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DETERMINATION OF PHENOLIC SUBSTANCES AND ANTIOXIDANT ACTIVITY IN SOME TREE BARK EXTRACTS

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Abstract

In this study, the total amount of phenolic substances and antioxidant activity were determined in extracts obtained from the barks of three coniferous (scots pine, spruce, cedar) and eight deciduous (poplar, chestnut, oak, pseudoacacia, beech, eucalyptus, iron and mimosa) tree species. The tree barks were extracted with alcohol: benzene (1: 2 v / v) solvent according to the TAPPI T 204 cm-07 standard. The antioxidant activities (AA) of the extracts were determined by the capture activity of the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. The total amount of phenol in the contents of the extracts (TPM (mg-GAE / g-DB)) was determined by the Folin-Ciocalteu method. This study also provides information on the antioxidant activity of the extract due to its total phenolic substance dependence on oxidation-reduction reactions, and because the total amount of phenolic substance in the bark extract has a linear relationship with TPM (mg-GAE / g-DB) and antioxidant activity (AA). In comparison to the coniferous tree barks, higher tannin content and antioxidant activity were determined in the deciduous tree (excluding beech) barks. The highest TPM (mg-GAE / g-DB) and AA were found especially in the extracts of the mimosa (*Acacia Dealbata* L.), iron (*Casuarina equisetifolia* L.), oak (*Quercus pontica* L.) and poplar (*Populus tremula* L.) species.

Keywords: Antioxidation activity, extraction, tree bark, total polyphenols analysis

1. Introduction

Wood is a natural bio-composite which consists of cellulose, hemicellulose, lignin and extractives (Rowell 2012). It has remained its significance throughout the history due to its unique properties. However, alternative materials such as iron, aluminium and steel have been tried as substitutes for wood. Its low maintenance cost, easy processing and higher quantity than these make wood a more valuable material. Moreover, as being one of the environmentally friendly materials, it has been mostly preferred for variety of applications, for example, construction, furniture, siding, decking, etc. (Rowell 2012).

As a result of natural processes, biomaterials have to decompose to complete their natural cycle (Schmidth 2006). However, this is undesirable for wood as it shortens its service life. Wood is treated with toxic chemicals, such as coper-based preservatives (CCA) to eliminate the negative effects on wood and enhance its service life (Temiz et al. 2006). These chemicals are both expensive and harmful for humans as well as the environment. The increasing susceptibility against the environment has initiated new investigations about natural preservatives. In recent years, wood bark extractives have received attention. Hundreds of studies have been carried out for this purpose (Onuorah 2000, Kartal et al. 2006, Yang 2009, Singh and Singh 2011, Tascioglu et al. 2013, Durmaz et al. 2015, Özgenc et al. 2016, Ozgenc and Durmaz 2016, Can et al., 2019).

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As wood constitutes up to 20% of trees, it is accepted as the most abundant forest residue (Fengel and Wegener 1989). Tree barks are usually left in the forest or used to obtained energy (Görcelioğlu 1973, Huş 1976). They have a complex structure consisting of main cell wall components (cellulose, hemicellulose and lignin) and extractives (Sillero et al. 2019). Meanwhile, tree barks are significantly rich in extractives in comparison to wood. Polyphenolic compounds are largely found in bark, leaves and heartwood (Hillis, 1987). Therefore, these compounds conserve the tree against external threats due to having biological activities, antioxidant activity and antifungal activity (Mihara et al. 2005). Tannins, flavonoids, lignans, stilbenes, terpenes and terpenoids have been recognised as components of extractives with high protective properties (Sing and Sing 2011, Tascioglu et al. 2013). While stilbenes have fungistatic and fungitoxic properties, tannins also inhibit fungal growth (Harun and Labosky 2007). Free radicals could inhibit wood decay. Extractives from lignocellulosic materials could be used in medical treatment or as food preservative, wood adhesive (Piccand et al. 2019, Sillero et al. 2019). Antioxidants restrained the aggregation of free radicals, therefore preventing cellular damage and aging (Saravanakumar et al. 2019).

