Bark Extractives and Suberin Monomers from *Arbutus andrachne* and *Platanus orientalis*

İlhami Emrah Dönmez,[a,*] Jarl Hemming,[b] and Stefan Willför [b]

Arbutus andrachne and *Platanus orientalis* grow naturally in Turkey. They do not occur in stands but can be seen as solitary trees. *A. andrachne* is seen in coastal parts of Anatolia, whereas *P. orientalis* can be found from west to the east of Turkey, mostly in river banks. Lipophilic extractives, hydrophilic extractives, and suberin monomers from *Arbutus andrachne* and *Platanus orientalis* bark was analyzed by chromatography. The total amount of lipophilic extractives was higher in *P. orientalis* (8.55 mg/g). However, the total amount of hydrophilic extractives had a bigger proportion, 100.86 mg/g, in *A. andrachne* bark. Dioic and hydroxy acids were the dominant group in the suberin monomers of both species of bark. Acid 1,18-dioic-18:0 and acid 18-hydroxy-18:1 were determined as the main compounds of suberin monomers in both samples. In addition, total amount of suberin monomers was determined to be 11.36 mg/g in *A. andrachne* and 15.95 mg/g in *P. orientalis* bark.

Keywords: Arbutus andrachne; Platanus orientalis; Lipophilics; Hydrophillics; Suberin monomers

Contact information: a: Süleyman Demirel University Faculty of Forestry Department of Forest Industrial Engineering, East Campus, 32100-Çünür, Isparta, Turkey; b: Laboratory of Wood and Paper Chemistry Åbo Akademi University, Porthansgatan 3–5, FI-20500 Åbo/Turku, Finland;
** Corresponding author: emrahdonmez@sdu.edu.tr*

INTRODUCTION

Wood and tree bark contain lignocellulosic materials—cellulose, hemicelluloses (polyoses), and lignin—as well as extractives. Because extractives can be utilized in the medicinal, pharmaceutical, and cosmetic fields, they have the potential to affect how trees are used. Extractives are lipophilic and hydrophilic components that can be recovered with neutral solvents (Fengel and Wegener 1989; Sjöström 1993; Holmbom 1999). They are a wide variety of non-structural, low molecular weight organic compounds. Extractives can protect wood from decay, add color and odor to wood, and enhance the strength properties of the wood. Extractives may also inhibit setting of concrete, glues, and finishes and cause problems in papermaking (Hillis 1987; Amusant *et al.* 2007; Hashemi *et al.* 2009). Extractives can be seen in both kraft and sulfite pulping as polyphenols in polymer fraction and fatty acids, terpenoids, steroids, lignans, and other compounds in monomer fractions (Holmbom 1999). Some of the extractives are unsaponifiable (hydrocarbons, sterols, various alcohols, aldehydes, *etc*.), and some are saponifiable (fats, sterols, esters). Phenols, one of the most important extractive substances group, are very interesting as antioxidants because of their natural origin and the ability to act as efficient free radical scavengers (Hertog *et al.* 1993, 1995; Langley-Evans 2000). There are a number of publications on the potential health benefits of polyphenols (Dreosti 1991; Friedman and Kimball 1986; Pietta 2000; Tiwari 2001; Lee *et al.* 2003; Modun *et al.* 2003). The current studies focus on natural antioxidants, especially plant polyphenolics (Skerget *et al.* 2005; Miranda *et al.*

2016). Utilization of polyphenolics in traditional folk medicine are gaining interest because of antioxidant properties of plants. Trees are an abundant source of phenolic compounds. These compounds can easily be isolated from wood by hydrophilic extraction after removing the lipophilic extractives by hexane extraction (Holmbom *et. al.* 2003; Willför *et al.* 2003). Polyphenolic compounds are ubiquitous in foods of plant origin, and thus they constitute an integral part of the human diet. Recent interest in polyphenols has greatly increased, since these phytochemicals have been implicated in suppressed rates of degenerative processes, such as cardiovascular disorders and cancer (Bravo 1998; Duthie *et al.* 2000). The amount of extractives and their composition vary with respect to the botanical families, tree species, growth region, tissue, and extraction methods. The tree species is probably the most important factor that affects the extractive content and component. Usually a higher extractive content is found in hardwoods than in softwoods (Hillis 1987).

