In vitro Bioactivity and Antimicrobial Activity of *Picea abies* and *Larix decidua* Wood and Bark Extracts

Mohamed Z. M. Salem,[a,*] Hosam O. Elansary,[b] Amr A. Elkelish,[c] Aleš Zeidler,[d] Hayssam M. Ali,[e,f] and Kowiyou Yessoufou [g]

Picea abies and *Larix decidua* were subjected to GC/MS analyses, and antimicrobial (fungi and bacteria) assays of their stem wood and bark extracts were investigated. *L. decidua* bark extract exhibited the highest antifungal and antibacterial activities against the microorganisms that were screened. The microbes *Penecillium ochrochloron* and *Aspergillus ochraceus* were the most sensitive to the extracts, whereas *Candida albicans* was the most resistant fungus. *L. decidua* wood and bark did not exhibit much variation in their antibacterial activities, except against *Micrococcus flavus* and *Pseudomonas aeruginosa*. The bacterium most sensitive to the extracts was *Escherichia coli*, whereas the most resistant was *M. flavus*. 13-epimanool and α-cedrol were the main components of *P. abies* wood extract. The main components in its bark were abietic acid, astringin, dehydroabietic acid, and α-terpineol. The main chemical compounds in *L. decidua* wood extract were abietic acid, oleanolic acid, duvatrienediol, and larixol. The main chemical compounds in its bark were (-)-2,9-dihydroxyverrucosane and larixol. The study revealed that *P. abies* and *L. decidua* stem wood and bark extracts contain several compounds that have antimicrobial activities towards diverse human pathogenic, food, and agricultural microbes. These results might guide in future searches for novel natural products with chemotherapeutic uses.

Keywords: Picea abies; Larix decidua; Antifungal activity; Antibacterial activity; GC/MS

Contact information: a: Department of Forestry and Wood Technology, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt; b: Molecular Biology Laboratory, Floriculture, Ornamental Horticulture and Garden Design Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt; c: Department of Botany, Faculty of Science, Suez Canal University, Ismailia 41522, Egypt; d: Department of Wood Processing, Faculty of Forestry and Wood Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic; e: Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; f: Department of Timber Trees Research, Sabahia Horticulture Research Station, Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt; g: Department of Environmental Sciences, University of South Africa, Florida campus, Florida 1710, South Africa;
** Corresponding author: zidan_forest@yahoo.com; mohamed-salim@alexu.edu.eg*

INTRODUCTION

Forestry residues such as tree barks and branches are important but underexplored supplies of biologically or pharmaceutically active compounds (Liimatainen *et al.* 2012; Si *et al.* 2012; Patinha *et al.* 2013). Tree bark, for example, provides not only a physical protective barrier for plants, but also an important chemical defense mediated through the secondary compounds they produce (Horsfall 1980; Merrill 1992; Alfredsen *et al.* 2008). Numerous studies recently conducted on bark extracts focus on tropical tree species (da Silva *et al.* 2013; Kusumoto *et al.* 2014; Salem *et al.* 2015a; Yessoufou *et al.* 2015). In the present study, we shift the focus onto Central European softwood species in the Pinaceae

family (Salem *et al.* 2015b). This family is a taxonomic group of high economic importance in the production of paper, timber, construction materials, chemicals, and essential oils used for perfume or pharmaceutical and aromatherapy purposes (Salem *et al.* 2015b).

Previous studies have been dedicated to analyzing the chemical composition of the essential oils and the antimicrobial activities of several parts of Pinaceae members (needles, branches, cones, seeds, and bark) (Grassmann *et al.* 2003; Nikolić *et al.* 2007; Tumen *et al.* 2010; Bier *et al.* 2016; Fattahi *et al.* 2016; Salem *et al.* 2016). Nevertheless, the majority of studies regarding the chemical composition of softwood species have related to North American and Central European species (Hennig *et al.* 1994; Danneyrolles *et al.* 2016; Greenberg *et al.* 2016; Krieg *et al.* 2016).

European larch (*Larix decidua* L.) is a deciduous tree that grows to 35 m and is indigenous to the Alps, the Sudetes, and the Carpathian mountains (von Bruchhausen *et al.* 1993; Pferschy-Wenzig *et al.* 2008). Larch wood is strong, water-resistant, and durable, but especially bendable in thin strips. The heartwood is extremely weather-proof due to its excessive resin content and hard machinability, and it is usually used as building timber (Pferschy-Wenzig *et al.* 2008). Larch sawdust is principally utilized in the production of pellet fuels, and the wood contains phenolic compounds such as lignans and flavonols (mainly dihydroquercetin and dihydrokaempferol) (von Bruchhausen *et al.* 1993). These compounds possess antioxidant and anti-inflammatory effects (Kolhir *et al.* 1996; Pietarinen *et al.* 2006). Polysaccharides such as arabinogalactan (also found in European larch) have been approved by the U.S. FDA as a source of fibers, have immunostimulant effects, and may be used in cancer therapy (Kelly 1999). Turpentine in *L. decidua*, often known as Venice turpentine (Pferschy-Wenzig *et al.* 2008), is made up of 15% essential oil, 50 to 65% resin acids, and approximately 15% non-saponifiable resin. The principal component of the resin fraction is the labdane type diterpene larixyl acetate (Kolhir *et al.* 1996). Larixol and larixyl acetate are distinctive compounds for larch resin, as they have been specifically found in *L. decidua* and *L. gmelinii*.

Norway spruce (*Picea abies*) is indigenous to the European Alps and ranges north towards Scandinavia and North Russia (Salem *et al.* 2013; Bianchi *et al.* 2014). *P. abies* wood is robust, soft, straight- and fine-grained, and simply worked (Cope *et al.* 2002). It is commonly used for building, pulp, furniture, and musical instrument manufacturing (Cope *et al.* 2002). The older trees are normally heavy with algae and possess shallow rounded scales which can be easily shed (Cope *et al.* 2002). In *P. abies* cells, the different soluble glycoside-bound types of phenolic acids make up ~ 85% of the overall content of the methanol extract, accompanied by the methanol-insoluble cell wall-bound phenolic esters (7 to 8%) (Malá *et al.* 2011). Some soluble methanol esters and free phenolic acids are very low, comprising ~2 and 4 to 5% of the entire phenolic content, respectively (Malá *et al.* 2011).

Additional information regarding the chemical structure of the bark tannins from *P. abies* and *L. decidua* is needed (Bianchi *et al.* 2014). Spruce bark tannins are recognized as procyanidins, with few incidences of prodelphinidins, having a mean polymerization degree of about 4.6 (Matthews *et al.* 1997). Similar results were reported for root bark (Pan and Lundgren 1995) and needle extracts (Behrens *et al.* 2003). Lignans and stilbenes, as well as their derivatives, were found in the oligomeric structure of condensed tannins of spruce (Steynberg *et al.* 1983; Bianchi *et al.* 2014). However, no characterization of condensed tannins from phloem or xylem tissues has yet been reported.

