Water-Soluble Components of *Pinus pinaster* Wood

Enma Conde,[a,b] Wenwen Fang,[b] Jarl Hemming,[b] Stefan Willför,[b] Andrés Moure,[a] Herminia Domínguez,[a] and Juan Carlos Parajó [a,*]

Aqueous fractionation of wood has been proposed as a suitable processing method for biorefineries. When treatments are performed under low severity conditions, water-soluble components (which could be detrimental in further processing stages) are removed, whereas polysaccharides, lignin, and other water-insoluble constituents remain in solid phase with little alteration. In order to explore the presence of added-value products in aqueous extracts from *Pinus pinaster* wood, different samples (heartwood and sapwood with and without knots) were extracted with water at 130 to 140 ºC, and the resulting solutions were assayed for yield and composition (by GC-FID, GC-MS, and HPLC). The major extract components, such as polysaccharide-derived products, simple phenolics, stilbenes, lignans, flavonoids, organic acids, jubaviones, steryl esters, and triglycerides, were identified and quantified. In order to assess a possible application of the extracts, their antioxidant activity was measured using the Trolox Equivalent Antioxidant Capacity assay.

Keywords: Pinus pinaster; Wood; Water extraction; Stilbenes; Flavonoids; Lignans

*Contact information: a: Chemical Engineering Department, University of Vigo, As Lagoas, 32004, Ourense, Spain / CITI-University of Vigo, Parque Tecnolóxico de Galicia, Rúa Galicia nº 2, 32900, Ourense, Spain; b: Process Chemistry Centre, Åbo Akademi University, Porthansgatan 3, FI-20500, Turku, Finland; *Corresponding author: jcparajo@uvigo.es*

INTRODUCTION

The selective separation of wood structural components (cellulose, hemicelluloses, and lignin) is a basic principle of biorefineries. For this purpose, wood processing with hot, compressed water (also known as autohydrolysis or hydrothermal treatments) under conditions of intermediate severity (usually at 160 to 210 ºC) lead to hemicellulose decomposition, which enables the separation of this fraction from cellulose and lignin (which remain in solid phase) (Gullón *et al*. 2012). Autohydrolysis has been proposed as a method for wood fractionation (Yáñez *et al*. 2009) since the resulting solids, which are almost free from hemicelluloses, can be further processed (for example, by delignification or enzymatic hydrolysis) to achieve a separate utilization of the three structural wood components (Gullón *et al*. 2012).

Besides hemicellulose-derived saccharides, the liquors from hydrothermal processing of wood contain other components (such as low molecular weight phenols or lipophilic compounds) that may be detrimental for the further purification and utilization of the soluble hemicellulose-derived saccharides. Because of this, an aqueous extraction under low severity conditions has been carried out prior to the hydrothermal stage in order to remove extractives (González-Muñoz *et al*. 2011 and 2012; Rivas *et al*. 2012). In this context, a wood biorefinery involving water extraction (for extractive removal) followed by further hydrothermal processing (for hemicellulose solubilization) could be

better implemented if the aqueous extracts contain value-added compounds that could contribute to the profitability of the whole process. For this purpose, reliable data on the composition of the aqueous extracts are necessary. In the same way, an experimental evaluation of the antioxidant activity of extracts is of interest in order to assess their potential for other key applications.

Softwoods are the dominant lignocellulosic materials in the Northern hemisphere (Galbe and Zacchi 2002). *Pinus pinaster* is a fast-growing species and is drought- and salt-resistant (Berthier *et al*. 2001). This species is abundant in the North-West of Spain, as well as in other Atlantic and Mediterranean regions.

The fractionation of pine wood with hot, compressed water or steam has been considered in the literature, which is oriented either to the removal of extractives or to the manufacture of soluble saccharides from hemicelluloses (Shahbazi *et al*. 2005; González-Muñoz *et al*. 2011 and 2012; Rivas *et al*. 2012; Yoon *et al*. 2008; Koell and Lenhardt 1987). The profitability of implementing stages to recover extractive-derived products in a biorefinery has been questioned (Van Ree and Annevelink 2007); however, it would be feasible if value-added products are present in the feedstock (or in process streams) above a given threshold, and can be separated efficiently. In the case of pine woods, phenolic stilbenes, flavonoids, and lignans are potential targets for biorefineries owing to their biological properties.

A wide scope of applications has been suggested or pinosylvins including anti-fungal and antibacterial agents (Lindberg *et al*. 2004; Lee *et al*. 2005; Celimene *et al*. 1999; Gref *et al*. 2000; Venäläinen *et al*. 2004) with activity towards *Listeria monocytogenes* (Gözü *et al*. 2010), cytotoxicity against a murine hepatic carcinoma cell line (Välimaa *et al*. 2007), antimetastatic activity (Park *et al*. 2012), antiinflammatory action based on the reduction of blood reactive species (Jančinová *et al*. 2012; Bauerova *et al*. 2011), and angiogenic effects (Kimura and Sumiyoshi 2011).

Flavonoids are known to exert biological, nutraceutical, and clinical effects (Maimoona *et al*. 2011), including *in vitro* antioxidant, anti-allergic, anti-inflammatory, anti-microbial, anti-cancer, and anti-diarrheal activities. Flavonoids can also be involved in plant defense mechanisms. Specifically, antioxidant activity has been reported for pinobanksin and pinocembrin (Neacsu *et al*. 2007), whereas the ability to modulate inflammatory responses *in vitro* has been claimed for the latter (Soromou *et al*. 2012). Pinocembrin protects neurons against beta-amyloid-induced toxicity (Liu *et al*. 2012) and has been predicted to have a number of biological activities, including anti-HIV action (Maridass *et al*. 2008). Other reported properties include bacteriostatic and antifungal activities (Villanueva *et al*. 1970; Shain and Miller 1982) and the ability to trigger the mitochondrial apoptosis in colon cancer cells (Kumar *et al*. 2007). Both pinocembrin and pinobanksin possess antimutagenic properties, in particular against ofloxacin-induced mutagenicity in *Euglena gracilis*; whereas pinobanksin is able to inhibit the peroxidation of low density lipoprotein and to scavenge peroxyl radicals (Ondrias *et al*. 1997; Neacsu *et al*. 2007). Taxifolin exerts a number of protective and anticancer effects (Lee *et al*. 2007; Luo *et al*. 2008; Rogovskii *et al*. 2010), and enhances the antibiotic activity in combined therapies (An *et al*. 2011). Antimicrobial activity (Ango *et al*. 2012), the ability to reduce reactive oxygen species (ROS) formation in polymorphonuclear cells (Kang *et al*. 2010), and activity to reduce lipid peroxidation (Redzynia *et al*. 2009) have been reported for dihydrokaempferol.

Lignans occurring in softwoods possess chemopreventive properties (Lampe 2003), present antioxidant and antitumor activities, cause neuroprotective effects (Li *et*

al. 2012), and can be employed cytotoxic antimicrobial agents (Willför *et al*. 2004). On the other hand, the associations between lignans and decreased risk of cardiovascular disease are promising, but they are not yet well established, perhaps due to low lignan intakes in habitual Western diets (Peterson *et al*. 2010). Nortrachelogenin has been proposed as a potential anti-malarial drug (Kebenei *et al*. 2011). The risk of certain types of breast cancer in premenopausal women is lowered by pinoresinol ingestion (Brown 2012). Pinoresinol presents activity against both human pathogenic fungi (Hwang *et al*. 2010) and Gram-positive bacteria (Céspedes *et al*. 2006). Secoisolariciresinol exhibited a significant antifungal activity on fungi of white rooting and wood staining (Céspedes *et al*, 2006).