Extractives in bark vary more than those from wood of the same tree. Moreover bark contains suberin. Suberin is a natural aliphatic-aromatic cross-linked polyester. It is found in the cell walls of all normal and wounded external tissues. Its main purpose is to protect the plant from environmental conditions (Kolattukudy and Espelie 1989; Kolattukudy 2001; Bernards 2002; Pollard *et al.* 2008). Its amount and composition in bark varies depending on the species and the isolation method (Gandini *et al.* 2006). Most of the studies have been focused on *Quercus suber* and *Betula pendula* because cork of *Q. suber* and outer bark of *B. pendula* include almost 50% suberin by weight (Pereira 1988; Lopes *et al.* 2000; Pinto *et al.* 2009). However, the composition of suberin studied in some tree bark such as *Pseudotsuga menziesii* (22 %), *Eucalyptus globulus* (less than 1%), and *Pinus sylvestris* (1.75 to 4.72 %) was presented previously (Dönmez *et al.* 2012; Miranda *et al.* 2013; Ferreira *et al.* 2015).

In many industries, bark is considered waste that is burned for energy production (Hemingway 1981). However, bark is a rich source of chemicals, for instance lipophilics, phytosterols, and other phenolic compounds (Kähkönen *et al.* 1999; Freire *et al.* 2002 a,b; Pietarinen *et al.* 2006; Ferreira *et al.* 2013). This makes the bark a possible raw material for new pharmaceutical and bioactive compounds, green polymers, and bio-based materials (Miranda *et al.* 2013; Baptista *et al.* 2013).

Arbutus andrachne is a woody plant that grows as a bush or a small tree. It is an evergreen tree with thick branches. *A. andrachne* has light-colored and bright bark that is peeled off as sheets like oriental plane (Anşin and Özkan 1993), and it has red-colored fruits that are harvested from the wild and consumed (Serçe *et al.* 2010). There is limited research on *A. andrachne*; most of the existing papers have been focused on the sugar composition, protein, ash content, and antioxidant activity of its fruits (Alarcao-E-Silva *et al.* 2001; Şeker and Toplu 2010; Serçe *et al.* 2010).

Platanus orientalis is a large deciduous tree known for its longevity and spreading crown. It is seen in the parks and landscapes as an ornamental plant (Khan *et al.* 2013). The phenolic composition of its leaves and buds and the effects of environmental stress have been studied, but there is limited knowledge of the chemical composition of *P. orientalis* bark. Most studies on its chemical composition have focused on anticancer or antiseptic properties and activity against urinary infections (Mitrocotsa *et al.* 1993; Mitrocotsa *et al.* 1999; Dimas *et al.* 2000; El-Alfy *et al.* 2008). To the best of our knowledge, there has been no research about extractive and suberin composition of *A. andrachne* and *P. orientalis* bark. It was aimed to determine the amount and the

composition of lipophilic, hydrophilic extractives and suberin monomers of *A. andrachne* and *P. orientalis* bark.

MATERIALS AND METHODS

Materials

Arbutus andrachne (L.) and *Platanus orientalis* (L.) were harvested from Isparta, Eğirdir, Aşağıgökdere, Turkey, where the altitude is 375 m. Cross-sections of the trees were taken according to the TAPPI T 257 cm-85 standard (1985). Bark was then separated from wood. All samples were stored at -24 °C. Bark was broken into small pieces, which were freeze-dried and ground by a Wiley mill to 1 mm (Ekman 1983). To remove volatile compounds, a second drying procedure was treated to the ground samples.

Extraction

Approximately 5 g of ground bark from each sample was sequentially extracted with an accelerated solvent extractor (ASE) apparatus (model ASE 200, Dionex Inc., Sunnyvale, CA, USA). Samples were successively extracted with n-hexane, acetone:water (95:5, v:v), and finally distilled water. After extraction, 100-mL aliquots of extractives were prepared with the same solvent used in extraction. For gravimetric analyses, 10 mL of the aliquot was evaporated completely, leaving a film of extractives in the solvent container. The containers were weighed before and after extraction to determine the extractive yield in mg/g. An internal standard (heneicosanoic acid and betulinol) was added to the hexane and acetone:water (95:5, v:v) extracts. The mixture was evaporated under nitrogen prior to silylation. Hexane and acetone:water (95:5, v:v) extracts were used for chromatography analysis.