In the present study, the chemical composition of wood and bark extracts of *P. abies* and *L. decidua* were identified using GC-MS, and their biological activities on diverse

bacteria, and fungi were investigated to elucidate the biochemical composition and the antifungal and antibacterial potentials for human health benefits, agricultural pest control, and wood industry enhancement.

EXPERIMENTAL

Plant Materials

Wood and bark samples of *Larix decidua* and *Picea abies* were supplied by the Department of Wood Processing (Czech University of Life Sciences, Czech Republic, February 2015), and vouchered (No. Zidan00971 to Zidan00974) at the Division of Forestry and Wood Technology, Alexandria University. The laboratory work was completed in March 2016. The ages of the trees were 28 and 41 years for *P. abies* and *L. decidua*, respectively. Heartwood and bark samples were air-dried at the laboratory conditions, and then the samples were prepared with a particle size of 0.4 to 0.6 mm.

Chemicals and cell cultures

Analytical/HPLC grade chemicals were bought from Sigma-Aldrich, Alexandria, Egypt. Fungi and bacteria were supplied by the Department of Plant Pathology and Department of Floriculture (Faculty of Agriculture, Alexandria University, Egypt).

Methods

Preparation of extracts

The extracts were prepared from ground air-dried (40 to 60 mesh) wood and bark samples, and extracted three times using 95% methanol over a water bath for one day at room temperature. All extracts were combined and then evaporated until dry in a vacuum at 40 °C. The yield of methanol extracts for *L. decidua* was 6.42% (heartwood), and 10.36% (bark) and for *P. abies* was 7.2% (heartwood), and 4.2% (bark), according to oven dry weight.

GC-MS analysis of extracts

The chemical composition of the wood and bark extracts of *L. decidua* and *P. abies* were analyzed using a Trace GC Ultra-ISQ mass spectrometer (Thermo Scientific, USA) with a TG-5MS capillary column (30 m x 0.25 mm x 0.25 µm film thickness) apparatus at Atomic and Molecular Physics Unit, Experimental Nuclear Physics Department, Nuclear Research Centre, Egyptian Atomic Energy Authority, Inshas, Cairo, Egypt. Helium was used as the carrier gas (flow rate of 1 mL/min), and the oven temperature program was set as follows: 45 °C to 165 °C (4 °C/min) and 165 °C to 180 °C (15 °C/min) with a post run (off) at 180 °C. Samples (1 µL) were injected at 250 °C using a split/split-less injector (50:1 split ratio) and a splitless mode flow of 10 mL/min. The solvent delay was 2 min, and diluted samples of 1 µL were injected automatically using an Auto-sampler AS3000 coupled with GC in the split mode. EI mass spectra were collected at 70 eV ionization voltages in the range of 40 to 550 m/z in full scan mode. The ion source and transfer line temperatures were set at 200 °C and 250 °C, respectively. The components were identified by comparing their retention times and mass spectra with those of the Wiley 09, mainlib, replib, and NIST 11 mass spectral database (Adams 2007).

Antifungal activities

The antifungal activities of wood and bark extracts were examined for several fungi, including *Penicillium funiculosum* (ATCC 56755), *P. ochrochloron* (ATCC 48663), *Aspergillus niger* (ATCC 6275), *A. flavus* (ATCC 9643), *A. ochraceus* (ATCC 12066), and *Candida albicans* (ATCC 12066). The cultures were renewed monthly and stored at 4 °C. The microdilution method (Espinel-Ingroff 2001) was used to determine the minimum inhibitory (MIC) and minimum fungicidal (MFC) concentrations using a spore suspension concentration of (1.0×10^5) dilutions in 96-well microtiter plates. Wood and bark extracts and isolated compounds were diluted to the desired concentrations in microplates containing Malt medium broth mixed with inoculum. The microplates were incubated at 28 °C for 72 h on a rotary shaker.

The lowest concentration that inhibits fungi growth at the binocular microscope level was defined as the MIC. The MFC was defined as the minimum concentration exhibiting no visible growth, indicating a 99.5% killing of the original inoculum. Serial sub-cultivations (2 µL) of wood and bark extracts incubated at 28 °C for 72 h in microtiter plates containing 100 µL of broth and inoculum were used to calculate the MIC. Positive controls were used, including fluconazole (FLZ) and ketoconazole (KLZ) (1 to 3500 µg/mL). The experiments were performed in triplicate.

Antibacterial activities

Gram-positive and Gram-negative bacteria were used for analyses. The Gram-positive bacteria included *Micrococcus flavus* (ATCC 10240), *Bacillus cereus* (clinical isolate), *Listeria monocytogenes* (ATCC 19113), and *Staphylococcus aureus* (ATCC 6538). The Gram-negative bacteria were *Pseudomonas aeruginosa* (D s0432-1), *Escherichia coli* (ATCC 35210), *Pectobacterium atrosepticum* (ATCC 33260), *Pectobacterium carotovorum* subsp. *carotovorum* (ATCC 15713), and *Dickeya solani* (D s0432-1). The micro-dilution method (Espinel-Ingroff 2001) was used to determine the minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations. The concentration of the bacteria was adjusted to 1.0×10^5 CFU/mL using sterile saline, and then stored at 4 °C. The wood and bark extracts were added to 100 µL Triptic Soy broth (TSB) containing a bacteria inoculum (1.0×10^5 CFU per well) in a microtitre plate, then the minimum inhibitory concentrations (MICs) and the minimum bacterial concentrations (MBCs) were determined. The microplates were incubated at 37 °C for 24 h in a rotary shaker.

A serial sub-cultivation of 2 µL was placed in microtitre plates containing 100 µL of TSB for each well and incubated for 24 h to determine the MIC and MBC. The optical density was measured using a microplate manager at 655 nm. Experiments were completed in triplicate. DMSO (5%) and streptomycin + ampicillin (1 mg/mL) were used as negative and positive controls, respectively.

RESULTS AND DISCUSSION

Chemical Composition of Wood and Bark Extracts *of Picea abies* and *Larix decidua*

P. abies wood extract contained the following main compounds: 13-epimanool (12.48%), *α*-cedrol (10.60%), 2,6-di-t-butyloctahydroazulene-3a,8-diol (8.32%), astringin (7.68%), (Z,Z,Z)-9,12,15-octadecatrienoic acid-methyl ester (7.12%), and (1,5,5,8-

tetramethylbicyclo[4.2.1]non-9-yl)acetic acid (6.07%) (Table 1). The main chemical compounds found in bark extract were abietic acid (26.80%), astringin (15.00%), dehydroabietic acid (5.90%), *α*-terpineol (4.67%), methyl sandaracopimarate (4.07%), and 9-desoxo-9-x-acetoxy-3,8,12-tri-o-acetylingol (3.79%) (Table 2).

