibited the highest FRAP, while black poplar the lowest overall FRAP. With the ABTS method, cherry showed an outstandingly high antioxidant capacity (533.3 ± 11.2 mg TE/g d.w.), which was almost double that of sweet chestnut (264.7 ± 13.9 mg TE/g d.w.). Larch and birch were also found to have excellent ABTS power, while black poplar, black locust, and hornbeam displayed the lowest values.

In scientific literature, researchers use various standard compounds for determining antioxidant capacity, and they can also indicate results in various differing units, which makes study comparisons quite difficult. Moreover, investigating inner and outer tree bark tissue

separately is quite common. Respecting these facts, the following, selected results found in the literature are presented and compared with the results of the present article. The examples are limited to those that are comparable in terms of standards and units of measurements.

Table 4. The DPPH, FRAP, and ABTS antioxidant capacities of the inner bark extracts

Species	Inner bark		
	DPPH (IC_{50}, μg/ml) *p < 0.05*	FRAP (mg AAE/ g d.w.) *p < 0.03*	ABTS (mg TE/g d.w.) *p < 0.05*
European hornbeam*	6.2 ± 0.26^{b}	30.1 ± 1.01^{b}	86.1 ± 0.81^{a}
Black locust	13.3 ± 1.88^{f}	13.6 ± 0.12^{a}	63.7 ± 2.72^{a}
Sessile oak	**4.6 ± 0.13^{a}**	44.5 ± 0.12^{c}	138.4 ± 7.91^{b}
Wild cherry	**4.7 ± 0.05^{a}**	**80.1 ± 3.98^{f}**	**533.3 ± 11.20^{g}**
Sweet chestnut	**4.8 ± 0.17^{a}**	**70.9 ± 3.47^{e}**	264.7 ± 13.91^{d}
Black poplar	44.0 ± 2.41^{g}	17.6 ± 0.27^{a}	94.7 ± 4.48^{a}
White poplar	8.8 ± 0.27^{e}	34.6 ± 0.40^{b}	143.2 ± 4.31^{b}
Silver birch	6.6 ± 0.22^{bc}	32.9 ± 2.23^{b}	**300.4 ± 10.53^{e}**
European larch	6.7 ± 0.04^{c}	**62.3 ± 3.58^{d}**	**345.6 ± 9.28^{f}**
Scots pine	7.2 ± 0.09^{d}	42.4 ± 2.66^{c}	219.0 ± 13.98^{c}

Results are expressed as average ± standard deviation. Within a given column, the different superscript letters indicate a significant difference at the given significance level. Bold numbers highlight the best antioxidant values within a method.

* Whole bark was investigated as it could not be separated into inner and outer parts. The results for the inner and outer bark correspond here to the same (whole bark) sample.

Hofmann et al. (2015a,b) determined 11.12 ± 0.90 μg/ml DPPH IC_{50} value for the methanolic extracts of inner bark from European beech, while Gao et al. (2007) measured inner and outer bark for Lawson cypress (*Chamaecyparis lawsoniana* (A. Murr.) Parl.) at 10.31 μg/ml and 19.87 μg/ml respectively. According to Noriega et al. (2015), the DPPH IC_{50} value of the ethanolic bark extract of red cinchona (*Cinchona pubescens* (Vahl)) was 42.00 μg/ml, which is comparable with the respective result of black poplar inner bark (44.0 ± 2.41 μg/ml). The DPPH IC_{50} values of the bark extracts of Swiss pine (*Pinus cembra* L.) (71,1 μg/ml, Apetrei et al. 2011) and Australian pine (*Casuarina equisetifolia* L.) (101,69 μg/ml, Zhang et al. 2010) are also poorer compared to the present study results.

According to the data of Hofmann et al. (2015b), the FRAP (36.42 ± 0.67 mg AAE/g d.w.) and ABTS (146.65 ± 2.48 mg TE/g d.w.) values of the extracts of European beech inner bark can be considered as average compared to the respective results of this article.

The above comparison indicates that the species investigated in this study had quite variable antioxidant capacities, with some showing excellent results when compared to the bark extracts of other species. However, it is also apparent from the data that DPPH, FRAP, and ABTS methods have different selectivities, which is reflected by difference sample order when comparing highest and lowest values. In order to simplify evaluation, a method that combines the results of different antioxidant assays is presented.