Hydrolysis of Suberin

As an internal standard, 0.5 g of cholesterol was dissolved in 50 mL acetone, and 1 mL of this solution was transferred to a 50-mL test tube. The solvent was evaporated in a water-bath under nitrogen. Extractive-free, ground bark (100 mg) and 10 mL of 0.5 M KOH in 90% ethanol were added to the same tube. Hydrolysis was performed at 70 °C for 1.5 h with continuous stirring. After hydrolysis, 1 mL of the solution was taken from liquid phase and placed in a 15-mL test tube, and 1 mL of distilled water and 2 to 3 drops of bromocresol green were added. The pH was adjusted to 3.5 with 2 to 3 drops of 0.25 M H_2SO_4, and 2 mL of MTBE (methyl *tert*-buthyl ether) was added. The tubes were shaken vigorously. The MTBE phase was moved to a new test tube and evaporated in a water bath under nitrogen prior to silylation (Ekman 1983).

Chromatography

Quantitative analyses (peak areas *vs.* internal standards) of both extractives and suberin monomers from the bark of *A. andrachne* and *P. orientalis* were performed with a Pelkin Elmer Autosystem XL gas chromatograph (GC; PerkinElmer Inc., Waltham, MA, USA) equipped with an HP-1 25 m × 0.2 mm (0.11 μm film thickness) column and flame ionization detector (FID). The carrier gas was H_2 at 0.8 mL/min, constant flow. The initial oven temperature was 120 °C (1 min hold), and was increased at a rate of 6 °C/min to 320 °C (15 min hold). The split-splitless injector was used in split mode (split ratio: 1:24). The injector temperature was 260 °C, and the FID temperature was 320 °C. One μL of each

sample was injected. Individual compounds were identified with an HP 6890-5973 gas chromatography/mass spectrometry (GC-MS) instrument (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with an identical HP-1 column and using the same temperature program, but using helium as carrier gas.

RESULTS AND DISCUSSION

Extractives

The lipophilic and hydrophilic extractives of *A. andrachne* and *P. orientalis* bark were studied by gravimetric measurements (Table 1) and by GC and GC-MS (Table 2). Although the hexane extracts of *P. orientalis* bark were more concentrated than in *A. andrachne*, the total amount of identified lipophilics were higher in *A. andrachne* bark (1.03 mg/g).

Similar to the gravimetric results, the total amount of identified hydrophilics (acetone:water 95:5, v:v extracts) were twofold higher in *A. andrachne* bark (61.17 mg/g) than in *P. orientalis* bark (38.42 mg/g).

Table 1. Gravimetric Amounts of Extractives from *A. andrachne* and *P. orientalis* Bark (mg/g to Dry Weight)

	Hexane extract (mg/g)	Acetone:water extract (mg/g)	Water extract (mg/g)
Arbutus andrachne	3.00	100.86	224.49
Platanus orientalis	8.55	57.06	98.61

Fatty acids and sterols were the main groups in both lipophilic extractives samples. Fatty acids composed 32.03% and 50.51% of the total lipophilic extractives in *A. andrachne* and *P. orientalis* bark, respectively. Palmitic acid was the most abundant fatty acid in both species. While it was determined as 0.22 mg/g in *A. andrachne* and 0.20 mg/g in *P. orientalis* bark, it was found 21.21% in *Robinia pseudoacacia* bark and 0.6 to 0.7 mg/kg in *Pseudotsuga menziesii* bark (Hosseinihashemi *et al.* 2016; Ferreira *et al.* 2015). Sitosterol was the only phytosterol detected in both samples, and it was observed to be within the range of 0.12 to 0.32 mg/g in both samples. Ferreira *et al.* (2015) studied the dichloromethane extracts of bark fractions of *Pseudotsuga menziesii*. β-sitosterol had the highest amount (43.3 to 78.8 mg/kg) in the sterols group.

Two phenolic compounds (3,4-dihydroxybenzoic acid and catechin) were found in both species. Catechin was the major component, with 1.35 mg/g in *A. andrachne* and 1.64 mg/g in *P. orientalis* bark. Catechin was also detected in other species barks and in medicinal and aromatic plats. It was detected as 5.5 mg/g in *Pinus pinaster* bark (Curkovic-Perica *et al.* 2015). Catechin is also a major constituent of black and green tea extract supplement that comprises 14.1 to 42.1 mg per dose (Henning *et al.* 2004). Catechin, derived from plant sources, is an important component to prevent heart diseases (Arts *et al.* 2001; Garbisa *et al.* 2001).