Table 1. Suggested Chemical Composition of Methanol Extract of *Picea abies* Wood Analyzed Using GC/MS

Compound	RT (min)	Area (%)
Docosane	2.86	4.62
(3*α*,5Z,7E)- 9,10-Secochola-5,7,10(19)- triene-3,24-diol	10.85	2.76
cis-Z-*α*-Bisabolene epoxide	12.12	4.60
(1,5,5,8-Tetramethyl-bicyclo[4.2.1]non-9-yl)-acetic acid	17.78	6.07
2,6-Di-t-butyloctahydroazulene-3a,8-diol	22.50	8.32
Astringin	22.81	7.68
13-Epimanool	23.22	12.48
d-Xylitol, pentaacetate	23.38	2.31
(3*α*,22E)-Ergosta-5,22-dien-3-ol, acetate	24.00	3.16
(Z,Z,Z)-9,12,15-Octadecatrienoic acid-methyl ester	24.54	7.12
1,2-Cinnolinedicarboxylic acid, 1,2,3,5,6,7,8,8a-octahydro-4-trimethylsilyloxy-,diethyl ester	24.70	2.25
Palustrol	34.51	2.07
α-Cedrol	34.76	10.60

Note: RT, retention time

Table 2 (Part 1). Suggested Chemical Composition of Methanol Extract of *Picea abies* Bark Analyzed Using GC/MS

Compound	RT (min)	Area (%)
Trans-Sabinene hydrate	2.48	1.21
2,4-Dibenzyloxy-5,7-di-tert-butylphenanthrene	2.86	1.34
Pyrano[4,3-b]benzopyran-1,9-dione,5a-methoxy-9a-methyl-3-(1-propenyl)perhydro-	3.34	0.42
α-D-methyl-2',3'-4'-tri-*O*-acetylglucopyranuronate	3.43	0.08
Quercetin-7,3',4'-trimethoxy	3.52	0.12
Glycodeoxycholic acid	3.97	0.26
(3*α*,24S)-stigmast-5-en-3-ol	4.18	0.13
Oxalic acid	4.27	0.21
D-Fenchyl alcohol	4.54	1.81
1-Borneol	5.42	2.36
α-Terpineol	5.75	4.67
17-acetoxy-4,4-dimethyl-3-methoxy-3-19-Epoxyandrost-8-en-7-ol	7.24	0.12
Docosahexaenoic acid,1,2,3-propanetriyl ester	7.32	0.15
Pyrano[4,3-b]benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl)perhydro-	9.05	0.12
Lycoxanthin	9.90	0.22
(5*α*)-Pregnane-3,11,20-trione	10.14	0.33
Phorbol-12,13-dihexanoate	11.47	0.13
(Z)-9-Octadecenoic acid-tetradecyl ester	11.99	0.13
7,14-Dibutyl-1,4-dioxa-7,14-diazacyclohexadecane-6,15-dione	14.02	0.21
Flavone 4'-OH,5-OH,7-di-*O*-glucoside	14.07	0.18
3*α*,5*α*-Cholan-24-oic acid,7,12-bis(acetyloxy)-3-methoxy-methyl ester	14.58	0.34

Table 2 (Part 2). Suggested Chemical Composition of Methanol Extract of *Picea abies* Bark Analyzed Using GC/MS

9,12,15-Octadecatrienoicacid,2-phenyl-1,3-dioxan-5-ylester	15.16	0.08
Astaxanthin	15.80	0.52
9-Desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol	18.29	0.25
Abietic acid	19.45	26.80
2,4,6,8,10-Tetradecapentae noic acid	21.03	0.58
1,3-Dioxolane	21.35	0.56
Astringin	22.79	15.00
DL-16-*α*-Hydroxy-norgestrel	23.06	0.25
17-Hydroxy-6,16*α*-dimethyl-Pregna-4,6-diene-3,20-dione	23.49	1.30
trans-1,3-Dioxane,5-(hexadecyloxy)-2-pentadecyl	23.53	0.61
Stearic acid,3-(octadecyloxy)propyl ester	23.57	0.57
9-Desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol	23.99	3.79
3-Acetoxy-7,8-epoxylanostan-11-ol	24.42	1.17
Irieol	24.57	0.58
4H-1-Benzopyran-4-one,8-*α*-D-glucopyranosyl-5,7-dihydroxy-2-(4-hydroxyphenyl)	26.49	0.88
2-Nonadecanone	28.25	0.33
Androsta-3,5,17-triol-6-one, 3,17-diacetate	28.55	1.75
5-*α*-Pregn-16-en-20-one,3*α*,12*α*-dihydroxy-diacetate	28.84	2.96
6-benzyl-3-*α*-hydroxy-6-Azacholest-4-en-7-one	30.97	0.45
Ingenol triacetate	31.04	0.25
Enmenol	31.50	0.23
Methyl sandaracopimarate	42.61	4.07
Dehydroabietic acid	42.92	5.90

Note: RT, retention time

Table 3. Suggested Chemical Composition of Methanol Extract of *Larix decidua* Wood Analyzed Using GC/MS

Compound	RT (min)	Area (%)
Decane	2.87	1.02
Menthoglycol	7.97	2.66
Squalene	15.56	9.13
13-Epimanool	23.22	5.07
Abietic acid	25.53	30.50
Duvatrienediol	26.48	15.79
Oleanolic acid	31.18	15.80
Larixol	42.22	14.55

Note: RT, retention time

The main chemical composition of *L. decidua* wood extract was abietic acid (30.50%), oleanolic acid (15.80%), duvatrienediol (15.79%), larixol (14.55%), squalene (9.13%), and 13-epimanool (5.07%) (Table 3). The main chemicals in the methanol extract of *L. decidua* bark were (-)-2,9-dihydroxyverrucosane (17.48%), larixol (15.80%), nonacosane (11.03%), cholan-24-oic acid,3,12-dihydroxy-(3α,5α,12α)- (8.77%), decane (8.63%), and 5-hydroxy-2,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone (5.95%) (Table 4).

Table 4. Suggested Chemical Composition of Methanol Extract of *Larix decidua* Bark Analyzed Using GC/MS

Compound	RT (min)	Area (%)
Decane	2.86	8.63
Nonacosane	4.43	11.03
5-Hydroxy-2,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone	8.71	5.95
3-Acetoxy-24-phenyl-25-norisopropyl-9,19-cyclolanostan-22-en-24-one	12.70	3.01
1-Amino-1-ortho-chlorophenyl-2-(2-quinoxalinyl)ethene	22.61	2.36
4-(Dimethylamino)azoestrone 3-methyl ether	23.05	2.60
3Beta,17beta-diacetoxy-5-chloro-6beta-nitro-5alpha-androstane	23.22	2.84
3-Acetoxy-24-phenyl-4,4,14-trimethyl-25-norisopropyl-9,19-cyclolanostan-22-en-24-one	23.28	0.94
4-Acetyloxyimino-6,6-dimethyl-3-methylsulfanyl-4,5,6,7-tetrahydro-benzo[c]thiophene-1-carboxylic acid methyl ester	24.11	0.94
2,9-dimethyl-1,10-Phenanthroline	24.20	1.01
2-[(2,5-Dichlorophenyl)amino]benzoic acid	25.16	2.61
Abietic acid	25.54	26.80
6-Oxo-spiro[adamantane-2,2'-[1,3]dithiolane]-1,5-dicarboxylic acid	26.27	2.18
(-)-2,9-Dihydroxyverrucosane	26.47	17.48
Astaxanthin	27.05	1.13
9-Thiocyanato-androst-4-en-11-ol-3,17-dione	27.71	1.19
Isoxazole,5-[3,3-dicyano-1-cyclohexylidene-2-morpholino-prop-2-enyl)]-3-p-methoxyphenyl	29.51	1.10
Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-	29.60	1.70
2-[(2,5-Dichlorophenyl)amino]benzoic acid	29.68	0.92
9-(Methylthio)-8H-acenaphtho[1,2-c]pyrrole-7-carboxylic Acid	32.42	2.03
9-Desoxo-9-x-acetoxy-3,8,12-tri-*O*-acetylingol	32.50	1.81
(25R)-2*α*-Hydroxy-5*α*-spirostan-3-one	32.59	1.27
Larixol	42.22	15.80