This article deals with the aqueous extraction of *Pinus pinaster* wood samples obtained at different positions of selected trees. Extractions were performed at 130 to 140 ºC, and extracts were assayed for yield, composition, and antioxidant activity. The experimental data provide information on the types and amounts of major extract components, as well as their potential antioxidant activities.

EXPERIMENTAL

Materials

Three healthy 30-year-old *Pinus pinaster* trees were felled near Ourense (NW Spain). Disks (5 cm height) were cut and the samples listed in Table 1 were milled, air-dried, milled to pass a 10-mesh screen, and stored at room temperature until use.

Table 1. Wood Samples and Nomenclature

Nomenclature	Material	Tree position
SW	Stem sapwood	1.5 m from the ground
HW	Stem heartwood	1.5 m from the ground
DK_SW	Sapwood/knotwood	Disc containing the knots of the first dead branch
DK_HW	Heartwood/knotwood	Disc containing the knots of the first dead branch
LK_SW	Sapwood/knotwood	Disc containing the knots of the first living branch
LK_HW	Heartwood/knotwood	Disc containing the knots of the first living branch

Methods

Aqueous extraction

Samples were extracted with water in a batch pressurized reactor equipped with a temperature controller (Parr Instr. Co., Moline, IL). Samples were heated in the reactor to the treatment temperatures of 130 or 140 ºC for prescribed times, and afterwards cooled immediately. Treatments were performed at a liquid:solid ratio (LSR) of 10:1 g:g (oven-dry solid basis). The maximum temperature of treatments was chosen on the basis of

literature data (González-Muñoz *et al*. 2012) and preliminary experimental results, as a compromise between high yield in soluble material and limited hemicellulose decomposition. After cooling, extracts were recovered by filtration and assayed for extraction yield, composition, and antioxidant activity.

Yield measurements and analytical methods

The aqueous extraction yield was measured gravimetrically by oven-drying at 105 ºC until constant weight. The compositional and antioxidant activity results are referred to the sample content of non-volatile components. Monosaccharides in extracts were determined by high performance liquid chromatography equipped with refractive index detector (HPLC-RI) as described by Garrote *et al*. (1999). Oligosaccharides were quantified as monosaccharides (using the same HPLC-RI method) by measuring the increase in sugar concentration caused by a quantitative acidic post-hydrolysis (Garrote *et al*. 1999). For measuring the concentrations of lipophilic and hydrophilic compounds in aqueous extracts, the samples were freeze-dried and re-extracted with acetone before silylation. Quantification and identification of components was done with GC-FID and GC-MS, respectively. Stilbenes, flavonoids, lignans, simple phenolics, juvabiones, resin acid, and free fatty acids were analyzed using a 25 m x 0.20 mm i.d. column coated with crosslinked methyl polysiloxane (HP-1, 0.11 μm film thickness) using heneicosanoic acid as an internal standard (Ekman and Holmbom 1989). Analysis of total steryl esters and triglycerides was performed on a short 6 m x 0.53 mm i.d. column (HP-1, 0.15 μm film thickness) using cholesteryl heptadecanoate and 1,3-dipalmiotyl-2-oleyl glycerol as internal standards (Örså and Holmbom 1994). The practical limit of quantification of the individual compounds was 1/100 of the internal standard amount in each sample, but compounds detected in smaller amounts were also identified. Identification of individual components was performed by GC-MS analysis of the silylated components using the HP-1 column cited above.

Trolox Equivalent Antioxidant Activity (TEAC)

The assay, based on the scavenging of 2,2´-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical (denoted ABTS), was performed according to Re *et al*. (1999). The ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting a 7 mM ABTS stock solution with 2.45 mM potassium persulfate. Results are expressed as Trolox equivalents using a standard curve (0 to 1.6 mM). Assays were performed in triplicate.

Average values and deviations

The data determined for yield, composition, and antioxidant activity for the corresponding samples of trees A, B, and C are reported in terms of average values and standard deviations. The standard deviations are largely due to between-tree variations, in agreement with the data reported for *Pinus sylvestris* in an extensive study (Willför *et al*. 2003a).

RESULTS AND DISCUSSION

Wood Processing and Aqueous Extraction Yield

Extraction of *Pinus pinaster* wood at 130 ºC has been proposed to remove water-soluble extractives while causing little hemicellulose decomposition (González-Muñoz *et*

al. 2011 and 2012; Rivas *et al*. 2012). Based on preliminary experimental data, extractions were also performed at 140 ºC in order to assess the expected benefits (increase in extraction yield) and disadvantages (increased hemicellulose solubilization) on a quantitative basis. The results in Table 2 showed significant differences between heartwood samples (HW, DK_HW and LK_HW, with average extraction yields in the range 80.7-84.5 g/kg g oven-dry wood) and sapwood samples (SW, DK_SW, and LK_SW, for which the extraction yield ranged from 12.8 up to 17.6 g/kg oven-dry wood). The extraction yield scarcely varied when the treatment temperature was increased from 130 to 140 ºC, confirming that limited incremental hemicellulose decomposition took place.

The major components contributing to the solid yield were soluble saccharides and non-saccharide compounds, as discussed in the following sections. In addition, the extraction treatment also efficiently removed inorganic salts and metals, which can be a great advantage in later upstream process stages.

Table 2. Aqueous Extraction Yields

Sample	Temperature (ºC)	Extraction Yield (g /kg oven-dry wood)
HW	130	81.2±30.4
	140	82.4±31.0
SW	130	12.8±0.4
	140	13.7±0.7
DK_HW	130	80.9±19.9
	140	84.5±22.2
DK_SW	130	15.2±2.3
	140	17.5±1.1
LK_HW	140	80.7±19.4
LK_SW	130	17.6±1.7
	140	17.5±2.0

Water-Soluble Saccharides

According to the literature (González-Muñoz *et al*. 2012), the hemicelluloses of *Pinus pinaster* wood show the following distribution of non-glucose anhydrosugars (expressed as % wt., oven-dry wood basis): mannosyl units, 10.5; xylosyl units, 4.30; galactosyl units, 2.39; and arabinosyl units, 1.71 (total, 18.9 % wt or 189 g/kg). Looking at the extraction yields shown in Table 2 and considering the extractive content (the ethanol-soluble fraction accounts for 28.4 g/kg, according to the same literature reference), it can be inferred that a significant part of the aqueous extracts must come from hemicelluloses (at least, with the heartwood samples). This was confirmed by the experimental data in Table 3, which lists the contents of samples in monosaccharides and in oligo- or polymeric saccharides. Whereas only small amounts of monosaccharides were observed in all cases (below 94 g monosaccharides/kg extract), oligo- and polymeric saccharides accounted for 366 to 459 g/kg extract from samples SW, DK_SW, and LK_SW, and for 780-964 g/kg extract from samples HW, DK_HW, and LK_HW. According to these findings, the separation of saccharides from non-saccharide compounds seems to be necessary in order to make profitable use of the extracts. The

necessary refining steps would lead to purified oligo- and/or polymeric-saccharides derived from the hemicelluloses, for which applications as immunostimulatory agents, prebiotics, or radical-scavengers have been reported (Rivas *et al*. 2012; Ebringerova *et al*. 2008).