Some mono- and disaccharide sugars and their alcohol derivatives were also detected. *Arbutus* species fruits have large amounts of sugars (Serçe *et al.* 2010), and similar results were observed in bark. Maltitol was determined as the largest amount in the

sugar group in *A. andrachne* bark (16.56 mg/g) whereas it was seen as the smallest (0.01 mg/g) in *P. orientalis* bark. Pinitol+d-glucose composed 12.8% of total sugar group of *A. andrachne* and 59.35% of total sugar group of *P. orientalis*. It was previously reported that the dominance of glucose, xylose, arabinose, and galactose were determined as the monomeric composition of polysaccharides of some tree species' bark (Miranda *et al.* 2016; Miranda *et al.* 2013; Ruiz-Aquinon *et al.* 2015). However in the present study, the dominant monosaccharides were pinitol+d-glucose (with 7.12 mg/g in *A. andrachne*, 6.60 mg/g in *P. orientalis* bark), sorbopyranose (5.68 mg/g in *A. andrachne*, 1.89 mg/g in *P. orientalis* bark), and d-fructose (4.45 mg/g in *A. andrachne* and 0.04 in *P. orientalis* bark).

In addition, betulinic acid and sinapyl alcohol were observed only in acetone:water (95:5, v:v) extracts, but lupeol was determined in both extracts of the species. Betulinic acid, a triterpenoid component, is found in the bark of several trees, especially birch.

Table 2. Extractives of *A. andrachne and P. orientalis* Bark (mg/g)

Compound	***A. andrachne* (L.)**		***P. orientalis* (L.)**	
	Hexane (mg/g)	Acetone:water (mg/g)	Hexane (mg/g)	Acetone:water (mg/g)
Fatty acids				
Palmitic	0.22	-	0.20	-
Stearic	0.01	-	0.02	-
Oleic	0.02	-	0.03	-
Linoleic	0.06	-	0.17	-
Linolenic	0.02	-	0.07	-
Phenolics				
3,4-Dihydroxybenzoic acid	-	0.04	-	0.07
Catechin	-	1.35	-	1.64
Sugar group				
d-Xylose	-	0.09	-	0.05
d-Fructose	-	4.45	-	0.04
Sorbopyranose	-	5.68	-	1.89
α-d-Glucopyranose	-	0.73	-	0.06
Glucitol	-	0.01	-	0.13
Pinitol+(d-glucose*)	-	7.12	-	6.60
Myo-inositol	-	0.05	-	0.11
l-Rhamnose	-	0.17	-	0.01
Mannose	-	0.09	-	0.06
Sucrose	-	3.76	-	0.01
Maltitol	-	16.56	-	0.01
Unidentified monosaccharides	0.06	19.10	0.15	2.15
Phytosterols				
Sitosterol	0.23	0.31	0.32	0.12
Others				
Betulinic acid	-	1.10	-	25.38
Sinapyl alcohol	-	0.03	-	0.03
Lupeol	0.41	0.53	0.01	0.06

*: Overlapped

Suberin Monomers

This report is the first to characterize the total amount and composition of suberin monomers from *A. andrachne* and *P. orientalis* bark. The total amount of suberin monomers was 11.36 mg/g in *A. andrachne* and 15.95 mg/g in *P. orientalis* bark. The

composition and the amount of suberin monomers are shown in in Table 3. The amount of suberin was previously reported to be present in small amounts in bark of Teak (1.9 %), Eucalypt (0.98 %), and larger amount in Birch bark (5.9 %) (Miranda *et al.* 2013; Baptista *et al.* 2013).

Alcohols 18:0 and 20:0 were detected in both samples. Alcohol 18:0 was the most abundant fatty alcohol; it was present at 0.11 mg/g and 0.09 mg/g in *A. andrachne* and *P. orientalis* bark, respectively.

Seven different fatty acids were found in the samples, comprising 15.15% of *A. andrachne* suberin monomers and 12.92% of *P. orientalis* bark. Acid 20:0 and acid 16:0 were the major constituents of saturated fatty acids in *A. andrachne* (0.51 mg/g) and *P. orientalis* (0.49 mg/g) bark. However, acid 18:2 was the most abundant unsaturated fatty acid in *A. andrachne* (0.46 mg/g) and in *P. orientalis* (0.33 mg/g).