Note: RT, retention time

Previously, five benzoic acid derivatives (syringic acids, p-hydroxybenzoic, vanillic, and anisic), two cinnamic acid derivatives, p-coumaric, and ferulic acids were identified in *P. abies* cells (Metsämuuronen and Siren 2014). Native ferulic acid and *p*-hydroxybenzoic acid glucoside were found in root extracts (Münzenberger *et al.* 1990). Various stilbenes and stilbenes glucosides have been previously observed in different parts of *P. abies* (Metsämuuronen and Siren 2014). Isorhapontin and astringin are the major constitutive stilbenes glycosides in *P. abies* (Viiri *et al.* 2001; Zeneli *et al.* 2006; Malá *et al.* 2011). Zeneli *et al.* (2006) found that astringin and isorhapontin represent 20.2% and 71.8% of sapwood phenolics, and 38.8% and 46.5% of bark phenolics in *P. abies* trees growing in Norway, respectively. The standard amount of extractable phenolic compound in *P. abies* wood is about 15% (w/w) (Metsämuuronen and Siren 2014), but higher values have also been reported up to 30% (w/w) (Eklund *et al.* 2004).

Wood phenolics also contain 5.1% piceid and bark phenolics, 7.7% piceid, and 0.4% piceatannol (Metsämuuronen and Siren 2014). These types of stilbenes aglycones and glucosides have previously been discovered in bark extracts (Pietarinen *et al.* 2006). Moreover, piceoside, piceatannol and its glucoside, and isorhapontin have been detected

in *P. abies* roots (Münzenberger *et al.* 1990; Latva-Mäenpää *et al.* 2014). However, various reports did not find stilbenes in the hydrophilic knotwood extractives of *P. abies* (Willför *et al.* 2003). Prior research discovered that more than a half of the knotwood extracts are lignans, with the remaining mostly oligolignans (Metsämuuronen and Siren 2014). One of the most substantial lignans was hydroxymatairesinol (Willför *et al.* 2003; Pietarinen *et al.* 2006), which comprised around 70% of the total lignans (Willför *et al.* 2003).

Diterpene acids from conifers are well known to provide several bioactivities (Rauha *et al.* 2000; Keeling and Bohlmann 2006). Many abietane acids exhibit antimicrobial activity against a wide range of microorganisms. Dehydroabietic acid derivatives have been proved to act as antiulcer agents (Sepúlveda *et al.* 2005), and several abietane acids have shown cardiovascular effects. Furthermore, several side effects of diterpene acids, such as resin acids within pulp and paper mill effluents, are well known to be noxious to aquatic organisms (Rissanen *et al.* 2003; Kamaya *et al.* 2005).

The oxidation products of resin acids appear to have a dermal allergenic potential (San Feliciano *et al.* 1993; Eriksson *et al.* 2004). Moreover, several studies have investigated the anti-inflammatory potential of diterpene acids. A combination of laevopimaric, abietic, neoabietic, palustric, and isopimaric acids along with triglycerides, have been prescribed for the external therapy of chronic diseases such as rheumatism and gout (Khare 2004). Abietic acid, which is also considered to be present in larch resin, has been identified as an inhibitor of soybean 5-lipoxygenase (Ulusu *et al.* 2002). The compound appears to inhibit PGE2 generation in lipopolysaccharide-treated macrophages *in vitro*, and prevent rat-paw and mouse-ear edema following oral or topical application *in vivo* (Fernández *et al.* 2001).

Antifungal and Antibacterial Activities

The MIC and MFC values of *P. abies* and *L. decidua* wood extracts were higher than their bark extracts (Table 5). *P. abies* wood extract showed the highest MIC and MFC for *Aspergillus ochraceus*, *Candida albicans*, and *Penicillium ochrochloron*, whereas the *L. decidua* wood extract showed the highest MIC and MFC for *A. flavus*, *A. niger*, and *P. funiculosum*. MIC from both commercial antifungal agents were lower than the examined wood and bark extracts, and ranged from 0.14 to 0.32 mg/mL and 0.11 to 3.71 mg/mL for FLZ and KTZ, respectively. The most sensitive fungus was *A. flavus* towards *P. abies* bark extract, and the most resistant fungus towards all wood and bark extracts was *C. albicans*.

In addition, the methanol extract of *P. abies* and *L. decidua* wood and bark were screened for their antibacterial activities against Gram-positive and Gram-negative bacteria. All of the examined wood or bark extracts for *P. abies* and *L. decidua* exhibited antibacterial activities against all bacteria. However, the antibacterial potential of the bark was much higher than the wood extract in general (Table 6). The MIC value of *P. abies* and *L. decidua* bark ranged from 0.008 to 1.15 mg/mL, whereas the MBC ranged from 0.19 to 2.30 mg/mL. While the MIC value of *P. abies* and *L. decidua* wood extract ranged from 0.06 to 1.20 mg/mL, the MBC ranged from 0.27 to 2.31 mg/mL. The extract that showed the highest antibacterial activity was found in *L. decidua* bark, with an MIC and MBC of 0.11 to 0.54 mg/mL and 0.36 to 0.96 mg/mL, respectively. This was followed by *L. decidua* wood extract, which exhibited an MIC and MBC of 0.13 to 0.54 mg/mL and 0.33 to 1.1 mg/mL, respectively. *P. abies* bark and wood did not exhibit much variation in antibacterial activities except against *S. aureus* and *E. coli*, where *P. abies* bark had the highest MIC in the whole data set (0.37 mg/mL). Furthermore, only *P. abies* wood had higher antibacterial activities than antibiotics (0.06 mg/mL), and most *P. abies* and *L.*

decidua wood and bark extracts were only slightly higher than antibiotics. In general, the most sensitive bacterium was *E. coli*, and the most resistant was *P. aeruginosa*.

These results indicated that the bark and wood extracts of *P. abies* and *L. decidua* represent potential antibacterial and antifungal resources and that the antibacterial activities of the bark and wood compounds are much higher than their antifungal activities. However, the bark of *L. decidua* demonstrated the highest antibacterial and antifungal activities.