Table 3. Hemicellulose-Derived Products in Water Extracts

		Monosaccharides (g/kg extract)				
Sample	T (ºC)	Glucose	Xylose	Galactose	Arabinose	Mannose
HW	130	1.60±0.85	2.99±1.77	2.03±0.81	8.85±4.31	0.66±0.59
	140	1.67±0.62	2.90±1.66	2.28±1.03	13.0±5.0	0.70±0.51
SW	130	8.28±7.40	29.3±15.6	6.44±2.57	20.0±8.0	13.0±6.8
	140	10.3±6.0	26.9±15.5	6.23±1.66	37.2±4.2	13.1±4.8
DK_HW	130	2.08±0.83	1.67±0.56	2.25±0.44	11.6±1.9	0.63±0.33
	140	2.15±0.62	1.64±0.52	2.04±0.33	13.8±1.7	0.66±0.34
DK_SW	130	8.70±1.47	12.8±5.9	5.62±1.61	21.5±1.3	4.05±2.16
	140	7.24±1.64	10.8±4.4	4.41±1.50	27.1±2.2	3.70±1.91
LK_HW	140	3.04±2.19	8.09±4.69	0.68±0.19	7.41±2.02	1.22±0.92
LK_SW	130	4.40±1.20	26.8±16.9	3.61±0.67	23.2±6.3	1.81±1.07
	140	9.94±4.29	24.1±17.7	3.33±0.95	28.6±4.2	1.86±0.57

		Oligo- and Polysaccharides (g/kg extract)				
Sample	T (ºC)	Glucosyl units	Xylosyl units	Galactosyl units	Arabinosyl units	Mannosyl units
HW	130	11.1±8.1	8.66±7.16	775.4±37.7	75.1±36.5	1.17±1.65
	140	9.75±7.62	8.04±6.50	767.2±6.6	77.2±40.9	1.67±2.36
SW	130	62.2±13.4	8.57±6.94	181.4±7.4	60.2±12.3	93.9±17.8
	140	60.5±11.5	8.43±3.79	153.3±42.1	52.5±7.3	91.4±19.8
DK_HW	130	9.36±3.1	23.9±1.6	813.2±20.1	99.8±7.2	15.3±4.2
	140	8.76±4.2	23.8±0.5	814.8±34.3	101.3±6.0	15.7±3.7
DK_SW	130	50.7±7.1	18.6±3.4	257.0±32.0	61.8±6.5	71.0±15.5
	140	51.6±7.8	16.9±3.1	224.1±28.9	58.6±10.7	75.3±19.7
LK_HW	140	12.1±2.8	19.4±1.1	648.5±72.0	85.9±4.3	14.3±4.8
LK_SW	130	42.5±2.2	12.1±2.1	223.7±49.2	57.7±12.3	63.5±11.4
	140	41.6±4.7	14.3±2.9	224.2±54.0	69.6±16.6	79.0±7.4

Phenolic Compounds

A variety of phenolic compounds were present in the extracts, including simple phenolics, phenolic stilbenoids, flavonoids, and lignans. The presence of similar phenolic fractions dominated by stilbenes, lignans, and flavonoids has earlier been reported for other pine species (Willför *et al*. 2003a and 2007; Pietarinen *et al*. 2006a). In the samples obtained in this work, the most abundant simple phenolics were isoferulic and 3,4-dihydroxycinnamic acids, which reached concentrations up to 2.3 to 1.3 g/kg in extracts from the sample DK_SW. Among the rest of the compounds, the most abundant simple

phenolics were vanillin, 3-hydroxybenzoic acid, 4-hydroxycinnamic acid, pinitol, and coniferyl alcohol, which appeared at average concentrations in the range 1.3 to 0.5 g/kg in extracts from samples DK_SW and/or LK_SW, DK_SW, and at lower concentrations in the rest. Vanillic alcohol, 4-hydroxybenzyl alcohol, vanillic acid, dihydroconiferyl alcohol, 3,4-dihydroxybenzoic acid, and 1-guaiacylglycerol were also found in some extracts, all of them at concentrations below 0.4 g/kg extract.

Phenolic stilbenoids (pinosylvin and its derivative pinosylvin monomethyl ether) were found in extracts at concentrations up to 0.5 and 0.4 g/kg, respectively (see Table 4). Studies have reported the occurrence and the extraction of pinosylvins from conifers such as *Picea abies*, *Pinus sylvestris* (Willför *et al*. 2003a, 2003b, 2004 and 2007; Pietarinen *et al*. 2006a; Hovelstad *et al*. 2006), *Pinus thunbergii* (Kokubo *et al*. 1990), *Pinus strobus* (Geraldo de Carvalho *et al*. 1996), *Pinus radiata* (Hillis and Inoue 1968), *Pinus contorta* (Loman 1970), *Pinus sibirica*, and *Pinus cembra* (Willför *et al*. 2003c). The occurrence of pinosylvins has also been reported for *Pinus jeffreyi* (Anderson 1956), *Pinus resinosa* (Simard *et al*. 2008), and *Pinus griffithii* (Mahesh and Seshadri 1954). In comparison, scarce information has been reported on the composition of *Pinus pinaster* wood. Pioneering studies byAlvarez Novoa *et al*. (1950) and Hata (1955) reported on the isolation of pinosylvin monomethyl ether from *Pinus pinaster* heartwood using a multistage extraction method.

According to the results in Table 4, both pinosylvin and pinosylvin monomethyl ether were more abundant in samples containing knots, and in most cases, the content of pinosylvin monomethyl ether was equal to or slightly higher than that of pinosylvin. Increased amounts of stilbenes in knotwood compared to stemwood have been reported for *Pinus sylvestris* (Willför *et al*. 2003a) and *Pinus radiata* (Hillis and Inoue 1968; Pietarinen *et al*. 2006a). Mass ratios of pinosylvin monomethyl ether/pinosylvin in the range 1.1 to 1.4 (near the values determined in this work) have been reported for *Pinus sylvestris* (Hovelstad *et al*. 2006).

