J Wood Sci (2006) 52:436–444
DOI 10.1007/s10086-005-0780-1

ORIGINAL ARTICLE

Suvi P. Pietarinen · Stefan M. Willför
Markku O. Ahotupa · Jarl E. Hemming
Bjarne R. Holmbom

Knotwood and bark extracts: strong antioxidants from waste materials

Received: April 27, 2005 / Accepted: October 26, 2005 / Published online: March 15, 2006

Abstract The antioxidant properties of hydrophilic extracts of knotwood of several industrially important tree species were evaluated by lipid-peroxidation inhibition and peroxyl-trapping capacity tests. The results were compared with the antioxidant properties of hydrophilic extracts of bark, and pure lignans and flavonoids isolated from knotwood extracts. The knot extracts from several tree species were stronger antioxidants than the bark extracts, which can, however, also be classified as strong antioxidants. In addition, the antioxidant properties of most of the knotwood extracts are stronger than the pure compounds. It is concluded that knotwood is a rich source of natural antioxidants.

Key words Knots · Bark · Lignans · Flavonoids · Antioxidants

Introduction

Knotwood occurs as branch stubs embedded in tree stems. In recent studies, we have found that knots of several softwoods (*Abies*,[1] *Picea*,[2] and *Pinus* spp.[3]) and hardwoods (*Populus*[4,5] and *Acacia* spp.[6]) contain exceptionally large amounts of polyphenols, mostly lignans and pinosylvins in softwoods, and flavonoids in hardwoods. For instance, the amount of extractable polyphenols can be up to 30% in the knotwood of *Picea abies*.[2] Polyphenols are enriched in knotwood probably due to their strong antioxidant[7] and antibacterial[8] effects. For instance, pinosylvin is known as both a fungicide and as a bactericide;[9] furthermore, it also has antioxidant properties.[10] These properties are needed in branch knots, because an open wound is formed when a branch is broken close to the stem, making the knot susceptible to attack by fungi. The motion of a branch also generates tension and free radicals in the branch base.

Polyphenols generally have three defense functions: as radical scavengers;[11] as biocides;[11] and metal chelators.[12] The radical scavenging activity is important because both white-rot and brown-rot fungi are believed to use radicals to disrupt cell walls.[13,14] Polyphenols occur in knotwood predominantly as free aglycones, while the glycosidic form is more abundant in other plant sources. Hopia and Heinonen[15] showed that the flavonoid aglycones are more potent antioxidants than their glycosylated equivalents. A recent study showed that some lignans isolated from branch knots possess strong antioxidant properties.[7] A number of flavonoids[16] and hydrolyzable and condensed tannins[17] efficiently chelate trace metals, which play an important role in oxygen metabolism. Even the lignan hydroxymatairesinol has been shown to chelate alkali metals.[18] Synergistic effects can be achieved when the knots contain mixtures of several types of extractives with complementary characteristics.[19]

Softwood knots are not preferred in pulping and papermaking; they are inferior as raw material due to their shorter fibers and large amount of extractives that consume pulping chemicals. In addition, because the knots are much harder than normal wood, extra energy is needed in chipping of the knots. However, knotwood can be removed from the oversized chip fraction prior to pulping and can then be used as a raw material for production of bioactive compounds.[20]

In pulp and paper production, large amounts of bark waste are produced. This waste material is usually used in energy production and is burned in large furnaces. However, barks of several tree species are known to contain bioactive chemicals that can give added value to this energy resource.[21–23] Pycnogenol is a commercial product of *Pinus maritima* (syn. *Pinus pinaster*) bark extract and it is proven to be an effective antioxidant.[24]

In this article, we show that industrial waste streams, such as knotwood and bark, are rich sources of potent

S.P. Pietarinen (✉) · S.M. Willför · J.E. Hemming · B.R. Holmbom
Åbo Akademi University, Process Chemistry Centre, Porthansgatan 3, Turku/Åbo FIN-20500, Finland
Tel. +358-2-215-4887; Fax +358-2-215-4868
Suvi.Pietarinen@abo.fi

M.O. Ahotupa
MCA Research Laboratory, Biocity B4, Tykistönkatu 6B, Turku/Åbo FIN-20520, Finland

antioxidants. We assessed the antioxidant potency and a radical scavenging capacity of 21 well-defined hydrophilic knotwood extracts; from both softwoods and hardwoods, and seven pure compounds; lignans, flavonoids, and pinosylvin, isolated from knotwood extracts. Structures of the isolated and identified compounds are presented in Figs. 1 and 2. Furthermore, the antioxidant potencies of hydrophilic bark extracts, including the commercially available Pycnogenol, were assessed and compared with the knotwood extracts.

Materials and methods

Sample preparation

Knotwood was sampled from full-grown trees of 21 tree species (Table 1). All sampled trees and knots were normal and healthy. The heartwood of the knots was splintered, freeze-dried, and ground.

Bark was sampled from the same trees as the knotwood. The outermost layers of outer bark were removed and the bark was cut into small pieces, freeze-dried, and ground. Pycnogenol was kindly provided by Bioteekki Oy, Finland.

Sequential extraction was carried out in an accelerated solvent extractor apparatus (ASE) (Dionex, Sunnyvale, CA).[2] The lipophilic extractives were first extracted with hexane, and thereafter the hydrophilic extractives with an acetone : water (95 : 5 v/v) mixture. After the extraction, the acetone : water mixture was evaporated to dryness and the remaining extractives were stored frozen. The gravimetrically determined total amounts of hydrophilic extracts are given in Table 1. The extracts were stored at –18°C. For isolation of pure polyphenols, a larger amount of knotwood was extracted with a Soxhlet apparatus, first with hexane and then with acetone.

Preparation of the pure compounds

Lariciresinol (structure *8* in Fig. 1), prepared as described in Willför et al.,[7] was converted to cyclolariciresinol (*1*) as described by Eklund et al.[25] *Acacia mangium* knotwood was sequentially extracted using the ASE apparatus. The hydrophilic extract was fractionated using flash chromatography on normal-phase silica gel columns with dichloromethane : ethanol, starting from 95% dichloromethane and increasing the ethanol ration gradually to 50%, yielding teracacidin (structure *25* in Fig. 2) [purity 82%, main impurity isoteracacidin (*27*), 7%].