The fungicidal activity of different components of the bark of coniferous trees has attracted much attention. Recently, Minova *et al.* (2015) confirmed that ethanol extracts of the bark of *P. abies* inhibit mycelial growth of *B. cinerea*, *C. acutatum*, *P. cactorum*, and *M. fragariae*. Bark extracts can reduce the sporulation of *B. cinerea*, *C. acutatum*, and *P. cactorum* (Minova *et al.* 2015). The bark extract of *P. abies* has the most efficient antioxidant activity within lipid peroxidation tests (Pietarinen *et al.* 2006). The extract of this bark is made up of stilbenes and stilbene glycosides, which are efficient in preventing lipid peroxidation (Mérillon *et al.* 1997). Similar to flavonoids, the glycosidation of stilbenes lowers antioxidant potency. Packer *et al.* (1999) evaluated the well-known antioxidative properties and chemical composition of Pycnogenol. The principle components could be divided into flavonoids (catechin, epicatechin, and taxifolin) and condensed tannins. Pycnogenol has been shown to have a higher biological capability as a mixture than its purified components, implying that its components act synergistically (Packer *et al.* 1999). The antioxidant potencies of *B. pendula*, Pycnogenol, and *P. menziensii* are most likely the result of condensed tannins (Pietarinen *et al.* 2006).

Five types of flavonoids (flavones, flavonols, flavanones, dihydro-flavonols, and flavans), reported by Rauha *et al.* (2000), were found in *P. abies*. Most antibacterial activity data concerns aglycones, and only small amounts of data on glycosides can be found (Metsämuuronen and Siren 2014). Out of all these flavonoids, aglycones, quercetin (Rice-Evans *et al.* 1996; Ibewuike *et al.* 1997; Puupponen-Pimiä *et al.* 2001), kaempferol (Rauha *et al.* 2000), and myricetin (Puupponen-Pimiä *et al.* 2001) have already been recognized to possess antibacterial activity. However, quercetin-3-glucoside (quercitrin) (Puupponen-Pimiä *et al.* 2001) has actually been found to be inactive. Zhou (2013) discovered that quercetin and myricetin-3-rhamnosides are inactive on *Proteus mirabilis* and *E. coli* at a concentration of 500 mg/L, and *S. aureus*, *S. epidermidis*, and *S. haemolyticus* at a concentration of 350 mg/L. This could be because the glycosylation of flavonoids minimizes their antibacterial activity compared to related aglycones.

The quantitative assay validated that the intensity of the antimicrobial activities of *P. abies* (wood and bark) are unique, and are determined by the tested microbial strain (Radulescu *et al.* 2011). It is possible that the most susceptible strains could be the Gram-positive ones (*Bacillus cereus* and *S. aureus*). If this is the case, it would confirm the suggested hypothesis that the outer membrane of Gram-negative bacteria could be an efficient barrier for the internalization of the active compounds of the wood and bark extract. The greater sensitivity of Gram-positive bacteria might be related to their outer layer chemical structure (peptidoglycan), which is not an efficient permeable barrier (Radulescu *et al.* 2011). The outer membrane of Gram-negative bacteria is normally negatively charged and hydrophilic, and because of its structural lipopolysaccharide components, may be less permeable to lipophilic substances. Although porins characterize a selective shield to high molecular weight hydrophilic compounds (Kaur and Arora 2009).

The *in vitro* study of Rautio *et al.* (2012) demonstrated that resin purified from the trunk of *P. abies* clearly shows a wide spectrum of antifungal activities. Moreover, the resin was highly microbicidal against all dermatophytes, but not against yeasts and

opportunistic fungi. The authors suggested that the mechanisms through which the resin inhibits the development of the microbes are "specific" (Rautio *et al.* 2012). Similarly, extracts of *P. abies* show microbicidal activity against Gram-positive bacteria, but not against Gram-negative bacteria (Rautio *et al.* 2007). Nevertheless, a different pattern was reported in the European Pharmacopoeia, with *P. abies* exerting a microbicidal activity against the Gram-negative bacteria, *E. coli*, and *Pseudomonas aeruginosa* (Sipponen and Laitinen 2011). This study showed the importance of using the extracts from heartwood and bark of *P. abies* and *L. decidua* as bioactive agents against the growth of some plant and human pathogens. Environmentally, the study suggested the save way in how to get rid of the woodworking residues (wood shavings and sawdust) resulted from the production of boards from the commercial softwoods to produce a bioactive extracts.

CONCLUSIONS

1. In this study, the methanol extracts from the wood and bark of *P. abies* and *L. decidua* were evaluated for their antibacterial and antifungal activity. Their chemical compositions were then analyzed using GC/MS.
2. The main chemical compounds of *P. abies* wood extract were 13-epimanool, α-cedrol, 2,6-di-t-butyloctahydroazulene-3a,8-diol, astringin, (Z,Z,Z)-9,12,15-octadecatrienoic acid-methyl ester, and (1,5,5,8-tetramethylbicyclo[4.2.1]non-9-yl)acetic acid. The main chemical compounds of *P. abies* bark were abietic acid, astringin, dehydroabietic acid, α-terpineol, methyl sandaracopimarate, and 9-desoxo-9-x-acetoxy-3,8,12-tri-o-acetylingol.
3. The main chemical compounds of *L. decidua* wood extract were abietic acid, oleanolic acid, duvatrienediol, larixol, squalene, and 13-epimanool. The main chemical compounds of *L. decidua* bark were (-)-2,9-dihydroxyverrucosane, larixol, nonacosane, cholan-24-oic acid,3,12-dihydroxy-(3*α*,5*α*,12*α*)-decane, and 5-hydroxy-2,3,3-trimethyl-2-(3-methyl-buta-1,3-dienyl)-cyclohexanone.
4. *P. abies* wood extract showed the highest MIC and MFC for *Aspergillus ochraceus*, *Candida albicans*, and *Penicillium ochrochloron*, whereas the *L. decidua* wood extract showed the highest MIC and MFC for *A. flavus*, *A. niger*, and *P. funiculosum*.
5. The examined wood or bark extracts for *P. abies* and *L. decidua* exhibited antibacterial activities against all bacteria. However, the antibacterial potential of the bark was much higher than the wood extract.
6. The results suggest that the wood and bark extracts from *P. abies* and *L. decidua* have a potential effect for use in food and/or pharmaceutical industries. The results showed that extracts have a good potential or human health benefits, agricultural pest control, and wood industry enhancement. However, further studies need to be undertaken to ascertain fully the bioactivity of the extracts.

ACKNOWLEDGMENTS

The authors would like to acknowledge the Deanship of Scientific Research at King Saud University for funding (Research grant NO. RG 1435-011).