3.3 Combined evaluation of antioxidant assays

Researchers usually apply at least three different antioxidant capacity methods to assess the antioxidant properties of plant extracts as none of the currently applied methods are alone suitable to determine overall antioxidant capacity. This is because each assay is specific to certain types of antioxidants; thus none of the methods evaluate the overall antioxidant power of a plant extract. The different selectivity of each method makes the combined evaluation of

different assays necessary (Hofmann et al. 2017). This was achieved in the present work through the use of a scoring system combining DPPH, FRAP and ABTS antioxidant assay results. Although in a broader sense the TPC method is also considered an antioxidant assay due to its reaction mechanism (Everette et al. 2010), it was excluded from the scoring evaluation in this study.

This combined multi-assay evaluation was completed as follows: samples were ordered according their antioxidant capacity value within each assay; a score of 1 was assigned to the highest antioxidant capacity; and a score of 0 was given to the lowest antioxidant capacity sample. Opposite scoring was used for the DPPH values because the lowest IC_{50} value (score: 1) represented the highest, while the highest IC_{50} (score: 0) represented the weakest antioxidant power. Scores were assigned proportionally in the range [1:0] for samples with intermediate antioxidant capacity values. Finally, scores were summed for each inner and outer bark sample. The maximum score was 3 (when a sample had the highest antioxidant capacity with all of the 3 methods, for example as with the inner bark of wild cherry). *Table 5* includes the calculated scores of the samples, a sum of scores for each sample, and the overall sum for species.

Table 5. Evaluation of the DPPH, FRAP, and ABTS antioxidant capacities of the inner and outer bark samples using the scoring system, sum of scores for each sample (species/tissue type), and the overall sum for species

Species	Inner bark				Outer bark				**Overall**
	DPPH	FRAP	ABTS	*Sum*	DPPH	FRAP	ABTS	*Sum*	
Sweet chestnut	0.99	0.86	0.43	*2.28*	1.00	1.00	0.83	*2.83*	**5.12**
European larch	0.95	0.73	0.60	*2.28*	0.89	0.56	1.00	*2.45*	**4.73**
Wild cherry	1.00	1.00	1.00	*3.00*	0.66	0.35	0.47	*1.48*	**4.48**
Silver birch	0.95	0.29	0.50	*1.74*	0.60	0.17	0.46	*1.24*	**2.98**
Sessile oak	1.00	0.46	0.16	*1.62*	0.96	0.26	0.08	*1.29*	**2.92**
White poplar	0.89	0.32	0.17	*1.38*	0.85	0.37	0.30	*1.52*	**2.89**
European hornbeam*	0.96	0.25	0.05	*1.25*	0.88	0.27	0.08	*1.22*	**2.48**
Scots pine	0.93	0.43	0.33	*1.70*	0.69	0.00	0.00	*0.69*	**2.39**
Black locust	0.78	0.00	0.00	*0.78*	0.92	0.12	0.13	*1.17*	**1.95**
Black poplar	0.00	0.06	0.07	*0.13*	0.00	0.10	0.30	*0.40*	**0.53**

* Whole bark was investigated as it could not be separated into inner and outer parts. The results for the inner and outer bark correspond here to the same (whole bark) sample.

According to *Table 5*, the best overall antioxidant capacity for the inner bark samples was found in wild cherry (3.00), sweet chestnut, and European larch (2.28), while for the outer bark extracts sweet chestnut (2.83) and European larch (2.45) showed outstanding scores. The inner bark of wild cherry had the best antioxidant power with all three applied assays.

Combining the results of the inner and outer bark sample (not considering here the size and ratio of bark tissues compared to each other within one species), the best performing samples overall were sweet chestnut > European larch > wild cherry. The species with the lowest activity were Scots pine > black locust > black poplar.

Further analysis on bark tissue composition is required to reveal which compounds or combination of compounds are responsible for the antioxidant effects in the samples with the best antioxidant properties.

4 CONCLUSIONS

The present article conducted a comparative investigation of bark tissue antioxidant capacities of major Hungarian forest trees, focusing on the composition of the inner and outer bark tissues separately. Results were combined and compared using a scoring system. The TPC levels did not always follow the values of the FRAP, DPPH, and ABTS assays. The different reaction mechanisms were a possible cause of this. According to the scores, inner bark showed higher scores compared to outer bark for most species; black poplar, black locust, white poplar, sweet chestnut, and European larch were the exceptions. The highest overall antioxidant capacities were determined in the inner bark of wild cherry and the outer bark of sweet chestnut. The species with the overall lowest scores were black locust and black poplar.

According to the results, the presented multi-assay based antioxidant capacity evaluation method was suitable for tracking complex antioxidant properties in wood bark, and can be also applied to other tissues such as cones or leaves. Samples with the best results need further evaluations to determine possible future uses.

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