Acacia crassicarpa knotwood was sequentially extracted using ASE. The hydrophilic extract was fractionated using flash chromatography as described above yielding a mixture consisting of 60% melacacidin (*28*) and 33% isomelacacidin (*29*).

Table 1. Sample definitions and gravimetric amounts of hydrophilic extractives

Samples	Source	Annual rings[a]	Extractives weight-%
Knotwood			
Acacia crassicarpa	Indonesia, Bakung langgam	7[b]	7.1
Acacia mangium	Indonesia, Bakung langgam	7[b]	5.3
Abies lasiocarpa	Ekenäs, Finland	59	3.5
Abies pectinata	Saint-Die, France	31	13
Eucalyptus globulus	Colunga, Asturias, Spain	11	4.0
Fagus sylvatica	Slovenia	141	2.0
Larix lariciana	Blandin Land, Itasca County, USA	44	3.0
Larix sibirica	Russia, St. Petersbug region	50	7.9
Picea glauca	Blandin Land, Itasca County, USA	49	9.2
Picea mariana	Ekenäs, Finland	63	5.0
Picea sitchensis	North Wales, UK	16	5.0
Pinus banksiana	Blandin Land, Itasca County, USA	52	6.8
Pinus resinosa	Blandin Land, Itasca County, USA	42	6.8
Pinus strobus	Cape Breton, Nova Scotia, Canada	53	19
Pinus sylvestris	Ekenäs, Finland	72	8.8
Populus grandidentata	Cape Breton, Nova Scotia, Canada	37	10
Populus tremula	Houtskär, Finland	31	6.3
Populus tremuloides	Cape Breton, Nova Scotia, Canada	58	5.2
Thuja plicata	Port Hardy, Vancouver islands, Canada	80	29
Tsuga canadensis	Cape Breton, Nova Scotia, Canada	196	29
Tsuga heterophylla	Port Hardy, Vancouver islands, Canada	80	17
Bark			
Abies lasiocarpa	Ekenäs, Finland		8.1
Betula pendula	Ekenäs, Finland		9.9
Picea abies	Ekenäs, Finland		12
Pinus banksiana	Blandin Land, Itasca County, USA		7.7
Populus tremula	Houtskär, Finland		18
Pseudotsuga menziensii	Ekenäs, Finland		13
Thuja occidentalis	Blandin Land, Itasca County, USA		5.9

[a] At height of 1.5 m
[b] Age in years

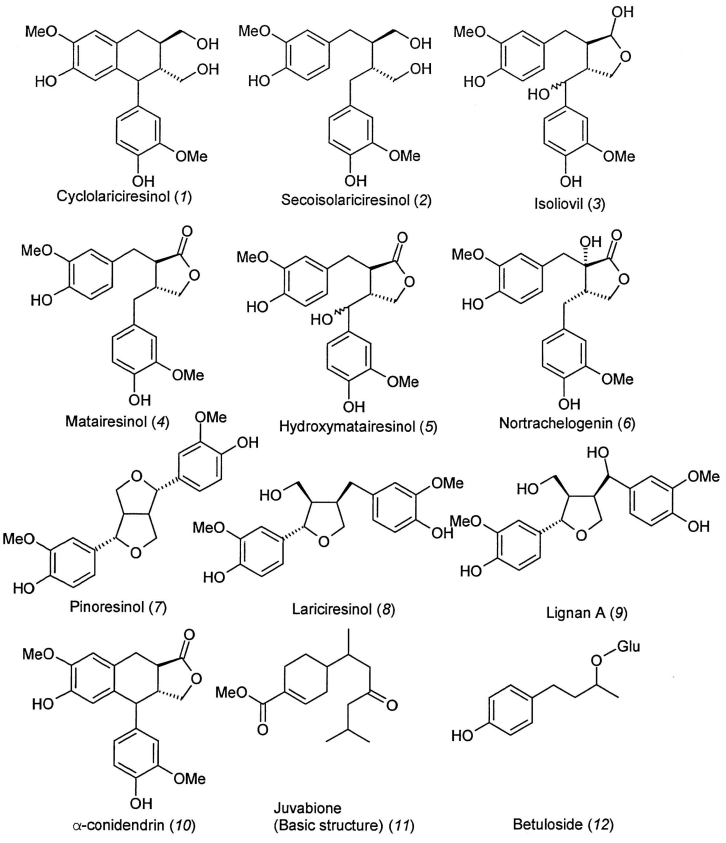

Fig. 1. Lignans (*1–10*) and juvabione (*11*) present in knotwood extracts, and betuloside (*12*) present in *Betula pendula* bark extract

Pinus cembra knotwood was sequentially extracted using ASE. The hydrophilic extract was fractionated using flash chromatography as described above yielding pinocembrin (*31*) (purity >95%).

Pinus sylvestris resin was hydrolyzed with strongly alkaline KOH solution, followed by filtration. The solid potassium salt of pinoresinol was acidified, and extracted with methyl-*tert*-butyl ether (MTBE). The MTBE extract was purified using flash chromatography as described above yielding pinoresinol (*7*) (purity >95%).

Pinus sylvestris knotwood was sequentially extracted, first with hexane and then with acetone, using a large Soxhlet apparatus. The hydrophilic extract was fractionated using flash chromatography on normal-phase silica gel columns as described above yielding pinosylvin (*13*) (purity >95%).

Populus tremula knotwood was sequentially extracted using ASE. The hydrophilic extract was fractionated using flash chromatography on normal-phase silica gel columns as described above to yield dihydrokaempferol (*21*) (purity >95%).