Table 5. Minimum Inhibitory (MICs) and Fungicidal (MFC) Concentration of Wood and Bark Extracts (mg/mL) of *Picea abies* and *Larix decidua*

Fungi	*Picea abies*				*Larix decidua*				FLZ		KTZ	
	Wood		Bark		Wood		Bark					
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
Aspergillus flavus	0.14 ± 0.01	0.27 ± 0.01	0.13 ± 0.03	0.25 ± 0.02	0.25 ± 0.02	0.51 ± 0.01	0.21 ± 0.01	0.46 ± .01	0.14 ± 0.01	0.23 ± 0.02	0.20 ± 0.01	0.41 ± 0.03
Aspergillus ochraceus	0.41 ± 0.01	0.87 ± 0.03	0.36 ± 0.01	0.71 ± 0.01	0.36 ± 0.01	0.83 ± 0.03	0.20 ± 0.01	0.45 ± 0.01	0.21 ± 0.01	0.31 ± 0.01	0.23 ± 0.01	0.40 ± 0.01
Aspergillus niger	0.31 ± 0.03	0.76 ± 0.05	0.32 ± 0.01	0.72 ± 0.03	0.56 ± 0.03	1.29 ± 0.09	0.43 ± 0.05	1.1 ± 0.07	0.14 ± 0.01	0.25 ± 0.01	0.11 ± 0.01	0.21 ± 0.01
Candida albicans	1.74 ± 0.1	3.52 ± 0.2	0.97 ± 0.03	2.36 ± 0.1	0.9 ± 0.07	2.1 ± 0.05	0.6 ± 0.03	1.34 ± 0.06	0.11 ± 0.01	0.21 ± 0.03	0.19 ± 0.01	0.39 ± 0.02
Penicillium funiculosum	0.29 ± 0.03	0.72 ± 0.04	0.34 ± 0.01	0.69 ± 0.05	0.40 ± 0.03	0.93 ± 0.05	0.35 ± 0.03	0.75 ± 0.02	0.14 ± 0.01	0.27 ± 0.03	2.13 ± 0.07	3.71 ± 0.05
Penicillium ochrochloron	0.23 ± 0.01	0.51 ± 0.03	0.19 ± 0.01	0.42 ± 0.03	0.21 ± 0.01	0.42 ± 0.01	0.17 ± 0.01	0.42 ± 0.03	0.23 ± 0.03	0.32 ± 0.01	0.20 ± 0.01	0.41 ± 0.01

Note: FLZ, Fluconazole; KLZ, Ketoconazole

Table 6. Minimum Inhibitory (MICs) and Bactericidal (MBCs) Concentrations of Wood and Bark Extracts (mg/mL) of *Picea abies* and *Larix decidua*

Bacteria	*Picea abies*				*Larix decidua*				Streptomycin		Ampicillin	
	Wood		Bark		wood		Bark					
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Micrococcus flavus	1.2 ± 0.01	2.31 ± 0.03	1.15 ± 0.1	2.30 ± 0.1	0.33 ± 0.01	0.53 ± 0.03	0.21 ± 0.01	0.43 ± 0.01	0.10 ± 0.01	0.20 ± 0.01	0.10 ± 0.01	0.20 ± 0.01
Pseudomonas aeruginosa	1.1 ± 0.02	1.82 ± 0.1	0.43 ± 0.08	0.91 ± 0.05	0.17 ± 0.003	0.33 ± 0.001	0.54 ± 0.03	0.96 ± 0.05	0.06 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.27 ± 0.03
Escherichia coli	0.06 ± 0.01	0.15 ± 0.01	0.08 ± 0.01	0.19 ± 0.01	0.54 ± 0.03	1.1 ± 0.08	0.33 ± 0.02	0.64 ± 0.03	0.10 ± 0.01	0.28 ± 0.02	0.22 ± 0.02	0.43 ± 0.05
Pectobacterium atrosepticum	0.28 ± 0.001	0.57 ± 0.003	0.37 ± 0.03	0.63 ± 0.05	0.27 ± 0.005	0.72 ± 0.03	0.22 ± 0.01	0.47 ± 0.03	0.07 ± 0.01	0.15 ± 0.03	0.26 ± 0.03	0.52 ± 0.05
P. c. subsp. carotovorum	0.13 ± 0.01	0.27 ± 0.01	0.8 ± 0.01	0.19 ± 0.02	0.26 ± 0.02	0.57 ± 0.03	0.21 ± 0.003	0.36 ± 0.001	0.07 ± 0.01	0.20 ± 0.03	0.25± 0.01	0.47 ± 0.03
Dickeya solani	0.35 ± 0.03	0.74 ± 0.05	0.34 ± 0.01	0.62 ± 0.03	0.27 ± 0.01	0.56 ± 0.05	0.21 ± 0.01	0.43 ± 0.03	0.22 ± 0.02	0.43 ± 0.05	0.31 ± 0.01	0.57 ± 0.03
Listeria monocytogenes	0.17 ± 0.01	0.29 ± 0.02	0.16 ± 0.01	0.33 ± 0.02	0.17 ± 0.01	0.53 ± 0.05	0.15 ± 0.02	0.48 ± 0.01	0.15 ± 0.01	0.31 ± 0.02	0.18 ± 0.01	0.31 ± 0.01
Staphylococcus aureus	0.15 ± 0.01	0.27 ± 0.03	0.13 ± 0.01	0.36 ± 0.01	0.24 ± 0.03	0.54 ± 0.01	0.21 ± 0.02	0.49 ± 0.03	0.22 ± 0.01	0.41 ± 0.05	0.11 ± 0.01	0.23 ± 0.03
Bacillus cereus	0.14 ± 0.02	0.31 ± 0.03	0.15 ± 0.01	0.28 ± 0.02	0.13 ± 0.00	0.54± 0.01	0.11 ± 0.01	0.42 ± 0.03	0.07 ± 0.01	0.16 ± 0.01	0.09 ± 0.01	0.19 ± 0.03

REFERENCES CITED

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/mass Spectroscopy* (4th Ed.), Allured Publishing, Carol Stream, IL, USA, p. 804.

Alfredsen, G., Solheim, H., and Slimestad, R. (2008). "Antifungal effect of bark extracts from some European tree species," *Eur. J. For. Res.* 127(5), 387-393**.** DOI: 10.1007/s10342-008-0222-x

Behrens, A., Maie, N., Knicker, H., and Kögel-Knabner, I. (2003). "MALDI-TOF mass spectrometry and PSD fragmentation as means for the analysis of condensed tannins in plant leaves and needles," *Phytochemistry* 62(7), 1159-1170. DOI: 10.1016/S0031-9422(02)00660-X

Bianchi, S., Gloess, A. N., Kroslakova, I., Mayer, I., and Pichelin, F. (2014). "Analysis of the structure of condensed tannins in water extracts from bark tissues of Norway spruce (*Picea abies* [Karst.]) and Silver fir (*Abies alba* [Mill.]) using MALDI-TOF mass spectrometry," *Ind. Crops Prod.* 61(November), 430-437. DOI: 10.1016/j.indcrop.2014.07.038

Bier, M. C. J., Medeiros, A. B. P., de Oliveira, J. S., Côcco, L. C., da Luz Costa, J., de Carvalho, J. C., and Soccol, C. R. (2016). "Liquefied gas extraction: A new method for the recovery of terpenoids from agroindustrial and forest wastes," *J. Supercrit Fluids* 110(April), 97-102. DOI: 10.1016/j.supflu.2015.12.016

Cope, J. A., Winch, F. E., and Cope, E. A. (2002). *Know Your Trees*, Cornell University Media and Technology Services Resource Center, Ithaca, NY, USA.