Flavonoids, characterized by a C_6–C_3–C_6 structure, are common phenolics in trees. In the water extracts obtained in this study, pinobanksins (including the dihydroflavonol pinobanksin and its derivative pinobanksin-3-acetate) were the most abundant flavonoids, reaching average concentrations in the range 1.4-2.0 and 2.0-2.3 g/kg in extracts from samples DK_HW and DK_SW, respectively. The presence of the flavanone pinocembrin (which can be converted into pinobanksin by hydroxylation), was also noticed, reaching higher average concentrations (0.5 to 1.6 g/kg extract) in samples containing knots than in HW or SW samples (for which the concentration range was 0.1 to 0.2 g/kg extract). Pinobanksin and pinocembrin were earlier identified in *Pinus pinaster, Pinus radiata, Pinus banksiana, Pinus contorta, Pinus griffithii, Pinus resinosa, Pinus parviflora*, and *Pinus morrisonicola* (Hata 1955; Alvarez-Novoa *et al*. 1950; Hillis and Inoue 1968; Willför *et al*. 2003c; Sinclair and Dymond 1973; Lindberg *et al*. 2004; Neacsu *et al*. 2007; Loman 1970; Simard *et al*. 2008; Mahesh and Seshadri 1954; Fang *et al*. 1987; Pietarinen *et al*, 2006a), but not in wood samples from *Pinus strobus* (Geraldo de Carvalho 1996). Pinocembrin was present in extracts from *Pinus sylvestris* and *Pinus jeffreyi* (Willför *et al*. 2003a; Anderson 1956). Pinobanksin was also found in *Pinus resinosa* wood (Simard *et al*. 2008).

The results in Table 4 also show the presence of minor amounts of taxifolin and dihydrokaempferol, which have been previously identified in extracts from pine (Neacsu *et al*. 2007; Lutskii *et al*. 1971) and from other softwoods (Pietarinen *et al*. 2006a; Willför *et al*. 2003c) with antioxidant activity (Willför *et al*. 2003c).

Table 4. Stilbenoids and Flavonoids in Water Extracts

		Stilbenoids (g/kg extract)		Flavonoids (g/kg extract)				
Sample	T (ºC)	PMME	PS	Pi	Pib	Pib-3	Dik	Ta
HW	130	0.02±0.01	0.02±0.02	0.12±0.07	0.59±0.42	0.02±0.01	0.13±0.08	0.12±0.10
	140	0.01±0.01	0.00±0.00	0.07±0.04	0.21±0.08	0.01±0.01	0.07±0.02	0.12±0.09
SW	130	0.02±0.00	0.01±0.00	0.06±0.03	0.10±0.07	0.10±0.11	0.04±0.00	0.33±0.14
	140	0.09±0.06	0.02±0.02	0.22±0.17	0.09±0.03	0.09±0.07	0.04±0.01	0.30±0.14
DK_HW	130	0.09±0.04	0.19±0.22	0.80±0.34	1.96±1.54	0.02±0.01	0.38±0.19	0.19±0.14
	140	0.06±0.01	0.05±0.04	0.50±0.28	1.38±1.18	0.02±0.02	0.24±0.13	0.12±0.08
DK_SW	130	0.43±0.19	0.54±0.34	1.58±0.56	1.96±0.66	0.30±0.14	0.48±0.13	0.43±0.14
	140	0.37±0.12	0.50±0.40	1.40±0.41	1.76±0.65	0.18±0.08	0.45±0.15	0.37±0.19
LK_HW	140	0.16±0.13	0.24±0.22	0.98±0.47	1.20±0.70	0.05±0.04	0.25±0.14	0.16±0.12
LK_SW	130	0.20±0.03	0.18±0.06	0.62±0.16	0.69±0.09	0.19±0.03	0.21±0.07	0.13±0.03
	140	0.23±0.12	0.17±0.10	0.71±0.48	0.73±0.19	0.18±0.05	0.24±0.17	0.12±0.04

PMME, Pinosylvin monomethyl ether; PS, Pinosylvin; Pic, Pinocembrin; Pib, Pinobanksin; Pib-3, Pinobanksin-3-acetate; Dik, Dihydrokaempferol; Ta, Taxifolin

Lignans, derived from phenylpropanoid precursors, are a large class of secondary metabolites in vascular plants, particularly conifers, where they reach higher concentrations in knots (Willför *et al*. 2003b and 2005a). Table 5 lists the results achieved in this work. Nortrachelogenin, a compound also found in extracts from *Pinus sylvestris* knotwood and other softwood species (Willför *et al*. 2005b), was the most abundant component, reaching average concentrations in the range 7.9 to 55.7 g/kg in extracts from samples containing knots (in comparison with 0 to 0.3 g/kg determined for samples HW and SW). Pinoresinol, a lignan widely distributed in plants, was the second most abundant lignan in extracts, reaching higher concentrations (up to 16.6 mg/g extract) in sample DK_SW. In comparison, secoisolariciresinol and isolariciresinol (which are typical softwood lignans reaching increased concentrations in knots) (Willför *et al*. 2005b), were found at lower proportions (0.3 to 2.2 g/kg extract); whereas todolactol (identified as a component of industrially important trees of the Pinaceae family) (Willför *et al*. 2005b) was found at limited concentrations (0 to 0.2 g/kg).

Other Compounds

Other extract components include resin acids, fatty acids, juvabiones, steryl esters, and triglycerides.

Resin acids are biologically active compounds whose occurrence in conifers is well known, including in *Pinus pinaster* wood (Hemingway *et al*. 1973; Nascimento *et al*. 1995) and bark. The fatty acid content of *Pinus pinaster* wood is known to vary widely with the age, growth rate, and sample position in the tree (Hemingway *et al*. 1973), a pattern confirmed by the data in Table 6. The average contents of native resin acids were below the threshold 2.5 g/kg extract in samples HW, SW, and DK_HW, and averaged for 3.8 to 5.7 g/kg extract from samples DK_SW, LK_HW, and LK_SW. The major individual resin acids in these extracts were dehydroabietic acid (accounting for 1.3 to 2.0 g/kg) and abietic acid (0.7 to 1.2 g/kg); whereas neoabietic acid and palustric

acid reached concentrations up to 0.9 and 0.8 g/kg. In comparison, pimaric and isopimaric acids presented maximum concentrations of 0.7 and 0.4 g/kg. Neoabietic acid and palustric acid, which reached limited contents in stemwood (0 to 0.3 g/kg in extracts from samples HW and SW), were not found in a previous study dealing with the composition of extracts from three *Pinus pinaster* Ait subspecies (Arrabal and Cortijo 1994). The high proportion of modified resin acids (including hydroxyabietic acid, 7-oxodehydroabietic acid, hydroxydehydroabietic acid, dihydroxy-dehydroabietic acid, and hydroxy-7-oxodehydroabietic acid) with respect to native resin acids was remarkable in extracts from samples DK_SW and LK_SW.