Table 3. Suberin Monomers in *A. andrachne* and *P. orientalis* Bark

Compound	***A. andrachne* (L.)** (mg/g)	***P. orientalis* (L.)** (mg/g)
Fatty Alcohols		
Alcohol 20:0	0.07	0.08
Alcohol 18:0	0.11	0.09
Fatty Acids		
Acid 16:0	0.29	0.49
Acid 18:1	0.10	0.21
Acid 18:2	0.46	0.33
Acid 18:0	0.17	0.33
Acid 20:0	0.51	0.29
Acid 22:0	0.12	0.32
Acid 18:3	0.11	0.12
Dioic and Hydroxy Acids		
1,16-Dioic-16:0	1.10	1.30
1,18-Dioic-18:0	0.51	0.72
1,18-Dioic-18:1	1.24	1.75
1,20-Dioic-20:0	0.67	1.13
16-Hydroxy-16:0	0.66	1.80
18-Hydroxy-18:0	0.41	0.83
18-Hydroxy-18:1	2.29	2.66
20-Hydroxy-20:0	0.83	1.56
22-Hydroxy-22:0	0.91	1.03
24-Hydroxy-24:0	0.80	0.91
Other		
Sitosterol	0.21	0.13
Ferulic acid	0.01	0.08
Vanillic acid	0.03	0.01

Dioic and hydroxy acids were the predominant groups in bark of both species. Dioic and hydroxy acids represent 81 to 84% of the total suberin monomers in both *A. andrachne* and *P. orientalis* bark. This is in agreement with the literature that dioic and hydroxy acids have the biggest proportion in cork of *Q. suber* and birch outer bark suberin (Ferreira *et al.* 2013). The most abundant compounds in both samples were 18-hydroxy-18:1 acid and 1,18-dioic-18:1 acid, which were also detected as the major constituents of suberin monomers in the bark of pines growing in Turkey (Hafızoğlu 1989; Dönmez *et al.* 2012).

CONCLUSIONS

1. Lipophilic and hydrophilic extractives as well as suberin monomers from the bark of *A. andrachne* and *P. orientalis* were analyzed by GC and GC-MS. The total amount of lipophilic extractives in *A. andrachne* bark (3.00 mg/g) was smaller than in *P. orientalis* (8.55 mg/g). However, twice the amount of hydrophilic extractives was found in *A. andrachne* (100.86 mg/g).
2. Sugars were the highest group in *A. andrachne* bark. Sugars were detected almost five-times more than in *P. orientalis* bark.
3. The dioic and hydroxy acids most prominent in both *A. andrachne* and *P. orientalis* bark were acid 1,18-dioic-18:0 and acid 18-hydroxy-18:1.
4. The largest amounts of fatty acids as suberin monomers in *A. andrachne* were acid 20:0, acid 18:2, and acid 16:0. The bark of *P. orientalis* exhibited the following main fatty acid compounds as suberin monomers: acid 16:0, acid 18:2, and acid 18:0.
5. Catechin was the most predominant constituents of phenolics detected in both samples. Because of catechin amount of the extracts, antioxidant properties of the barks can be studied.

ACKNOWLEDGEMENTS

The authors thank Dr. Annika Smeds, Laboratory of Wood and Paper Chemistry, Åbo Akademi University, Turku, Finland, for her help with chromatographic analyses.

REFERENCES

Alarcao-E-Silva, M. L. C. M. M., Leitao, A. E. B., Azinheira, H. G., and Leitao, M. C. A. (2001). "The *Arbutus* berry: Studies on its color and chemical characteristics at two mature stages," *Journal of Food Composition and Analysis* 14, 27-35. DOI: 10.1006/jfca.2000.0962

Amusant, N., Moretti, C., Richard, B., and Prost, E. (2007). "Chemical compounds from *Eperua falcata* and *Eperua grandiflora* heartwood and their biological activities against wood destroying fungus (*Coriolus versicolor*)," *Holz Roh Werkst.* 65(1), 23-28. DOI: 10.1007/s00107-006-0120-1

Anşin, R., and Özkan, Z. C. (1993). *Seed Plants* (*Spermatophyta*) *Woody Taxa* (in Turkish: *Tohumlu Bitkiler* (*Spermatophyta*) *Odunsu Taksonlar*), Black Sea Technical University Faculty of Forestry Publications, Trabzon, Turkey.

Arts, I. C.W., Hollman, P. C. H., Feskens, E. J. M., Mesquita, H. B. B., and Kromhout, D. (2001). "Catechin intake might explain the inverse relation between tea consumption and ischemic heart disease: The Zutphen Elderly Study," *The American Journal of Clinical Nutrition* 74, 227-232.