Analyses of the extracts

Lignans, stilbenes, flavonoids, and juvabiones were analyzed by gas chromatography on a 25 m × 0.20 mm i.d., 0.11 μm HP-1 capillary column coated with cross-linked methyl polysiloxane (Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was a Perkin Elmer AutoSystem XL instrument (Perkin Elmer, Boston, MA, USA). The analysis conditions were: column oven 120°C ramped at 6°C/min to 300°C (held for 10 min); carrier gas H_2 (0.6 ml/min); split injector (1:20) 260°C; flame ionization detector (FID) 300°C; injection volume 1 μl. The solvent was evaporated and the extractives were silylated by addition of 80 μl bis-(trimethylsilyl)-trifluoroacetamide, 20 μl trimethylchlorosilane, and 20 μl pyridine. The reaction was completed by keeping the test tubes in an oven at 70°C for 1 h. Heneicosanoic acid and betulinol were used as internal standards. A correction factor of 1.2 was used for the lignans that were calculated against betulinol.[2] Oligolignans, biflavonoids, and flavonoid glycosides were quantified on a short 6 m × 0.53 mm i.d., 0.15 μm HP-1 column using cholesteryl heptadecanoate and 1,3-dipalmitoyl-2-oleyl glycerol as internal standards.[2] The gas chromatograph was a Varian 3400 instrument (Varian, Palo Alto, CA, USA). The analysis conditions were: column oven 100°C (held for 1.5 min) ramped at 12°C/min to 340°C (held for 5 min); carrier gas H_2 (20 ml/min); septum-equipped programmable injector 80°C (held for 0.5 min) ramped at 200°C/min to 340°C (held for 18 min); FID 340°C; injection volume 0.4 μl. Identification of individual components was performed by GC-MS analysis of the silylated components with an HP 6890-5973 GC-quadrupole-MSD instrument. Both a similar 25-m HP-1 GC column as above and a 15 m × 0.25 mm i.d., 0.1 μm MXT-65TG column (Restek, USA), which allowed elution of the silylated oligolignans,[2] biflavonoids,[6] and flavonoid glycosides,[5] were used. The molar-mass distribution of the extracts was determined by high-performance size-

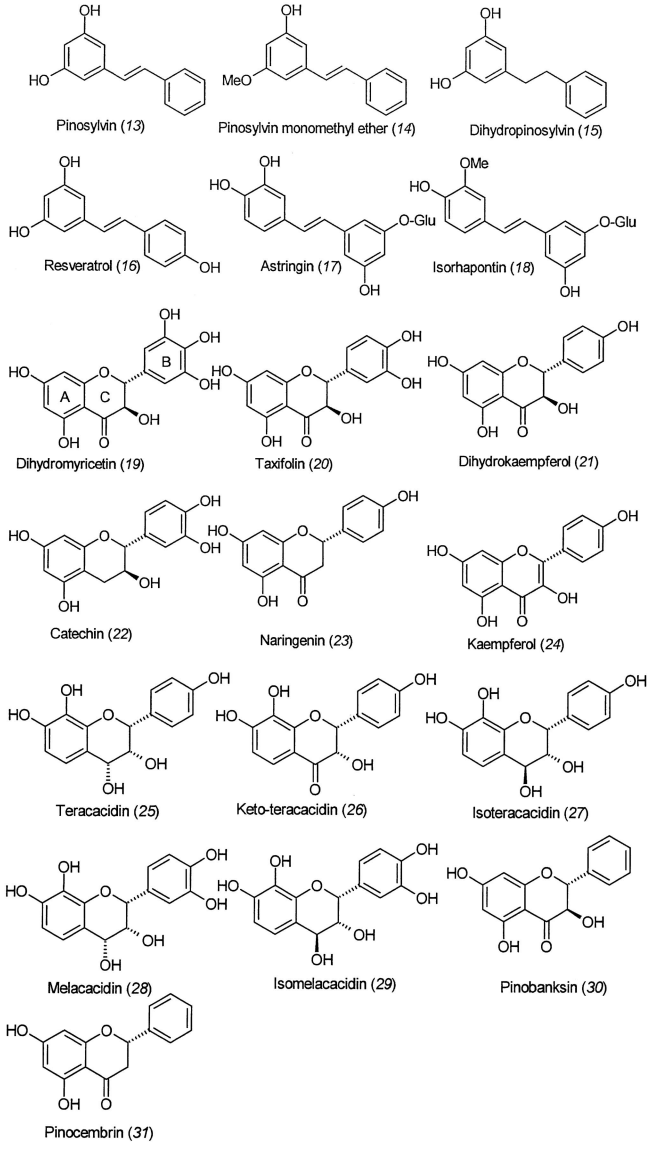

Fig. 2. Stilbenes (*13–18*) and flavonoids (*19–31*) present in knotwood and bark extracts. Flavonoid ring nominations are (*A–C*) shown on dihydromyricetin (*19*)

exclusion chromatography (HPSEC) in order to detect substances with larger molecular sizes than biflavonoids and flavonoid glycosides, such as tannins as described by Willför et al.[1–3] and Pietarinen et al.[4–6]

Determination of the antioxidant activity and radical trapping capacity

The extracts and pure compounds were dissolved in 5–12 ml ethanol, giving a concentration of 3–26 mg dry extract/ml, and then filtered using 0.2-μm syringe filters. The antioxidant properties of the extracts and the pure compounds were estimated for their potency to inhibit *tert*-butylhydroperoxide (*t*-BuOOH) induced lipid peroxidation in rat liver microsomes in vitro[26] as described by Willför et al.[7] The lipid peroxidation was detected by luminol-enhanced chemiluminescence. The tests were repeated two to four times on separate days. Variation between assays was less than 10%.

The capacity of the extracts and the pure compounds to trap peroxyl radicals was estimated by chemiluminescence-based methodology[26] as also described by Willför et al.[7] Peroxyl radicals were generated by thermal decomposition of 2,2′-azobis(2-amidinopropane) hydrochloride. Chemiluminescence in triplicate cuvettes at 37°C was measured until a peak value for each sample was detected. The halfpeak time point defined the peroxyl radical trapping capacity.