Danneyrolles, V., Arseneault, D., and Bergeron, Y. (2016). "Pre-industrial landscape composition patterns and post-industrial changes at the temperate-boreal forest interface in western Quebec, Canada," *J. Veg. Sci.* 27(3), 470-481. DOI: 10.1111/jvs.12373

da Silva, A. R., Monteiro Pastore, T. C., Batista Braga, J. W., Davrieux, F., Arakaki Okino, E. Y., Rauber Coradin, V. T., Alves Camargos, J. A., and do Prado, A. G. S. (2013). "Assessment of total phenols and extractives of mahogany wood by near infrared spectroscopy (NIRS)," *Holzforschung* 67(1), 1-8. DOI: 10.1515/hf-2011-0207

Eklund, P. C., Willför, S. M., Smeds, A. I., Sundell, F. J., Sjöholm, R. E., and Holmbom, B. R. (2004). "A new lariciresinol-type butyrolactone lignan derived from hydroxymatairesinol and its identification in spruce wood," *J. Nat. Prod.* 67(6), 927-931. DOI: 10.1021/np0340706

Eriksson, K., Wiklund, L., and Larsson, C. (2004). "Dermal exposure to terpenic resin acids in Swedish carpentry workshops and sawmills," *Ann. Occup. Hyg.* 48(3), 267-275. DOI: 10.1093/annhyg/meh013

Espinel-Ingroff, A. (2001). "*In vitro* fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dematiaceous fungi," *J. Clin. Microbiol.* 39(3), 954-958. DOI: 10.1128/JCM.39.3.954-958.2001

Fattahi, B., Nazeri, V., Kalantari, S., Bonfill, M. and Fattahi, M. (2016). "Essential oil variation in wild-growing populations of *Salvia reuterana Boiss*. collected from Iran using GC-MS and multivariate analysis," *Ind. Crops Prod.* 81(March), 180-190. DOI: 10.1016/j.indcrop.2015.11.061

Fernández, M. A., Tornos, M. P., Garcia, M. D., Heras, B. D. L., Villar, A. M., and Saenz, M.T. (2001). "Anti-inflammatory activity of abietic acid, a diterpene isolated

from *Pimenta racemosa var. grissea*," *J. Pharm. Pharmacol.* 53(6), 867-872. DOI: 10.1211/0022357011776027

Grassmann, J., Hippeli, S., Vollmann, R., and Elstner, E. F. (2003). "Antioxidative properties of the essential oil from *Pinus mugo*," *J. Agric. Food Chem.* 51(26), 7576-7582. DOI: 10.1021/jf030496e

Greenberg, C. H., Collins, B. S., McNab, W. H., Miller, D. K., and Wein, G. R. (2016). "Introduction to natural disturbances and historic range of variation: Type, frequency, severity, and post-disturbance structure in central hardwood forests," in: *Natural Disturbances and Historic Range of Variation*, C. H Greenberg and B. S. Collins (eds.), Springer International Publishing, Cham, Switzerland, pp. 1-32.

Hennig, P., Steinborn, A., and Engewald, W. (1994). "Investigation of the composition of *Pinus peuce* needle oil by GC-MS and GC-GC-MS," *Chromatographia* 38(11), 689-693. DOI: 10.1007/BF02269622

Horsfall, J. G. (ed.) (1980). "5: How plants defend themselves," in: *Plant Disease: An Advanced Treatise*, Academic Press, New York, NY.

Ibewuike, J. C., Ogungbamila, F. O., Ogundaini, A. O., Okeke, I. N., and Bohlin, L. (1997). "Antiinflammatory and antibacterial activities of C-methylflavonols from *Piliostigma thonningii*," *Phytother. Res.* 11(4), 281-284. DOI: 10.1002/(SICI)1099-1573(199706)11:4<281::AID-PTR281>3.0.CO;2-9

Kamaya, Y., Tokita, N., and Suzuki, K. (2005). "Effects of dehydroabietic acid and abietic acid on survival, reproduction, and growth of the crustacean *Daphnia magna*," *Ecotoxicol. Environ. Saf.* 61(1), 83-88. DOI: 10.1016/j.ecoenv.2004.07.007

Kaur, G. J., and Arora, D. S. (2009). "Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi*," *BMC Complement. Altern. Med.* 9, 30. DOI: 10.1186/1472-6882-9-30

Kelly, G. S. (1999). "Larch arabinogalactan: Clinical relevance of a novel immune-enhancing polysaccharide," *Altern. Med. Rev.* 4(2), 96-103.

Keeling, C. I., and Bohlmann, J. (2006). "Diterpene resin acids in conifers," *Phytochemistry* 67(22), 2415-2423. DOI: 10.1016/j.phytochem.2006.08.019

Khare, C. P. (ed.) (2004). *Indian Herbal Remedies*, Springer, Berlin, Germany.

Kolhir, V. K., Bykov, V. A., Baginskaja, A. I., Sokolov, S. Y., Glazova, N. G., Leskova, T. E., Sakovich, G. S., Tjukavkina, N. A., Kolesnik, Y. A., and Rulenko, I. A. (1996). "Antioxidant activity of a dihydroquercetin isolated from *Larix gmelinii* (Rupr.) Rupr. wood," *Phytother Res.* 10(6), 478-482. DOI: 10.1002/(SICI)1099-1573

Krieg, O. D., Schwinn, T., and Menges, A. (2016). "Integrative design computation for local resource effectiveness in architecture," in: *Urbanization and Locality*, F. Wang and M. Prominski (eds.), Springer, Berlin, Germany, pp. 123-143.

Kusumoto, N., Zhao, T., Swedjemark, G., Ashitani, T., Takahashi, K., and Borg-Karlson, A. K. (2014). "Antifungal properties of terpenoids in *Picea abies* against *Heterobasidion parviporum*," *For. Pathol.* 44(5), 353-361. DOI: 10.1111/efp.12106

Latva-Mäenpää, H., Laakso, T., Sarjala, T., Wähälä, K., and Saranpää, P. (2014). "Root neck of Norway spruce as a source of bioactive lignans and stilbenes," *Holzforschung* 68(1), 1-7. DOI: 10.1515/hf-2013-0020

Liimatainen, J., Karonen, M., Sinkkonen, J., Helander, M., and Salminen, J. P. (2012). "Characterization of phenolic compounds from inner bark of *Betula pendula*," *Holzforschung* 66(2), 171-181. DOI: 10.1515/HF.2011.146

Münzenberger, B., Heilemann, J., Strack, D., Kottke, I., and Oberwinkler, F. (1990). "Phenolics of mycorrhizas and non-mycorrhizal roots of Norway spruce," *Planta* 182(1), 142-148. DOI: 10.1007/BF00239996

Malá, J., Hrubcová, M., Máchová, P., Cvrcková, H., Martincová, O., and Cvikrová, M. (2011). "Changes in phenolic acids and stilbenes induced in embryogenic cell cultures of Norway spruce by two fractions of *Sirococcus strobilinus* mycelia," *J. For. Sci.* 57(1), 1-7.