Baptista, I., Miranda, I., Quilho, T., and Pereira, H. (2013). "Characterisation and fractoning of *Tectona grandis* bark in view of its valorization as a biorefinery raw-material," *Industrial Crops and Products* 50, 166-175. DOI: 10.1016/j.indcrop.2013.07.004

Bernards, M. A. (2002). "Demystifying suberin," *Canadian Journal of Botany* 80, 227-240. DOI: 10.1139/b02-017

Bravo, L. (1998). "Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance," *Nutrition Reviews* 56(11), 317-333. DOI: 10.1111/j.1753-4887.1998.tb01670.x

Ćurković-Perica, M., Hrenović. J., Kugler, N., Goić-Barišić, I., and Tkalec, M. (2015) "Antibacterial activity of *Pinus pinaster* bark extract and its components against multidrug-resistant clinical isolates of *Acinetobacter baumannii*," *Croatica Chemica Acta* 88(2), 133-137. DOI: 10.5562/cca2548

Dimas, K., Demetzos, C., Mitaku, S., Marselos, M., Tzavaras, T., and Kokkinopoulos, D. (2000). "Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines *in vitro*," *Pharmacological Research* 41(1), 83-86. DOI: 10.1006/phrs.1999.0562

Dönmez, I. E., Hafızoğlu, H., Kılıç, A., Hemming, J., and Eckerman, C. (2012). "Effect of altitude on the composition of suberin monomers in the outer bark of Scots pine (*Pinus sylvestris* (L.))," *Industrial Crops and Products* 37, 441-444. DOI: 10.1016/j.indcrop.2011.07.012

Dreosti, I. E. (1991). "*Trace Elements, Micronutrients, and Free Radicals*," Totowa: Human Press, pp. 149-199. DOI: 10.1007/978-1-4612-0419-0

Duthie, G. G., Duthie, S. J., and Kyle, J. A. M. (2000). "Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants," *Nutrition Research Reviews*, 13, 79-106. DOI: 10.1079/095442200108729016

Ekman, R. (1983). "The suberin monomers and triterpenoids from the outer bark of *Betula verrucosa* (Ehrh.)," *Holzforschung* 37(4), 205-211. DOI: 10.1515/hfsg.1983.37.4.205

El-Alfy, T. S., El-Gohary, H., Sokkar, N. M., Sleem, A. A., and Al-Mahdy, D. A. (2008). "Phenolic constituents of *Platanus orientalis* L. leaves," *Natural Product Communications* 3, 1-5.

Fengel, D., and Wegener, G. (1989). *Wood: Chemistry, Ultrastructure, Reactions*, Walter de Gruyter, Berlin, pp. 240-267.

Ferreira, R., Garcia, H., Sousa, A. F., Freire, C. S. R., Silvestre, A. J. D., Rebelo, L. P. N., and Pereira, C. S. (2013). "Isolation of suberin from birch outer bark and cork using ionic liquids: A new source of macromonomers" *Industrial Crops and Products* 44, 520-527. DOI: 10.1016/j.indcrop.2012.10.002

Ferreira, J. P. A., Miranda, I., Gominho, J., and Pereira, H. (2015). "Selective fractioning of *Pseudotsuga menziesii* bark in view of an integrated valorization," *Industrial Crops and Products* 74, 998-1007. DOI:10.1016/j.indcrop.2015.05.065

Freire, C. S. R., Silvestre, A. J. D., and Neto, C. P. (2002a). "Identification of new hydroxy fatty acids and ferulic acid esters in the wood of *Eucalyptus globulus*," *Holzforschung* 56, 143-149. DOI: 10.1515/HF.2002.024

Freire, C. S. R., Silvestre, A. J. D., Neto, C. P., and Cavaleiro, J. A. S. (2002b). "Lipophilic extractives of the inner and outer barks of *Eucalyptus globulus*," *Holzforschung* 56, 372-379. DOI: 10.1515/HF.2002.059

Friedman, L. A., and Kimball, A. W. (1986). "Coronary heart disease mortality and alcohol consumption in Framingham," *American Journal of Epidemiology* 24, 481-489.

Gandini, A., Pascoal Neto, C., and Silvestre, A. J. D. (2006). "Suberin: A promising renewable resource for novel macromolecular materials," *Progress in Polymer Science* 31, 878-892. DOI: 10.1016/j.progpolymsci.2006.07.004

Hafızoğlu, H. (1989). "Studies on the wood and bark constituents of *Pinus pinea* (L.)," *Holzforschung* 43, 41-43. DOI: 10.1515/hfsg.1989.43.1.41

Hashemi, S. K. H., Parsapajouh, D., Khademi-eslam, H., Mirshokraeai, S. A., and Hemmasi A. H. (2009). "Identification of chemical compounds within north of Iran's walnut heart wood extractives by GC/MS method," *Iranian Journal of Agriculture Science* 12(4), 939.

Hemingway, R. W. (1981). "Bark: Its chemistry and prospects for chemical utilization," in: *Organic Chemicals from Biomass*, Goldstein, I. S. (ed.), CRC Press, Boca Raton.