Results and discussion

Extract composition

Lignans and oligolignans dominated in the *Abies*, *Larix*, *Picea*, and *Tsuga* knotwood extracts (Table 2). The most abundant lignan was secoisolariciresinol (structure *2* in Fig. 1) in the *Abies* and *Larix* extracts, while hydroxymatairesinol (*5*) was predominant in the *Picea* and *Tsuga* extracts. Pinosylvins (structures *13–15* in Fig. 2) were predominant in the *Pinus strobus* and *Pinus resinosa* extracts, whereas the *Pinus banksiana* and *Pinus sylvestris* extracts contained large amounts of lignans (*1*,*3*,*6*,*8*) in addition to pinosylvins. The *Thuja plicata* extract contained unidentified lignans related to thujaplicatin and 4-*O*-demethylyatein. The *Populus* and *Acacia* extracts, as well as the *Fagus sylvatica* extract had flavonoids as their predominant compounds (Table 3). Dihydrokaempferol (*21*) dominated in all *Populus* extracts, while teracacidin (*25*) and melacacidin (*28*) dominated in the *Acacia mangium* and *Acacia crassicarpa* extracts, respectively. The predominant compound group in *Eucalyptus globulus* was tannins.

The major components, according to GC-MS analysis, in the bark extracts were identified, but not quantified. Quantitative analysis was not performed because the extracts contained large amounts of material that was not eluted in GC analysis, i.e., tannins, and the separation obtained by HPSEC did not allow accurate quantification. The total amounts of extractives in bark samples are presented in Table 1. The *Abies lasiocarpa*, *Thuja occidentalis*, *Pinus banksiana*, *Picea mariana*, and *Pseudotsuga menziensii* bark extracts contained large amounts of sugars (Table 4). The *Pinus* extracts and *Pseudotsuga menziensii* extracts contained flavonoids (*19*,*20*,*22)* as major polyphenols. The main component in *Betula pendula* was betuloside (*12*). *Abies lasiocarpa* contained resin acids while *Thuja occidentalis* and *Picea abies* contained stilbenes (*16–18*). All bark samples contained tannin.

Antioxidant properties of the pure compounds

The tested pure lignans, cyclolariciresinol and pinoresinol, possessed strong antioxidant properties in the lipid peroxidation test; they were even stronger than the lignans tested in the earlier study[7] (Table 5). Melacacidin was proven

Table 2. Main component groups and components in the softwood hydrophilic knotwood extracts

Abies lasiocarpa	
Lignans	7%
Secoisolariciresinol	2%
Todolactol A + isoliovil	2%
Hydroxymatairesinol	1%
Oligolignans	16%
Juvabiones	5%
Abies pectinata	
Lignans	42%
Secoisolariciresinol	23%
Hydroxymatairesinol	5%
Todolactol A + isoliovil	5%
Lariciresinol	4%
Matairesinol	2%
Oligolignans	22%
Juvabiones	6%
Larix lariciana	
Lignans	22%
Secoisolariciresinol	15%
Cyclolariciresinol	3%
Todolactol A + isoliovil	2%
Oligolignans	12%
Flavonoids	13%
Taxifolin	11%
Larix sibirica	
Lignans	24%
Secoisolariciresinol	15%
Lariciresinol	4%
Todolactol A + isoliovil	2%
Nortrachelogenin	1%
Oligolignans	15%
Flavonoids	4%
Taxifolin	3%
Picea glauca	
Lignans	60%
Hydroxymatairesinol	45%
Secoisolariciresinol	6%
Matairesinol	3%
Todolactol A + isoliovil	2%
α-Conidendrin	1%
Oligolignans	10%
Picea mariana	
Lignans	36%
Hydroxymatairesinol	23%
Todolactol A + isoliovil	5%
Conidendrin	2%
Lignan A	2%
Secoisolariciresinol	2%
Lariciresinol	1%
Oligolignans	22%
Picea sitchensis	
Lignans	12%
Todolactol A + isoliovil	5%
Hydroxymatairesinol	3%
Lignan A	1%
Oligolignans	19%
Pinus banksiana	
Lignans	21%
Nortrachelogenin	18%
Oligolignans	5%
Flavonoids	18%
Pinocembrin	8%
Pinobanksin	8%
Pinosylvins	12%
Pinosylvin monomethyl ether	8%
Pinosylvin	4%
Pinus strobus	
Lignans	5%
Lariciresinol	4%
Cyclolariciresinol	1%
Flavonoids	8%
Pinobanksin	4%
Pinocembrin	2%
Pinosylvins	45%
Pinosylvin monomethyl ether	28%
Pinosylvin	10%
Dihydropinosylvin	3%
Dihydropinosylvin monomethyl ether	4%
Pinus resinosa	
Lignans	8%
Todolactol A + isoliovil	4%
Nortrachelogenin	1%
Oligolignans	8%
Pinosylvins	38%
Pinosylvin monomethyl ether	22%
Pinosylvin	16%
Pinus sylvestris	
Lignans	31%
Nortrachelogenin	30%
Oligolignans	6%
Pinosylvins	38%
Pinosylvin	22%
Pinosylvin monomethyl ether	16%
Tsuga canadensis	
Lignans	44%
Hydroxymatairesinol	35%
α-Conidendrin	2%
Matairesinol	1%
Lignan A	1%
Todolactol A + isoliovil	1%
Oligolignans	9%
Tsuga heterophylla	
Lignans	44%
Hydroxymatairesinol	40%
Todolactol A + isoliovil	2%
Lignan A	2%
Oligolignans	10%
Flavonoids	1%
Thuja plicata	
Thujalignans	35%
Lignans	1%

to be a strong antioxidant whereas the other tested flavonoids had similar or lower capacities than the studied lignans.

The capacity of pure polyphenols to trap peroxyl radicals was estimated by chemiluminescence-based methodology. Of the studied flavonoids, melacacidin and teracacidin were found to effectively trap peroxyl radicals (Table 6). Lignan cyclolariciresinol (12 mmol/g) was a more effective radical trap than Trolox (8 mmol/g) and pinoresinol (7.8 mmol/g) showed similar large trapping capacity as Trolox whereas the flavonoids dihydrokaempferol (0.78 mmol/g) and pinocembrin (0.49 mmol/g) and the stilben pinosylvin (0.78 mmol/g) had relatively low trapping capacities.

The antioxidant potencies of the studied flavonoids follow the values reported by Pietta.[16] The low value of pinocembrin is expected due to a lack of hydroxyl groups in the B-ring. In a similar manner, the potency of dihydrokaempferol is weaker than that of taxifolin because taxifolin has an additional hydroxyl group in the B-ring when compared with the structure of dihydrokaempferol. The same is true for teracacidin and melacacidin and leads to stronger antioxidant potency of melacacidin. The strong potencies of melacacidin and teracacidin when compared with the other studied flavonoids might be due to the 7,8-hydroxylation pattern in the A-ring.