Table 5. Lignans in Water Extracts

		Lignans (g/kg extract)				
Sample	T (ºC)	NTG	Pic	Sir	Ir	To
HW	130	0.05±0.02	0.05±0.05	0.09±0.08	0.34±0.29	0.07±0.05
	140	0.04±0.01	0.04±0.05	0.08±0.07	0.29±0.24	0.06±0.04
SW	130	0.28±0.22	0.18±0.19	0.05±0.01	0.65±0.29	0.04±0.02
	140	0.14±0.10	0.02±0.01	0.02±0.01	0.70±0.45	0.03±0.01
DK_HW	130	10.6±8.4	1.64±2.05	0.60±0.56	1.11±1.06	0.10±0.06
	140	7.92±6.60	1.13±1.40	0.44±0.40	0.78±0.73	0.08±0.04
DK_SW	130	55.7±17.8	16.6±4.2	1.77±0.77	2.23±0.73	0.18±0.03
	140	46.8±19.1	13.9±4.6	1.60±0.69	1.92±0.48	0.11±0.01
LK_HW	140	18.3±16.4	9.85±8.50	0.41±0.33	1.50±1.19	0.19±0.16
LK_SW	130	31.9±13.0	4.39±3.08	1.29±0.27	0.83±0.35	0.07±0.05
	140	26.7±15.6	3.16±2.38	1.03±0.13	0.70±0.22	0.03±0.02

NTG, Nortrachelogenin; Pi, Pinoresinol; Sir Secoisolariciresinol; Ir, Isolariciresinol; To, Todolactol

Table 6. Other Components in Extracts from *Pinus pinaster* Wood

		Resin acids (g/kg extract)		Fatty acids (g/kg extract)	Juvabiones (g/kg extract)
Sample	T (ºC)	Native resin acids	Modified resin acids		
HW	130	0.57±0.21	0.25±0.14	0.05±0.02	0.01±0.00
	140	0.70±0.25	0.21±0.11	0.08±0.07	0.00±0.00
SW	130	1.52±0.29	1.32±0.92	0.26±0.06	0.04±0.03
	140	2.52±1.30	1.10±0.61	1.22±0.93	0.04±0.01
DK_HW	130	1.39±0.26	0.77±0.61	0.19±0.09	0.63±0.44
	140	1.27±0.09	0.62±0.51	0.12±0.02	0.47±0.18
DK_SW	130	4.55±0.88	5.64±0.58	1.87±0.46	6.92±4.06
	140	5.66±0.77	4.95±0.99	1.94±0.41	5.59±3.06
LK_HW	140	4.29±3.32	1.09±0.86	0.63±0.41	1.29±0.91
LK_SW	130	3.79±0.51	4.14±0.38	3.19±0.28	4.41±2.43
	140	5.06±2.13	3.68±0.60	3.09±0.61	3.53±2.04

In comparison, the fatty acids (caprylic acid, palmitic acid, and oleic acid) presented limited average concentrations in extracts (0 to 1.2 g/kg, except in samples DK_SW and LK_SW, for which the concentrations fell in the range 1.9 to 3.2 g/kg).

Epijuvabione was present in extracts as the only member of the juvabione family, which includes sesquiterpenes commonly found in conifers involved in plant defense with insect-juvenilizing properties (Phillips *et al*. 2006; Bohlmann *et al*. 1998).

Finally, little added value seems to be achievable from steryl esters and triglycerides, owing to their limited concentrations in samples (0.1 to 0.6 and 0.2 to 1.5 kg/kg, respectively) and the complexity of the media.

Antioxidant Activity

From the experimental information summarized in this work it can be seen that the complex composition of the extracts would make the individual separation of a single compound difficult and that the manufacture of multicomponent, active concentrates could be a more favourable approach to their valorisation. This philosophy has been followed in literature studies, in which applications such as technical antioxidants, functional foods, pharmaceuticals, natural biocides, and wood preservatives have been suggested for crude extracts or concentrates (Moure *et al*. 2005; Willför *et al*. 2003c, 2005c; Pietarinen *et al*. 2006b).

In order to assess the value of extracts for a representative potential application, and considering that a number of the extract components are active antioxidants, experimental work was carried out to measure the antioxidant activity of extracts. Interestingly, the synergistic effects among active extract components may result in higher antioxidant activity than the one of the dominating compounds in pure form (Willför *et al*. 2003c; Pietarinen *et al*. 2006b).

In this study, the performance of extracts was measured using the Trolox Equivalent Antioxidant Capacity (TEAC) method, which led to the experimental results listed in Table 7. This assay was selected owing its suitability for assessing both the hydrophilic and lipophilic antioxidant capacities of the target compounds in multiple media. The antioxidant activities were higher in sapwood samples (0.24 to 0.93 g Trolox/ g extract) than in heartwood samples (0.09 to 0.28 g Trolox/ g extract). The highest radical scavenging capacity (0.92 to 0.93 g Trolox/ g extract) corresponded to the extract from sample DK_SW, and was consistent with its phenolic content (higher than the ones of other extracts). The experimental result corresponded to 17.6 to 20.9% of ABTS radical scavenging activity at 200 mg/L; an activity similar to the that observed for the extract from sample DK_HW sample (16.0 to 20.1%) but at much higher concentration (800 mg/L).

It can be noted that the antioxidant potential of the extracts are influenced not only by the individual contributions of the major phenolic compounds (stilbenes, flavonoids, lignans, simple phenols) and accompanying components (polysaccharide-derived and lipophilic compounds), but also by synergisms among them. In related studies, high radical scavenging capacities have also been reported for hydrophilic extracts from knots of softwoods and hardwoods (Willför *et al*. 2003c). As it has been shown for lignans, the strength of such compounds also rely on the capability to scavenge a large number of radicals (Eklund *et al*. 2005), although the reaction kinetics might be slower than for related synthetic compounds.

Table 7. TEAC of Extracts

Sample	T (ºC)	TEAC (g Trolox/g extract)
HW	130	0.09±0.04
	140	0.10±0.03
SW	130	0.24±0.05
	140	0.28±0.07
DK_HW	130	0.27±0.14
	140	0.26±0.14
DK_SW	130	0.93±0.30
	140	0.92±0.25
LK_HW	140	0.28±0.13
LK_SW	130	0.47±0.06
	140	0.41±0.10

CONCLUSIONS

1. Hemicellulose-derived saccharides (mainly of oligomeric or polymeric nature) were the major components of all extracts obtained in this work from *Pinus pinaster* wood by water extraction at 130 to 140 ^{o}C.
2. A variety of phenolic compounds were present in the extracts, including simple phenolics, phenolic stilbenoids, flavonoids, and lignans.
3. The most abundant simple phenolics were isoferulic and 3,4-dihydroxycinnamic acids.
4. Pinosylvin and its derivative pinosylvin monomethyl ether were the only phenolic stilbenoids found in the extracts.
5. The dihydroflavonol pinobanksin, its derivative pinobanksin-3-acetate, and the flavanone pinocembrin were found in extracts in measurable concentrations. Taxifolin and dihydrokaempferol were found at smaller concentrations.
6. The lignans identified were nortrachelogenin, pinoresinol, secoisolariciresinol and isolariciresinol.
7. Other components of extracts (resin acids, fatty acids, juvabiones, steryl esters, and triglycerides) seem to add little value to the extracts.
8. Extracts from samples containing knots presented remarkable radical scavenging activities.

ACKNOWLEDGMENTS

The authors are grateful to the Spanish "Ministry of Science and Innovation" for supporting this study, in the framework of the research project "Development and evaluation of processing methods for biorefineries" (reference CTQ2011-22972), and to

Xunta de Galicia (INBIOMED project) for additional financial support. Both projects were partially funded by the FEDER Program of the European Union ("Unha maneira de facer Europa"). Ms. Sandra Rivas thanks the Ministry for her predoctoral grant. Dr. Enma Conde thanks the COST Action FP0901 and the Process Chemistry Centre - Åbo Akademi University for the funding received through the Short Term Scientific Mission 090412-016530 and the Johan Gadolin Scholarship, respectively. Docent Annika Smeds is acknowledged for help with the MS analyses. The research leading to these results has received funding from the WoodWisdom-Net Research Programme, which is a transnational R&D programme jointly funded by national funding organizations within the framework of the ERA-NET WoodWisdom-Net 2. This work was also part of the activities at the Process Chemistry Centre at Åbo Akademi University.