Henning, S. M., Niu, Y., Lee, H. N., Thames, G. D., Minutti, R. R., Wang, H., Go, V. L. W., and Heber, D. (2004). "Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement," *The American Journal of Clinical Nutrition* 80, 1558-1564.

Hertog, M. G. L., Hollman, P. C. H., and Van de Putte, B. (1993). "Content of potentially anticancerogenic flavonoids of tea infusions, wines and fruit juices," *Journal of Agricultural and Food Chemistry* 41, 1242-1246.

Hertog, M. G. L., Kromhout, D., Aravanis, C., Blackburn, H., Buzina, R., and Fidanza, F. (1995). "Flavonoid intake and longterm risk of coronary heart disease and cancer in the seven countries study," *Archives of Internal Medicine* 155, 381-386. DOI: 10.1021/jf00032a015

Hillis, W. E. (1987). "*Heartwood and Tree Exudates*," Springer-Verlag, Berlin, Germany, 26 pp. DOI: 10.1007/978-3-642-72534-0

Holmbom, B., Eckerman, C., Eklund, P., Hemming, J., Nisula, L., *et al*. (2003). "Knots in trees – A new rich source of lignans," *Phytochem. Rev.* 2, 331-340. DOI: 10.1023/B:PHYT.0000045493.95074.a8

Holmbom, B. (1999). "Extractives," in: *Analytical Methods in Wood Chemistry, Pulping, and Papermaking*, Sjöström, E., and Alén, R. (eds.), Springer-Verlag, Berlin, pp. 125-148. DOI: 10.1007/978-3-662-03898-7_5

Hosseinihashemi, S. K., HosseinAshrafi, S. K., Goldeh, A. J., and Salem, M. Z. M. (2016). "Antifungal and antioxidant activities of heartwood, bark, and leaf extracts of *Robinia pseudoacacia*," *BioResources* 11(1), 1634-1646.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., and Heinonen, M. (1999). "Antioxidant activity of plant extracts containing phenolic compounds," *Journal of Agricultural and Food Chemistry* 47, 3954-3962. DOI: 10.1021/jf990146l

Khan, I., Sangwan, P. L., Dar, A. A., Rafiq, R. A., Farrukh, M. R., Dhar, J. K., Tasduq, S. A., and Koul, S. (2013). "A validated high-performance thin-layer chromatography

method for the identification and simultaneous quantification of six markers from *Platanus orientalis* and their cytotoxic profiles against skin cancer cell lines," *Journal of Separation Science* 36, 2602-2610. DOI: 10.1002/jssc.201300380

Kolattukudy, P. E. (2001). "Polyesters in higher plants," in: *Advances in Biochemical Engineering/Biotechnology*: *Biopolyesters* (Vol. 71), Babel, W., and Steinbüchel, A. (eds.), Springer-Verlag, Berlin, pp. 1-50. DOI: 10.1007/3-540-40021-4_1

Langley-Evans, S. C. (2000). "Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay," *International Journal of Food Science and Nutrition* 51, 181-188. DOI: 10.1080/09637480050029683

Lee, S. E., Hyun, J. H., Ha, J.-S., Jeong, H. S., and Kim, J. H. (2003). "Screening of medicinal plant extracts for antioxidant activity," *Life Sciences* 73, 167-179. DOI: 10.1016/S0024-3205(03)00259-5

Kolattukudy, P. E., and Espelie, K. E. (1989). "Chemistry biochemistry and function of suberin and associated waxes," in: *Natural Products of Woody Plants: Chemicals Extraneous to the Lignocellulosic Cell Wall*, Rowe, J. (ed.), Springer-Verlag, Berlin, pp. 67-304. DOI: 10.1007/978-3-642-74075-6_11

Lopes, M. H., Gill, A. M., Silvestre, A. J. D., and Neto, C. P. (2000). "Composition of suberin extracted upon gradual alkaline methanolysis of *Quercus suber* L. cork," *Journal of Agricultural Food Chemistry* 48(2), 383-391. DOI: 10.1021/jf9909398

Miranda, I., Gominho, J., Mirra, I., and Pereira, H. (2013). "Fractioning and chemical characterization of barks of *Betula pendula* and *Eucalyptus globulus*," *Industrial Crops and Products* 41, 299-305. DOI: 10.1016/j.indcrop.2012.04.024

Miranda, I., Lima, L., Quilhó, T., Knapic, S., and Pereira, H. (2016). "The bark of *Eucalyptus sideroxylon* as a source of phenolic extracts with anti-oxidant properties," *Industrial Crops and Products* 82, 81-87. DOI: 10.1016/j.indcrop.2015.12.003

Mitrocotsa, D., Bosch, S., Mitaku, S., Dimas, C., Skaltsounis, A. L., Harval, C., Briand, G., and Roussakis, C. (1999). "Cytotoxicity against human leukemic cell lines, and the activity on the expression of resistance genes of flavonoids from *Platanus orientalis*," *Anticancer Research Journal* 19, 2085-2088.