In the case of stilbenes, the antioxidant activity is strongly dependent on the position of the hydroxyl groups. In pinosylvin the hydroxyl groups are in the *meta*-position, which indicates that the antioxidant potency is strongest in alkaline environments,[10] while a hydroxyl group in *para*-position would indicate high potency in an acidic environment. Resveratrol, found in *Picea abies* bark, has a combined effect because it has both *meta*- and *para*-hydroxyl groups. When compared with the results that Willför et al.[7] obtained for the antioxidant potency and trapping capacity of a mixture of pinosylvin monomethyl ether and dihydropinosylvin monomethyl ether, pure pinosylvin was found to possess higher antioxidant potency but a smaller trapping capacity. A methyl ether group may stabilize the activity of pinosylvin in radical trapping reactions, while in lipid peroxidation it may create a steric hindrance.

Table 3. Main component groups and components in the hardwood hydrophilic knotwood extracts

Acacia crassicarpa		*Populus grandidentata*	
Flavonoids	54%	Flavonoids	31%
Melacacidin	24%	Dihydrokaempferol	13%
Isomelacacidin	18%	Catechin	9%
Biflavonoids	9%	Naringenin	7%
		Taxifolin	3%
Acacia mangium		Flavonoid glycosides	34%
Flavonoids	36%		
Teracacidin	25%	*Populus tremula*	
Ketoteracacidin	3%	Flavonoids	21%
Biflavonoids	8%	Dihydrokaempferol	17%
		Naringenin	3%
Fagus sylvatica		Flavonoid glycosides	8%
Flavonoids	7%		
Catechin	6%	*Populus tremuloides*	
		Flavonoids	23%
Eucalyptus globulus		Dihydrokaempferol	12%
Tannins	19%	Naringenin	10%
Tannin monomers	5%	Kaempferol	1%
Ellagic acid	3%	Taxifolin	1%
Gallic acids	2%	Flavonoid glycosides	24%

Table 4. Main component groups and components in the hydrophilic bark extracts

Thuja occidentalis	*Picea abies*
Sugars	Isorhapontin
Catechin	Astringin
Isorhapontin	Resveratrol-glycoside
Astringin	Tannins
Tannins	
	Abies lasiocarpa
Pinus banksiana	Sugars
Sugars	Resin acids
Taxifolin	Tannins
Dihydromyrcetin	
Tannins	*Populus tremula*
	Unidentified
glycosides	Tannins
Betula pendula	
Betuloside	
Catechin	*Pinus mariana*
Sugars	Sugars
Tannins	Catechin
	Tannins
Pseudotsuga menziensii	
Sugars	
Taxifolin	
Catechin	
Tannins	

The structural features that affect radical scavenging activity of lignans are the degree of methoxylation in the phenolic hydroxyls (the activity decreases with higher level of methoxylation), the butanediol structure (enhances activity), and higher degree of oxidation at the benzylic positions (decreases activity).[27] All these studied lignans had the same degree of methoxylation. Cyclolariciresinol (*1*) and secoisolariciresinol (*2*) contain a butanediol structure and were the two most effective lignans in the radical scavenging test. Between results presented in Eklund et al.[27] and the present work, there are differences in the lignan activity order. For example, pinoresinol (*7*) had weaker radical trapping capacity than hydroxymatairesinol (*5*) and lariciresinol (*8*) in the work by Eklund et al.,[27] while in the present study the order was the opposite. This is, most likely, due to different testing methods.

The total phenolic content is often used to explain antioxidant activity; however, it is unlikely to account for the total antioxidant capacity and activity in the extracts.[22,28] Therefore, the knowledge obtained for the individual compounds is important in evaluating antioxidant activities of the extracts.

Antioxidant properties of the branch knot extracts

The antioxidant potency measured with the lipid peroxidation test varied widely among the knotwood extracts (Table 5). The *Acacia crassicarpa* knotwood extract possessed the strongest potency to inhibit lipid peroxidation (19 μg/l) of the studied knotwood extracts. All three studied knotwood extracts of the *Picea* species had IC_{50} values lower than 40 μg/l as well as both the *Tsuga* species and *Larix sibirica*, while the knotwood extracts of *Larix lariciana* had slightly weaker potency. The inhibition potency differed among the *Pinus* species: the best value was found for *P. sylvestris* (40 μg/l) while *P. strobus* showed four times weaker potency (159 μg/l) and the antioxidant potencies for the *P. banksiana* (48 μg/l) and *P. resinosa* (61 μg/l) extracts were close to that of *P. sylvestris*. Related behavior was observed for the *Abies* extracts: *Abies pectinata* (21 μg/l) was shown to be the second strongest antioxidant of all studied extracts, while *A. lasiocarpa* (59 μg/l) had a weaker potency. *Populus tremula* (317 μg/l) and *Populus tremuloides* (135 μg/l) had very modest antioxidant potencies, while that of *Populus grandidentata* (61 μg/l) was stronger.

The *Acacia crassicarpa* knotwood extract was found to be an effective radical scavenger with a capacity (21 mmol/g) that was clearly larger than that of Trolox (8.0 mmol/g) (Table 6). The capacities of the other knotwood extracts varied between 7.8 and 2.7 mmol/g, except *P. banksiana* (1.9 mmol/g) and *P. strobus* (1.1 mmol/g) that had slightly smaller capacities, and *P. tremula* (0.39 mmol/g) and *P. tremuloides* (0.29 mmol/g) that had very small trapping capacities. The order of the extracts was almost the same in this test as in the lipid peroxidation test, except for *E. globulus*, which was the second most efficient extract to trap peroxyl radicals, probably due to its large tannin concentration.