The objective of this study was to examine the antioxidant properties of bark extracts. The total amount of phenolic substances and antioxidant activities of bark extractives were determined in this study. The extracts of a total of 11 tree species (scots pine, spruce, cedar, poplar, chestnut, oak, pseudoacacia, beech, eucalyptus, iron and mimosa) were investigated for this purpose. Alcohol: benzene solvent was used for extraction.

2. Materials and Methods

2.1. Tree Barks

In this study, eleven tree species barks which were 30-40 years old in Turkey, including scots pine (*Pinus sylvestris* L.) from Trabzon, cedar (*Cedrus libani* L.) from Antalya, spruce (*Picea orientalis* L.) from Gümüşhane, poplar (*Populous tremula* L.) from Giresun, chestnut (*Cestanea sativa* L.) from Aydın, acacia (*Robinia pseudoacacia* L.) from Trabzon, oak (*Quercus pontica* L.) from Trabzon, beech (*Fagus orientalis* L.) from Gümüşhane, eucalyptus (*Eucalyptus globulus* L.) from Antalya, iron (*Casuarina equisetifolia* L.) from Trabzon and mimosa (*Acaccia dealbata* L.) from Trabzon were provided. The tree barks were dried at room temperature (~25°C).

2.2. Bark Extraction

Tree air-dried tree barks were ground with a laboratory-scale Willey mill to obtain 40-60-mesh bark powders. The bark powders were subjected to extraction with alcohol: benzene (1:2 v/v) solution in a Soxhlet extractor for 6 hours for the softwood species and for 4 hours for the hardwood species. The solvents from the extracts were concentrated with a rotary evaporator at 50°C and stored in sealed flasks at 4°C until use.

2.3. Determination of Total Phenolic Content

The total phenolic content of extracts was determined by the Folin-Ciocalteu method at 765 nm with a spectrophotometer (PG Instruments, T60/Leicestershire, the United Kingdom). A 0.1 ml aliquot of extract was combined with 0.1 ml Folin-Ciocalteu reagent, 2 ml of 2% (w/v) sodium carbonate and 2.8 ml deionised water.

2.4. Determination of antioxidant activity

The antioxidant activities of the extracts were evaluated with 2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Yu et al. (2005). The following equation was used to determinate the inhibition of DPPH.

$$\% \text{ Inhibition} = (X_{control} - X_{sample}) / X_{control} \times 100 \qquad (1)$$

Where Xcontrol is the absorbance of control at 517 nm and Xsample is the absorbance of sample at 517 nm. Antioxidant capacity was stated as mg Trolox/g dried bark extractives (mg-TE/g-DB). Three repetitions were made for each experiment.

3. Results

The total phenolic content (TPC) and antioxidant activity (AA) of the tree bark extracts of three different coniferous species and eight different deciduous species were examined. As seen in Table 1, the TPC and AA of the bark extracts were very impressive. It is shown that the total phenolic content of the bark extracts was mostly compatible with antioxidant activity. The variety of compounds especially stocked in heartwood influence the durability of wood (Schmidth 2006). Tannins included in the class of phenolics are important components of tree barks (Aydın and Üstün 2007). In general, flavonoid monomers up to 20 constitute tannins (Pizzi et al. 1986). Flavonoids are also effective on antioxidant activity (Yu et al. 2005).

According to the obtained results, the antioxidant activity of extracts was found to be higher in the deciduous tree bark extracts than the coniferous species. The highest AA activity was found to be in mimosa (91.30) for the deciduous species, while it was in spruce (81.52) for the coniferous species. Indeed, there was a gap between the coniferous and deciduous species. Meanwhile, the lowest AA was found from cedar bark extract for the coniferous species, while it was found from beech tree bark extract for the deciduous species.