REFERENCES CITED

Alvarez-Novoa, J. C., Erdtman, H., and Lindstedt, G. (1950). "Constituents of pine heartwood. XIX. The heartwood of *Pinus pinea* L., *Pinus pinaster* Aiton, *Pinus halepensis* Mill., and *Pinus nigra* Arnold var. *calabrica* (Loudon) Scheneider," *Acta Chemica Scandinavica* 4(3), 444-447.

An, J., Zuo, G. Y., Hao, X. Y., Wang, G. C., and Li, Z. S. (2011). "Antibacterial and synergy of a flavanonol rhamnoside with antibiotics against clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA)," *Phytomedicine* 18(11), 990-993.

Anderson, A. B. (1956). "Increasing extractive content in trees for rosin production. Extract stimulation of Jeffrey pine," *Tappi* 39, 55-59.

Ango, P. Y., Kapche, D. W. F. G., Kuete, V., Ngadjui, B. T., Bezabih, M., and Abegaz, B. M. (2012). "Chemical constituents of *Trilepisium madagascariense* (Moraceae) and their antimicrobial activity," *Phytochemistry Letters* 5(3), 524-528.

Arrabal, C., and Cortijo, M. (1994). "Fatty and resin acids of Spanish *Pinus pinaster* Ait. Subspecies," *J. Am. Oil Chem. Soc.* 71(9), 1039-1040.

Bauerova, K., Ponist, S., Mihalova, D., Drafi, F., and Kuncirova, V. (2011). "Utilization of adjuvant arthritis model for evaluation of new approaches in rheumatoid arthritis therapy focused on regulation of immune processes and oxidative stress," *Interdisc. Toxicol*. 4(1), 33-39.

Berthier, S., Kokutse, A., Stokes, A., and Fourcad, T. (2001). "Irregular heartwood formation in maritime pine (*Pinus pinaster* Ait): Consequences for biomechanical and hydraulic tree functioning," *Annals of Botany* 87(1), 19-25.

Bohlmann, J., Crock, J., Jetter, R., and Croteau, R. (1998). "Terpenoid-based defenses in conifers: cDNA cloning, characterization, and functional expression of wound-inducible (E)-alpha-bisabolene synthase from grand fir (*Abies grandis*)," *Proc. Natl. Acad. Sci. USA* 95(12), 6756-6761.

Brown, D. J. (2012). "Dietary lignan intake and breast cancer risk," *Integrative Medicine Alert* 15(8), 94.

Celimene, C. C., Micales, J. A., Ferge, L., and Young, R. A. (1999). "Efficacy of pinosylvins against white-rot and brown-rot fungi," *Holzforschung* 53(5), 491-497.

Céspedes, C. L., Avila, J. G., García, A. M., Becerra, J., Flores, C., Aqueveque, P., Bittner, M., Hoeneisen, M., Martinez, M., and Silva, M. (2006). "Antifungal and

antibacterial activities of *Araucaria araucana* (Mol.) K. Koch heartwood lignans," *Z. Naturforsch C.* 61(1-2), 35-43.

Ebringerová, A., Hromádková, Z., Hríbalová, V., Xu, C., Holmbom, B., Sundberg, A., and Willför, S. (2008). "Norway spruce galactoglucomannans exhibiting immunomodulating and radical-scavenging activities," *Int. J. Biol. Macromol.* 42(1), 1-5.

Eklund, P. C., Långvik, O. K., Wärnå, J. P., Salmi, T. O., Willför, S. M., and Sjöblom, R. (2005). "Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans," *Org. Biomol. Chem.* 3(18), 3336-3347.

Ekman, R., and Holmbom, B. (1989). "Analysis by gas chromatography of the wood extractives in pulp and water samples from mechanical pulping of spruce," *Nord. Pulp Pap. Res. J*. 4(1), 16-24.

Ekman, R., Willför, S., Sjöholm, R., Reunanen, M., Mäki, J., Lehtilä, R., and Eckerman, C. (2002). "Identification of the lignan nortrachelogenin in knot and branch heartwood of Scots pine (*Pinus sylvestris* L.)," *Holzforschung* 56(3), 253-256.

Fang, J. M., Chang, C. F., and Cheng, Y. S. (1987). "Flavonoids from *Pinus morrisonicola*," *Phytochemistry* 26(9), 2559-2561.

Galbe, M., and Zacchi, G. (2002). "A review of the production of ethanol from softwood," *Appl. Microbiol. Biotechnol*. 59(6), 618-628.

Garrote, G., Domínguez, H., and Parajó, J. C. (1999). "Mild autohydrolysis: an environmentally friendly technology for xylooligosaccharide production from wood," *J. Chem. Technol. Biotechnol*. 74(11), 1101-1109.

Geraldo de Carvalho, M., Cranchi, D. C., and Geraldo de Carvalho, A. (1996). "Chemical constituents from *Pinus strobus* var. chiapensis," *J. Braz. Chem. Soc.* 7(3), 187-191.

González-Muñoz, M. J., Santos, V., and Parajó, J. C. (2011). "Purification of oligosaccharides obtained from *Pinus pinaster* hemicelluloses by diafiltration," *Desal. Water Treatm.* 27(1-3), 48-53.

González-Muñoz, M. J., Alvarez, R., Santos, V., and Parajó, J. C. (2012). "Production of hemicellulosic sugars from *Pinus pinaster* wood by sequential steps of aqueous extraction and acid hydrolysis," *Wood Sci. Technol*. 46(1-3), 271-285.

Gözü, B. B., Komulainen, H., Hyvönen, P., and von Wright, A. (2010). "The impact of pinosylvin on the development of *Listeria monocytogenes* in the salted rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) stored at different temperatures," *J. FisheriesSciences.com* 4(4), 419-426. (www.fisheriessciences.com).

Gref, R., Hakansson, C., Henningsson, B., and Hemming, J. (2000). "Influence of wood extractives on brown and white rot decay in scots pine heart-, light- and sapwood," *Mat. Organism*. 33(2), 119-128.

Gullón, P., Romaní, A., Vila, C., Garrote, G., and Parajó, J. C. (2012). "Potential of hydrothermal treatments in lignocellulose biorefineries," *Biofuels Bioprod. Bioref.* 6(2), 219-232.

Hata, K. (1955). "Chemical properties of *Pinus pinaster* wood from the Ehime District Trans," *J. Japan Forest. Soc*. 36(Special Issue), 335-337.

Hemingway, R. W., Hills, W. E., and Lau, L. S. (1973). "Extractives in *Pinus pinaster* wood," *Svensk Papperstidning* 76(10), 371-376.

Hillis, W. E., and Inoue, T. (1968). "The formation of polyphenols in trees – IV. The polyphenols formed in *Pinus radiata* after *Sirex* attack," *Phytochem.* 7(1), 13-22.