The two studied *Acacia* extracts exhibited strong antioxidant potency and trapping capacity (Table 6). The extracts contained large amounts of flavonoids (melacacidin and teracacidin) and biflavonoids (proanthocyanidins, promelacacidins, and proteracacidins).[6] The molar trapping capacity of proanthocyanidins increases with the degree of polymerization; if the degree of polymerization is higher there are more hydroxyl groups in the molecule, and, therefore, more radicals can be scavenged per molecule.[29] The trapping capacity of melacacidin (20 mmol/g) was similar to that of *A. crassicarpa* (21 mmol/g), and teracacidin (7.8 mmol/g) was an even more effective radical scavenger than the *A. mangium* (6.8 mmol/g) knotwood extract. In the lipid peroxidation test, the melacacidin (36 μg/l) and teracacidin

Table 5. Inhibition of lipid peroxidation in vitro, expressed as IC_{50} values, by pure polyphenols, knotwood extracts, and bark extracts

Pure substances	IC_{50} (μg/l)	IC_{50} (μM)	Knotwood extracts	IC_{50} (μg/l)	Bark extracts	IC_{50} (μg/l)
Trolox (reference)	5	0.02	*Acacia crassicarpa*	19		
Cyclolariciresinol	17	0.05				
Pinoresinol	20	0.06				
Melacacidin	36	0.12	*Abies pectinata*	21		
Secoisolariciresinol[a]	37	0.10	*Picea glauca*	24		
			Acacia mangium	24		
			Tsuga canadensis	27		
			Picea sitchensis	28		
			Tsuga heterophylla	28		
			Larix sibirica	29		
			Picea mariana	38		
			Pinus sylvestris	40		
Taxifolin[a]	46	0.15	*Larix lariciana*	44	*Picea abies*	49
Pinosylvin	50	0.23	*Pinus banksiana*	48		
Teracacidin	50	0.17	*Thuja plicata*	52		
Nortrachelogenin[a]	53	0.14	*Eucalyptus globulus*	57		
Hydroxymatairesinol[a]	58	0.15	*Abies lasiocarpa*	59		
Matairesinol[a]	99	0.28	*Populus grandidentata*	61	*Betula pendula*	81
			Pinus resinosa	61	Pycnogenol	84
			Fagus sylvatica	91	*Pseudotsuga menziensii*	84
Lariciresinol[a]	126	0.35	*Populus tremuloides*	135	*Thuja occidentalis*	131
Dihydrokaempferol	488	1.7	*Pinus strobus*	159	*Pinus banksiana*	143
Pinocembrin	1135	4.4	*Populus tremula*	317	*Populus tremula*	213
					Abies lasiocarpa	316

IC_{50}, concentration of extract that inhibits lipid peroxidation by 50%
[a] According to Willför et al.[7]

Table 6. Scavenging of peroxyl radicals in vitro, expressed as the trapping capacity and as the stoichiometric factor by pure polyphenols, knotwood extrats, and bark extracts

Pure substances	Trapping capacity (mmol/g)	Stoichiometric factor (mol/mol)	Knotwood extracts	Trapping capacity (mmol/g)	Bark extracts	Trapping capacity (mmol/g)
Melacacidin	20	6.2	*Acacia crassicarpa*	21		
Taxifolin[a]	16	4.7				
Cyclolariciresinol	12	4.2				
Secoisolariciresinol[a]	8.5	3.1	*Eucalyptus globulus*	7.8		
Trolox (reference)	8.0	2.0	*Picea glauca*	7.8		
Pinoresinol	7.8	2.8	*Abies pectinata*	6.8		
Teracacidin	7.8	2.2	*Tsuga canadensis*	6.8		
Nortrachelogenin[a]	5.9	2.2	*Acacia mangium*	6.8		
Hydroxymatairesinol[a]	5.6	2.1	*Tsuga heterophylla*	5.8		
			Larix lariciana	5.8		
			Larix sibirica	5.8		
			Picea mariana	5.8		
Matairesinol[a]	2.9	1.0	*Picea sitchensis*	4.9	*Pseudotsuga menziensii*	4.9
Lariciresinol[a]	2.7	1.0	*Pinus sylvestris*	4.9	Pycnogenol	4.9
			Thuja plicata	3.9	*Pinus banksiana*	3.1
			Populus grandidentata	3.9	*Betula pendula*	2.9
			Fagus sylvatica	2.9	*Picea abies*	2.9
			Abies lasiocarpa	2.7	*Thuja occidentalis*	1.9
			Pinus resinosa	2.7		
			Pinus banksiana	1.9		
			Pinus strobus	1.1		
Dihydrokaempferol	0.78	0.20	*Populus tremuloides*	0.39	*Abies lasiocarpa*	0.58
Pinosylvin	0.78	0.16	*Populus tremula*	0.29	*Populus tremula*	0.29
Pinocembrin	0.49	0.12				

Trapping capacity is expressed as the number of peroxy radicals in millimoles that are scavenged per gram of extract. Stoichiometric factor is expressed as the number of moles of peroxy radicals scavenged per mole of compound
[a] According to Willför et al.[7]

(50 μg/l) were not as potent as their source knotwood extracts *A. crassicarpa* (19 μg/l) and *A. mangium* (24 μg/l). Not only the knots but also the heartwood of these two *Acacia* species contain large amounts of flavonoids and biflavonoids[6] and could be utilized for production of antioxidants.

Secoisolariciresinol, which is a potent antioxidant (37 μg/l) and an efficient radical scavenger (8.5 mmol/g), is the predominant lignan in the knotwood extracts of the *Abies* and *Larix* species. The different antioxidant potencies for the two extracts of *Abies* species can be explained by the difference in the extract components: the *A. pectinata* knotwood extract contains 42% lignans and, in addition, 22% oligolignans, while the *A. lasiocarpa* extract contains only 7% lignans and 16% oligolignans. The *A. pectinata* (21 μg/l) and *L. sibirica* (29 μg/l) knotwood extracts showed even stronger potency than pure secoisolariciresinol, and even the knotwood extracts of *L. lariciana* (44 μg/l) and *A. lasiocarpa* (59 μg/l) possessed stronger potency than what their secoisolariciresinol concentrations (15% and 2% respectively) would predict. The trapping capacity of pure secoisolariciresinol is larger than that of the knotwood extracts of the *Abies* (6.8 and 2.7 mmol/g) and *Larix* (5.8 mmol/g both) species. The values obtained for the two studied *Larix* species were on the same level in the trapping capacity test, while the *L. sibirica* knotwood extract showed stronger antioxidant potency than *L. lariciana.* The composition and amounts of lignans in the extracts were similar, whereas the *L. lariciana* extract contained 11% of taxifolin in addition to the lignans. Taxifolin is a strong antioxidant and the antioxidant potency of *L. lariciana* should be increased, not reduced, by this flavonoid.