Mitrocotsa, D., Mitaku, S., Demetzos, C., Harvala, C., Mentis, A., Perez, S., and Kokkinopoulos, D. (1993). "Bioactive compounds from the buds of *Platanus orientalis* and isolation of a new kaempferol glycoside," *Planta Medica* 59(6), 517-520. DOI: 10.1055/s-2006-959751

Modun, D., Musić, I., Katalinić, V., Salamunić, I., and Boban, M. (2003). "Comparison of protective effects of catechin applied in vitro or in vivo on ischemia-reperfusion injury in the isolated rat hearts," *Croatian Medical Journal* 44(6), 690-696.

Pereira, H. (1988). "Chemical composition and variability of cork from *Quercus suber* L.," *Wood Science and Technology* 22(3), 211-218. DOI: 10.1007/BF00386015

Pietarinen, S. P., Willför, S. M., Athotupa, M. O., Hemming, J. E., and Holmbom, B. R. (2006). "Knotwoods and bark extracts: Strong antioxidants from waste materials," *Journal of Wood Science* 52, 436-444. DOI: 10.1007/s10086-005-0780-1

Pietta, P. G. (2000). "Flavonoids as antioxidants," *Journal of Natural Products* 63, 1035-1042. DOI: 10.1021/np9904509

Pinto, P. C. R. O., Sousa, A. R., Silvestre, A. J. D., Neto, C. P., Gandini, A., Eckerman, C., and Holmbom, B. (2009). "*Quercus suber* and *Betula pendula* outer barks as renewable sources of oleochemicals: A comparative study," *Industrial Crops and Products* 29(1), 126-132. DOI: 10.1016/j.indcrop.2008.04.015

Pollard, M., Beisson, F., Li, Y., and Ohlrogge, J. B. (2008). "Building lipid barriers: Biosynthesis of cutin and suberin," *Trends in Plant Science* 13(5), 236-246. DOI: 10.1016/j.tplants.2008.03.003

Ruiz-Aquino, F., Gonzalez-Pena, M. M., Valdez-Hernandez, J. I., Revilla, U. S., and Romero-Manzanares, A. (2015). "Chemical characterization and fuel properties of wood and bark of two oaks from Oaxaca, Mexico," *Industrial Crops and Products* 65, 90-95. DOI: 10.1016/j.indcrop.2014.11.024

Serçe, S., Özgen, M., Torun, A. A., and Ercişli, S. (2010). "Chemical composition, antioxidant activities, and total phenolic content of *Arbutus andrachne* (L.) (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey," *Journal of Food Composition and Analysis* 23, 619-623. DOI: 10.1016/j.jfca.2009.12.007

Sjöström, E. (1993). *Wood Chemistry Fundamentals and Applications* (2nd Ed.), Academic Press, San Diego.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A. N., Simonic, M., and Knez, Z. (2005) "Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities," *Food Chemistry* 89, 191-198. DOI: 10.1016/j.foodchem.2004.02.025

Şeker, M., and Toplu, C. (2010). "Determination and comparison of chemical characteristics of *Arbutus unedo* L. and *Arbutus andrachne* L. (Family Ericaceae) fruits," *Journal of Medicinal Food* 13(4), 1013-1018. DOI: 10.1089/jmf.2009.0167

TAPPI T 257 cm-85. (1985). "Sampling and preparing wood for analyses," TAPPI Press, Atlanta, GA.

Tiwari, A. K. (2001). "Imbalance in antioxidant defense and human diseases: Multiple approach of natural antioxidant therapy," *Current Science* 8, 1179-1187.

Willför, S., Hemming, J., Reunanen, M., and Holmbom, B. (2003). "Phenolic and lipophilic extractives in Scots pine knots and stemwood," *Holzforschung* 57, 359-372. DOI: 10.1515/hf.2003.054

Article submitted: September 28, 2015; Peer review completed: December 18, 2015; Revised version received and accepted; December 31, 2015; Published: February 2, 2016.
DOI: 10.15376/biores.11.1.2809-2819