The total phenolic content of the bark extracts is almost all in parallel with their antioxidant activity. Likewise, the TPC values of the deciduous tree bark extracts were higher than the coniferous tree bark extracts. Similarly, a big difference was also seen in the TPC results. It was seen that the highest total phenolic contents were found from the mimosa, iron, oak and poplar tree bark extracts, respectively. On the contrary, the lowest phenolic contents were in the beech, cedar, and pine tree bark extracts, respectively.

Table 1: Total Phenolic Content (TPC) and antioxidant activity (AA) of bark extracts.

Tree Species	TPC (mg-GAE/g-DB)	AA (%)
Pinus sylvestris L.	56.6 ± 2.2	68.9 ± 1.4
Picea orientalis L.	86.6 ± 2.6	81.5 ± 2.2
Cedrus libani L.	46.6 ± 2.1	63.6 ± 1.8
Populus tremula L.	117.1 ± 2.5	89.3 ± 2.2
Castanea sativa L.	79.8 ± 1.6	90.6 ±2.3
Queercus pontica L.	119.7 ± 3.1	91.1 ± 2.1
Robinia pseudoacacia L.	71.3 ± 1.8	87.2 ± 2.0
Fagus orientalis L.	35.6 ± 1.4	41.5 ± 1.6
Eucalyptus globulus L.	69.0 ± 1.7	88.5 ± 1.9
Casuarina equisetifolia L.	121.5 ± 2.6	90.5 ± 1.9
Acaccia dealbata L.	215.5 ± 2.8	91.3 ± 2.0

4. Discussion

Natural durability is defined as resistance against bacteria, fungi, beetles and marine borers without any treatment of wood (Schimdth 2006). At this point, it may be stated that wood extractives are responsible for natural durability. The main component of extractives is phenols such as terpenoids, flavonoids, stilbenes and tannins. These components have an effect on inhibiting fungal activity. For this purpose, the phenolic content of extractives is the most important parameter, if it is desired to evaluate them as wood preservatives. As known, plant extractives, having a potential, have been investigated to be used as natural wood preservatives in recent years (Kartal et al. 2006, Mohan et al. 2008, Yang 2009, Tascioglu et al. 2013).

In this study, the TPC contents of the tree barks were found to be low as in comparison to those reported by Sillero et al. (2019). They examined the barks of six different species and found these values to vary between 178.11 to 635.08 mg GAE/g. Piccand et al. (2019) determined the antioxidant activity of different extractives with the DPPH assay whose effectiveness is restricted in case of non-polar extractives. Therefore, they stated that the AA of the extracts was found to be low, which was compatible with our results. The solvent plays an important role to determine the TPC and AA of bark extractives.

Deciduous wood species had the highest phenolic contents. Especially the mimosa bark extractives had the highest TPC. It is known that phenolic components play a protective role against UV light (Volf et al. 2014). Therefore, the wood species which have the highest TPC and AA may be used to obtain wood preservatives, UV absorbing agents, etc. This study highlighted the importance of tree bark extracts which can be utilised in the preservative industry as well as the medicine, cosmetic and food industries.

5. Conclusion

Tree bark is regarded to be forest residue which is abandoned to decay in the forest. In this study, a total of 11 tree species were evaluated to determine the phenolic content and antioxidant activity of their barks. Therefore, it was aimed to propose various application fields. According to the obtained results, the total phenolic content and antioxidant activity of the extracts were found to be considerably high. TPC and AA results were obtained from the deciduous tree species. In particular, the best results were obtained from the mimosa, iron, oak and poplar tree bark extracts. The AA and TPC values of the coniferous species were low as in comparison to those of the deciduous species.

Deciduous tree species with high TPC have a potential. Phenols are some of the important components of tree extracts. They are evaluated in various applications since ancient times. Tannins are prevalently used in medicine due to having antiviral, antimicrobial and antioxidant properties. Moreover, they are also important for the paint industry, ink production, anticorrosion, clarifier for beer and wine, various chemical applications and the adhesive industry.

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