Hydroxymatairesinol is the predominant lignan in *Picea* and *Tsuga* species. In both the antioxidant potency and trapping capacity tests, the values measured for pure hydroxymatairesinol (58 μg/l and 5.6 mmol/g) showed weaker antioxidant properties than the values measured for the *Picea* (24–38 μg/l and 7.8–5.8 mmol/g) and *Tsuga* (24–28 μg/l and 6.8–5.8 mmol/g) knotwood extracts. The knotwood extract of *Picea sitchensis* that contained the smallest amount of lignans (12%) also had the weakest antioxidant potency of the studied *Picea* species. *Tsuga canadensis* and *Tsuga heterophylla*, had similar extract composition and, accordingly, similar antioxidant potencies and trapping capacities.

The *Pinus* species that had the largest lignan amount, i.e., *P. sylvestris* (31%), and the second largest amount, i.e., *P. banksiana* (21%), also had the strongest (40 μg/l) and second strongest (48 μg/l) antioxidant potencies within the *Pinus* species. *Pinus sylvestris*, which contained the largest total amount of polyphenols (75%), had the largest radical trapping capacity (4.9 mmol/g), while the other three studied *Pinus* species contained similar amounts of polyphenols (54%–58%) and had similar radical trapping capacities (1.1–2.7 mmol/g).

The composition of the *Thuja plicata* extract differed remarkably from the other studied species. Thujalignans were not tested here as pure substances and their contribution to the antioxidant capacity cannot be evaluated. However, compared with results obtained by Willför et al.,[7] it can be concluded that the knotwood extract of *T. plicata* is a stronger antioxidant and more effective radical scavenger (52 μg/l and 3.9 mmol/g) than the *T. occidentalis* knotwood extract (447 μg/l and 2.4 mmol/g), and, indeed, *T. plicata* contains a larger amount of thujalignans (35%) than *T. occidentalis* (25%).

The *E. globulus* knotwood extract had moderate antioxidant potency in the lipid peroxidation test; however, it showed a large peroxyl radical trapping capacity. The main components in the extract are ellagitannins, which are known to be potent antioxidants. The antioxidant activity of ellagitannins depends on the number of hydroxyl groups.[30] In addition, ellagitannins are capable of chelating metals.[30]

All *Populus* species had weak antioxidant potencies and small trapping capacities. Dihydrokaempferol, the predominant compound in the *Populus* knotwood extracts, is a weaker antioxidant and radical trap than the studied lignans, and melacacidin and teracacidin. Therefore, the antioxidant potencies and trapping capacities of the knotwood extracts of *Populus* species cannot be expected to be as strong as those of the lignan-rich species or *Acacias*. In addition, a major part of the flavonoids observed in the knotwood extracts of *Populus* species are glycosylated, which lowers the antioxidant properties of the flavonoids.[15] The *P. grandidentata* knotwood extract was a stronger antioxidant than the knotwood extracts of *P. tremula* and *P. tremuloides*, most likely due to taxifolin and catechin found in the *P. grandidentata* knotwood extract.

Antioxidant properties of the bark extracts

Bark extract of *Picea abies* had the strongest antioxidant potency in the lipid peroxidation tests of the bark extracts. *Picea abies* bark extract contains stilbenes and stilbene glycosides that are known for their potency to inhibit lipid peroxidation.[31] Like in flavonoids, glycosidation of stilbenes lowers the antioxidant potency.

Packer et al.[24] have reviewed the well-known antioxidative properties and chemical composition of Pycnogenol. The main constituents can be broadly divided into flavonoids (catechin, epicatechin, and taxifolin) and condensed tannins. Pycnogenol has been shown to possess greater biologic potency as a mixture than its purified components do, indicating that the components are acting synergistically.[24] The antioxidant potencies of *B. pendula*, Pycnogenol, and *P. menziensii* are probably mostly due to condensed tannins.

In the trapping capacity test, Pycnogenol and *P. menziensii* bark extract had the largest capacities. The free radical scavenging activity of Pycnogenol is due to condensed tannin and, in particular, flavonoids.[24] *Pseudotsuga menziensii* contains biflavonoids and condensed tannins that probably are responsible for the trapping capacity.[22] The bark extracts did not contain any lignans. Because lignans were found to possess strong antioxidant activity and large radical trapping capacity, it is, indeed, reasonable that the lignan-rich knotwood extracts are stronger antioxidants than bark extracts. However, the large sugar amounts that were

observed in the bark extracts, but not in the branch knots, lowered the measured antioxidant activities and trapping capacities of the bark extracts because the measurements were made according to the gravimetric amounts of extracts.

Conclusions

Even though the main part of the antioxidant activity of the extracts can be explained by the predominant compounds, there are still more factors affecting the activity. This can be because of synergism of the polyphenols or there may be compounds in minor amounts that strongly contribute to the activity. The correlation between decay resistance and the total extractive content can be poor, suggesting that more subtle differences in extractive composition and distribution may be important.[32]

It can be concluded that most lignans are powerful antioxidants, with effects as strong as or even stronger than most flavonoids. However, the flavonoid melacacidin possessed the largest trapping capacity of the studied polyphenols. Furthermore, the knotwood extracts of several tree species contain valuable antioxidants with stronger effects than the individual compounds. The bark can also provide an interesting source for strong antioxidants.

Acknowledgments We thank Sune Forsström at Sydväst Polytechnic, Dave Hensel at Blandin Paper, Chuck Barber and Dompak M. Siregar at Riau Andalan Pulp and Paper, Indonesia, S.M. Krutov at St. Petersburg Forest Technical Academy, Donald and Peter MacNeil, Edgar Sjövall, Janja Zule, and Markku Reunanen. This work is part of the activities at the Åbo Akademi Process Chemistry Centre within the Finnish Centre of Excellence Programme (2000–2005) selected by the Academy of Finland and is a part of the project "NanOx" funded by the National Technology Agency of Finland (Tekes).