Hovelstad, H., Leirset, I., Oyaas, K., and Fiksdahl, A. (2006). "Screening analyses of pinosylvin stilbenes, resin acids and lignans in Norwegian conifers," *Molecules* 11(1), 103-114.

Hwang, B., Lee, J., Liu, Q. H., Woo, E. R., and Lee, D. G. (2010). "Antifungal effect of (+)-pinoresinol isolated from *Sambucus williamsii*," *Molecules* 15(5), 3507-3516.

Jančinová, V., Perečko, T., Nosáľ, R., Harmatha, J., Smidrkal, J., and Drábiková, K. (2012). "The natural stilbenoid pinosylvin and activated neutrophils: Effects on oxidative burst, protein kinase C, apoptosis and efficiency in adjuvant arthritis," *Acta Pharmacol. Sin.* 33(10), 1285-1292.

Kang, J., Li, Z., Wu, T., Jensen, G. S., Schauss, A. G., and Wu, X. (2010). "Anti-oxidant capacities of flavonoid compounds isolated from acai pulp (*Euterpe oleracea* Mart.)," *Food Chem*. 122(3), 610-617.

Kebenei, J. S., Ndalut, P. K., and Sabah, A. O. (2011). "Anti-plasmodial activity of Nortrachelogenin from the root bark of *Carissa edulis* (vahl)," *Int. J. Appl. Res. Nat. Prod.* 4(3), 1-5.

Kimura, Y., and Sumiyoshi, M. (2011). "Antitumor actions of anthraquinone- and stilbene-related natural products through anti-angiogenic action," *Curr. Topics Phytochem.* 10, 75-96.

Koell, P., and Lenhardt, H. (1987). "Degradation of hemicellulose-rich biological materials with water in a flow reactor," *Macromol. Chem. Phys.* 188(4), 749-762.

Kokubo, R., Sakai, K., and Imamura, H. (1990). "Secondary metabolites in cell cultures of woody plants. II. Formation of pinosylvin and its monomethyl ether in callus and the effect of UV irradiation on their contents," *J. Japan Wood Res. Soc.* 36(12), 1084-1088.

Kumar, M. A., Nair, M., Hema, P. S., Mohan, J., and Santhoshkumar, T. R. (2007). "Pinocembrin triggers Bax-dependent mitochondrial apoptosis in colon cancer cells," *Mol. Carcinog.* 46(3), 231-241.

Lampe, J. W. (2003). "Isoflavonoid and lignan phytoestrogens as dietary biomarkers," *J. Nutr.* 133(3), 956S-964S.

Lee, S. B., Cha, K, H., Selenge, D., Solongo, A., and Nho, C. W. (2007). "The chemopreventive effect of taxifolin is exerted through ARE-dependent gene regulation," *Biol. Pharm. Bull.* 30(6), 1074-1079.

Lee, S. K., Lee, H. J., Min, H. Y., Parka, E. J., Lee, K. M., Ahn, Y. H., Cho, Y. J., and Pyee, J. H. (2005). "Antibacterial and antifungal activity of pinosylvin, a constituent of pine," *Fitoterapia* 76(2), 258-260.

Li, X. B., Yang, Z. X., Yang, L., Chen, X. L., Zhang, K., Yang, Q., Wu, Y. M., Liu, S. B., Tao, K, S., and Zhao, M. G. (2012). "Neuroprotective effects of flax lignan against NMDA-induced neurotoxicity *in vitro*," *CNS Neurosci. Ther.* 18(11), 927-933.

Lindberg, L. E., Willför, S. M., and Holmbom, B. R. (2004). "Antibacterial effects of knotwood extractives on paper mill bacteria," *J. Ind. Microbiol. Biotechnol.* 31(3), 137-147.

Liu, R., Wu, C. X., Zhou, D., Yan, F., Tian, S., Zhang, L., Zhang, T. T., and Du, G. H. (2012). "Pinocembrin protects against β-amyloid-induced toxicity in neurons through inhibiting receptor for advanced glycation end products (RAGE)-independent signaling pathways and regulating mitochondrion-mediated apoptosis," *BMC Medicine* 10(Sept.), 105, 22 pp. (http://www.biomedcentral.com/bmcmed/content/10/September/2012).

Loman, A. A. (1970). "Effect of heartwood fungi of *Pinus contorta* var. *latifolia* on pinosylvin, pinosylvinmonomethyl ether, pinobanksin, and pinocembrin," *Can. J. Bot.* 48(4), 737-747.

Luo, H., Jiang, B. H., King, S. M., and Chen, Y. C. (2008). "Inhibition of cell growth and VEGF expression in ovarian cancer cells by flavonoids," *Nutr. Cancer* 60(6), 800-809.

Lutskii, V. I., Gromova, A. S., and Tyukavkina, N. A. (1971). "Aromadendrin, apigenin, and kaempferol from the wood of *Pinus sibirica*," *Chem. Nat. Comp.* 7(2), 197-198.

Mahesh, V. B., and Seshadri, T. R. (1954). "Chemical components of commercial woods and related plant materials. II. The heartwood of *Pinus griffithii*," *J. Sci. Ind. Res.* 13B, 835-841.

Maimoona, A., Naeem, I., Saddiqe, Z., and Jameel, K. (2011). "A review on biological, nutraceutical and clinical aspects of French maritime pine bark extract," *J. Ethnopharmacol.* 133(2), 261-277.

Maridass, M., Raju, G., Thangavel, K., and Ghanthikumar, S. (2008). "Prediction of anti-HIV activity of flavanoid constituents through PASS," *Ethnobotanical Leaflets* 12, 954-994. (http://opensiuc.lib.siu.edu/cgi/viewcontent.cgi?article=1165&context=ebl&sei-redir=1).

Moure, A., Domínguez, H., and Parajo, J. C. (2005). "Antioxidant activity of liquors from aqueous treatments of *Pinus radiata* wood," *Wood Sci. Technol.* 39(2), 129-139.

Nascimento, E. A., Morais, S. A. L., Vallejo, M. C. G., Fernandez-Vega, F. I., and Varela, P. N. (1995). "The composition of wood extracts form Spanish *Pinus pinaster* and Brazilian *Pinus caribaea*," *J. Braz. Chem. Soc.* 6(4), 331-336.

Neacsu, M., Eklund, P. C., Sjoeholm, R. E., Pietarinen, S. P., Ahotupa, M. O., Holmbom, B. R., and Willför, S. M. (2007). "Antioxidant flavonoids from knotwood of Jack pine and European aspen," *Holz als Roh- und Werkstoff* 65(1), 1-6.

Ondrias, K., Stasko, A., Hromadova, M., Suchy, V., and Nagy, M. (1997). "Pinobanksin inhibits peroxidation of low density lipoprotein and it has electron donor properties reducing α-tocopherol radicals," *Pharmazie* 52(7), 566-567.

Örså, F., and Holmbom, B. (1994). "A convenient method for the determination of wood extractives in papermaking process waters and effluents," *J. Pulp. Pap. Sci.* 20(12), J361-J366

Park, E. J., Park, H. J., Chung, H. J., Shin, Y., Min, H. Y., Hong, J. Y., Kang, Y. J., Ahn, Y. H., Pyee, J. H., and Lee, S. K. (2012). "Antimetastatic activity of pinosylvin, a natural stilbenoid, is associated with the suppression of matrix metalloproteinases," *J. Nutr. Biochem.* 23(8), 946-952.

Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., and McCullough, M. L. (2010). "Dietary lignans: Physiology and potential for cardiovascular disease risk reduction," *Nutr. Rev.* 68(10), 571-603.

Phillips, M. A., Bohlmann, J., and Gershenzon, J. (2006). "Molecular regulation of induced terpenoid biosynthesis in conifers," *Phytochem. Rev.* 5(1), 179-189.

Pietarinen, S. P., Willför, S. M., Ahotupa, M. O., Hemming, J., and Holmbom B. R. (2006b). "Knotwood and bark extracts: strong antioxidants from waste materials," *J. Wood Sci.* 52(5), 436-444.

Pietarinen, S. P., Willfor, S. M., Vikstrom, F. A., and Holmbom, B. R. (2006a). "Aspen knots, a rich source of flavonoids," *J. Wood Chem. and Technol.* 26(3), 245-258.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., and Rice-Evans, C. (1999). "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Free Radic. Biol. Med.* 26(9-10), 1231-1237.

Redzynia, I., Ziolkowska, N. E., Majzner, W. R., Willför, S., Sjoholm, R., Eklund, P., and Bujacz, G. D. (2009). "Structural investigation of biologically active phenolic compounds isolated from European tree species," *Molecules* 14(10), 4147-4158.

Rivas, S., Gullón, B., Gullón, P., Alonso, J. L., and Parajó, J. C. (2012). "Manufacture and properties of bifidogenic saccharides derived from wood mannan," *J. Agric. Food Chem.* 60(17), 4296-4305.

Rogovskiĭ, V. S., Matiushin, A. I., Shimanovskiĭ, N. L., Semeĭkin, A. V., Kukhareva, T. S., Koroteev, A. M., Koroteev, M. P., and Nifant'ev, E. E. (2010). "Antiproliferative and antioxidant activity of new dihydroquercetin derivatives," *Eksp. Klin. Farmakol.* 73(9), 39-42.

Shahbazi, A., Li, Y., and Mims, M. R. (2005). "Application of sequential aqueous steam treatments to the fractionation of softwood," *Appl. Biochem. Biotechnol*. 121-124, 973-987.

Shain, L., and Miller, J. B. (1982). "Pinocembrin: An antifungal compound secreted by leaf glands of eastern cottonwood," *Phytopathology* 72(7), 877-880.

Simard, F., Legault, J., Lavoie, S., Mshvildadze, V., and Pichette, A. (2008). "Isolation and identification of cytotoxic compounds from the wood of *Pinus resinosa*," *Phytother. Res.* 22(7), 919-922.

Sinclair, G. D., and Dymond, D. K. (1973). "Distribution and composition of extractives in jack pine trees," *Can. J. Forest Res.* 3(4), 516-521.

Soromou, L. W., Chu, X., Jiang, L., Wei, M., Huo, M., Chen, N., Guan, S., Yang, X., Chen, C., and Feng, H. (2012). "*In vitro* and *in vivo* protection provided by pinocembrin against lipopolysaccharide-induced inflammatory responses," *Int. Immunopharmacol.* 14(1), 66-74.

Välimaa, A. L., Honkalampi-Hämäläinen, U., Pietarinen, S., Willför, S., Holmbom, B., and von Wright, A. (2007). "Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms," *Int. J. Food Microbiol.* 115(2), 235-243.

Van Ree, R., and Annevelink, B. (2007). *Status Report Biorefinery 2007*, Wageniningen, (http://www.biorefinery.nl/fileadmin/biorefinery/docs/publications/StatusDocumentBiorefinery2007final211107.pdf).

Venäläinen, M., Harju, A. M., Saranpää, P., Kainulainen, P., Tiitta M., and Velling. P. (2004). "The concentration of phenolics in brown-rot decay resistant and susceptible Scots pine heartwood," *Wood Sci. Tech.* 38(2), 109-118.

Villanueva, V. R., Barbier, M., Gonnet, M., and Lavie, P. (1970). "Les flavonoids de la propolis isolement d'une nouvelle substance bacteriostatique: La pinocembrine," *Ann. Inst. Pasteur* 118(1), 84-87.

Willför, S. M., Hemming, J., Reunanen, M., and Holmbom, B. (2003a). "Phenolic and lipophilic extractives in Scots pine knots and stemwood," *Holzforschung* 57(4), 359-372.

Willför, S. M., Hemming, J., Reunanen, M., Eckerman, C., and Holmbom, B. (2003b). "Lignans and lipophilic extractives in Norway spruce knots and stemwood," *Holzforschung* 57(1), 27-36.

Willför, S. M., Ahotupa, M. O., Hemming, J. E., Reunanen, M. H. T., Eklund, P. C., Sjöholm, R. E., Eckerman, C. S. E., Pohjamo, S. P., and Holmbom, B. R. (2003c).

"Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species," *J. Agr. Food Chem*. 51(26), 7600-7606.

Willför, S., Nisula, L., Hemming, J., Reunanen, M., and Holmbom, B. (2004). "Bioactive phenolic substances in industrially important tree species. Part 1: Knots and stemwood of different spruce species," *Holzforschung* 58(4), 335-344.

Willför, S. M., Sundberg, A. C., Rehn, P. W., Holmbom, B. R., and Saranpaeae, P. T. (2005a). "Distribution of lignans in knots and adjacent stemwood of *Picea abies*," *Holz als Roh- und Werkstoff* 63(5), 353-357.

Willför, S., Eklund, P., Sjoholm, R., Reunanen, M., Sillanpaa, R., von Schoultz, S., Hemming, J., Nisula, L., and Holmbom, B. (2005b). "Bioactive phenolic substances in industrially important tree species. Part 4: Identification of two new 7-hydroxy divanillyl butyrolactol lignans in some spruce, fir, and pine species," *Holzforschung* 59(4), 413-417.

Willför, S., Eckerman, C., Hemming, J., Holmbom, B., Pietarinen, S., and Sundberg, A. (2005c). "Use of knotwood extracts as antioxidant," *PCT Int. Appl.* WO 2005047423 A1 20050526.

Willför, S., Hafizoğlu, H., Tümen, I., Yazici, H., Arfan, M., Ali, M., and Holmbom, B. (2007). "Extractives of Turkish and Pakistani tree species," *Holz als Roh- und Werkstoff* 65(3), 215-221. (http://link.springer.com/article/10.1007%2Fs10616-012-9502-x).

Yáñez, R., Romaní, A., Garrote, G., Alonso, J. L., and Parajó, J. C. (2009). "Processing of *Acacia dealbata* in aqueous media: A first step of wood biorefinery," *Ind. Eng. Chem. Res*. 48(14), 6618-6626.

Yoon, S. H., Macewan, K., and van Heiningen, A. (2008). "Hot-water pre-extraction from loblolly pine (*Pinus taeda*) in an integrated forest products biorefinery," *TAPPI J.* 7(6), 27-32.

Article submitted: January 9, 2013; Peer review completed: February 16, 2013; Revised version received and accepted: February 22, 2013; Published: February 28, 2013.