References

1. Willför S, Nisula L, Hemming J, Reunanen M, Holmbom B (2004) Bioactive phenolic substances in important tree species. Part 2. Knots and stemwood of fir species. Holzforschung 58:650–659
2. Willför S, Hemming J, Reunanen M, Eckerman C, Holmbom B (2003) Lignans and lipophilic extractives in Norway spruce knots and stemwood. Holzforschung 57:27–36
3. Willför S, Hemming J, Reunanen M, Holmbom B (2003) Phenolic and lipophilic extractives in Scots pine knots and stemwood. Holzforschung 57:359–372
4. Pietarinen S, Willför S, Vikström F, Holmbom B (2004) Aspen knots, a rich source of flavonoids. XXII International Conference on Polyphenols, Helsinki, Finland, pp 669–670
5. Pietarinen SP, Willför SM, Vikström, FA, Holmbom BR (2006) Aspen knots, a rich source of flavonoids. J Wood Chem Technol (in press)
6. Pietarinen S, Willför S, Sjöholm R, Holmbom B (2005) Bioactive phenolic substances in important tree species. Part 3. Knots and stemwood of *Acacia crassicarpa* and *A. mangium*. Holzforschung 59:94–101
7. Willför SM, Ahotupa MO, Hemming JE, Reunanen MH, Eklund PC, Sjöholm RE, Eckerman CS, Pohjamo SP, Holmbom BR (2003) Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species. J Agric Food Chem 51:7600–7606
8. Lindberg L, Willför S, Hemming J, Holmbom B (2004) Antibacterial effects of hydrophilic knotwood extracts on papermill bacteria. J Ind Microbiol Biotechnol 31:137–147
9. Bergman J, Lindgren BO, Svahn CM (1965) Triterpenes and 4-alpha-methylsterols in birch wood. Acta Chem Scand 19:1661–1666
10. Stojanovic S, Brede O (2002) Elementary reactions of the antioxidant action of trans-stilbene derivatives: resveratrol, pinosylvin and 4-hydroxystilbene. Phys Chem Chem Phys 4:757–764
11. Schultz TP, Nicholas DD (2000) Naturally durable heartwood: evidence for a proposed dual defensive function of the extractives. Phytochemistry 54:47–52
12. Schultz TP, Nicholas DD (2002) Development of environmentally benign wood preservatives based on combination of organic biocides with antioxidants and metal chelators. Phytochemistry 61:555–560
13. Backa S, Gierer J, Reitberger T, Nilsson T (1992) Hydroxyl radical activity in brown-rot fungi studied by a new chemiluminescence method. Holzforschung 46:61–67
14. Backa S, Gierer J, Reitberger T, Nilsson T (1993) Hydroxyl radical activity associated with the growth of white-rot fungi. Holzforschung 47:181–187
15. Hopia A, Heinonen M (1999) Antioxidant activity of flavonoid aglycones and their glycosides in methyl linoleate. J Am Oil Chem Soc 76:139–144
16. Pietta P (2000) Flavonoids as antioxidants. J Nat Prod 63:1035–1042
17. Scalbert A (1991) Antimicrobial properties of tannins. Phytochemistry 30:3875–3883
18. Taskinen A, Eklund P, Sjöholm E, Hotokka M (2004) The molecular structure and some properties of hydroxymatairesinol. An ab initio study. J Mol Struct 667:113–124
19. Becker EM, Nissen LR, Skibsted LH (2004) Antioxidant evaluation protocols: food quality or health effects. Eur Food Res Technol 219:561–571
20. Holmbom B, Eckerman C, Eklund P, Hemming J, Nisula L, Reunanen M, Sjöholm R, Sundberg A, Sundberg K, Willför S (2004) Knots in trees – a new rich source of lignans. Phytochem Rev 3:331–340
21. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M (1999) Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47:3954–3962
22. Polya GM, Foo LY (1994) Inhibition of eukaryote signal-regulated protein kinases by plant-derived catechin-related compounds. Phytochemistry 35:1399–1405
23. Tan YM, Yu R, Pezzuto JM (2003) Betulinic acid-induced programmed cell death in human melanoma cell involves mitogen-activated protein kinase activation. Clin Cancer Res 9:2866–2875
24. Packer L, Rimbach G, Virgili F (1999) Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radical Biol Med 27:704–724
25. Eklund P, Sillanpää R, Sjöholm R (2002) Synthetic transformation of hydroxymatairesinol from Norway spruce (*Picea abies*) to 7-hydroxysecoisolariciresinol, (+)-lariciresinol and (+)-cyclolariciresinol. J Chem Soc Perkin Trans 1:1906–1910
26. Ahotupa M, Mäntylä E, Kangas L (1997) Antioxidant properties of the triphenylethylene antiestrogen drug toremifene. Naunyn-Schmiederberg's Arch Pharmacol 356:297–302
27. Eklund PC, Långvik OK, Wärnå JP, Salmi TO, Willför SM, Sjöholm RE (2005) Chemical studies on antioxidant mechanisms and free radical scavenging properties of lignans. Org Biomol Chem 3:3336–3347
28. Heinonen M, Rein D, Satue-Gracia MT, Huang S-W, German JB, Frankel EN (1998) Effect of protein on the antioxidant activity of phenolic compounds in a lecithin–liposome oxidation system. J Agric Food Chem 46:917–922
29. De Bruyne T, Pieters L, Deelstra H, Vlietinck A (1999) Condensed vegetable tannins: biodiversity in structure and biological activities. Biochem Syst Ecol 27:445–459
30. Shimizu K, Kondo R, Sakai K (2002) Antioxidant activity of heartwood extracts of Papua New Guinean woods. J Wood Sci 48:446–450
31. Merillon J, Fauconneau B, Teguo PW, Barrier L, Vercauteren J, Huguet F (1997) Antioxidant activity of the stilbene astringin, newly extracted from *Vitis vinifera* cell cultures. Clin Chem 43:1092–1093
32. Taylor AM, Gartner BL, Morrell JJ (2002) Heartwood formation and natural durability – a review. Wood Fiber Sci 34:587–611