Matthews, S., Mila, I., Scalbert, A., and Donnelly, D. M. X. (1997). "Extractable and non-extractable proanthocyanidins in barks," *Phytochemistry* 45(2), 405-410. DOI: 10.1016/S0031-9422(96)00873-4

Mérillon, J. M., Fauconneau, B., Teguo, P. W., Barrier, L., Vercauteren, J., and Huguet, F. (1997). "Antioxidant activity of the stilbene astringin, newly extracted from *Vitis vinifera* cell cultures," *Clin. Chem*. 43(6), 1092-1093.

Merrill, W. (1992). "Mechanisms of resistance to fungi in woody plants: A historical perspective," in: *Defense Mechanisms of Woody Plants against Fungi*, R. A. Blanchette, A. R. Biggs (eds). Springer, Berlin, Germany, pp. 1-12.

Metsämuuronen, S., and Siren, H. (2014). "Antibacterial compounds in predominant trees in Finland: Review," *J. Bioprocess Biotech.* 4(5), 167. DOI: 10.4172/2155-9821.1000167

Minova, S., Sešķēna, R., Voitkāne, S., Metla, Z., Daugavietis, M., and Jankevica, L. (2015). "Impact of pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.) bark extracts on important strawberry pathogens," *Proc. Latv. Acad. Sci. Sect. B. Nat. Exact. Appl. Sci.* 69(1-2), 62-67. DOI: 10.1515/prolas-2015-0008

Nikolić, B., Ristić, M., Bojović, S., Marin, P. D. (2007). "Variability of the needle essential oils of *Pinus heldreichii* from different populations in Montenegro and Serbia," *Chem. Biodivers.* 4(5), 905-916. DOI: 10.1002/cbdv.200790079

Packer, L., Rimbach, G., and Virgili, F. (1999). "Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol," *Free Radic. Biol. Med.* 27(5-6), 704-724. DOI: 10.1016/S0891-5849(99)00090-8

Pan, H., and Lundgren, L. N. (1995). "Phenolic extractives from root bark of *Picea abies*," *Phytochemistry* 39(6), 1423-1428. DOI: 10.1016/0031-9422(95)00144-V

Patinha, D. J. S., Domingues, R. M. A., Villaverde, J. J., Silva, A. M. S., Silva, C. M., Freire, C. S. R., Neto, C. P., and Silvestre, A. J. D. (2013). "Lipophilic extractives from the bark of *Eucalyptus grandis* x *globulus*, a rich source of methyl morolate: Selective extraction with supercritical CO_2," *Ind. Crops Prod.* 43(May), 340-348. DOI: 10.1016/j.indcrop.2012.06.056

Pferschy-Wenzig, E. M., Kunert, O., Presser, A., and Bauer, R. (2008). "*In vitro* anti-inflammatory activity of larch (*Larix decidua* L.) sawdust," *J. Agric. Food Chem.* 56(24), 11688-11693. DOI: 10.1021/jf8024002

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

Puupponen-Pimiä, R., Nohynek, L., Meier, C., Kähkönen, M., Heinonen, M., Hopia, A., and Oksman-Caldentey, K. M. (2001). "Antimicrobial properties of phenolic compounds from berries," *J. Appl. Microbiol.* 90(4), 494-507. DOI: 10.1046/j.1365-2672.2001.01271.x

Radulescu, V., Saviuc, C., Chifiriuc, C., Oprea, E., Ilies, D. C., Marutescu, L., and Lazar, V. (2011). "Chemical composition and antimicrobial activity of essential oil from shoots spruce (*Picea abies* L.)," *Revista De Chimie –Bucharest* 62(1), 69-72.

Rauha, J. P., Remes, S., Heinonen, M., Hopia, A., Kähkönen, M., Kujala, T., Pihlaja, K., Vuorela, H., and Vuorela, P. (2000). "Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds," *Int. J. Food Microbiol.* 56(1), 3-12. DOI: 10.1016/S0168-1605(00)00218-X

Rautio, M., Sipponen, A., Lohi, J., Lounatmaa, K., Koukila-Kähkölä, P., and Laitinen, K. (2012). "*In vitro* fungistatic effects of natural coniferous resin from Norway spruce (*Picea abies*)," *Eur. J. Clin. Microbiol. Infect. Dis.* 31(8), 1783-1789. DOI: 10.1007/s10096-011-1502-9

Rautio, M., Sipponen, A., Peltola, R., Lohi, J., Jokinen, J. J., Papp, A., Carlson, P., and Sipponen, P. (2007). "Antibacterial effects of home-made resin salve from Norway spruce (*Picea abies*)," *APMIS* 115(4), 335-340. DOI: 10.1111/j.1600-0463.2007.apm_548.x

Rice-Evans, C. A., Miller, N. J., and Paganga, G. (1996). "Structure-antioxidant activity relationships of flavonoids and phenolic acids," *Free Radic. Biol. Med.* 20(7), 933-956. DOI: 10.1016/0891-5849(95)02227-9

Rissanen, E., Krumschnabel, G., and Nikinmaa, M. (2003). "Dehydroabietic acid, a major component of wood industry effluents, interferes with cellular energetics in rainbow trout hepatocytes," *Aquat. Toxicol. Amst. Neth.* 62(1), 45-53. DOI: 10.1016/S0166-445X(02)00066-8

Salem, M. Z. M., Zeidler, A., Böhm, M., and Srba, J. (2013). "Norway spruce (*Picea abies* [L.] Karst.) as a bioresource: Evaluation of solid wood, particleboard and MDF technological properties and formaldehyde emission," *BioResources* 8(1), 1199-1221. DOI: 10.15376/biores.8.1.1199-1221

Salem, M. Z. M., Nasser, R. A., Zeidler, A., Elansary, H. O., Aref, I. M., Böhm, M., Ali, H. M., and Ahmed, A. I. (2015a). "Methylated fatty acids from heartwood and bark of *Pinus sylvestris*, *Abies alba*, *Picea abies*, and *Larix decidua*: Effect of strong acid treatment," *BioResources* 10(4), 7715-7724. DOI: 10.15376/biores.10.4.7715-7724

Salem, M. Z. M., Zeidler, A., Böhm, M., Mohamed, M. E., and Ali, H. M. (2015b). "GC/MS analysis of oil extractives from wood and bark of *Pinus sylvestris*, *Abies alba*, *Picea abies*, and *Larix decidua*," *BioResources* 10(4), 7725-7737. DOI: 10.15376/biores.10.4.7725-7737

Salem, M. Z. M., Zidan, Y. E., Mansour, M. M., El Hadidi, N. M., and Elgat, W. A. A. (2016). "Antifungal activities of two essential oils used in the treatment of three commercial woods deteriorated by five common mold fungi," *Int. Biodeterior. Biodegrad.* 106(January), 88-96. DOI: 10.1016/j.ibiod.2015.10.010

San Feliciano, A., Gordaliza, M., Salinero, M. A., and Miguel del Corral, J. M. (1993). "Abietane acids: Sources, biological activities, and therapeutic uses," *Planta Med.* 59(6), 485-490. DOI: 10.1055/s-2006-959744

Sepúlveda, B., Astudillo, L., Rodríguez, J. A., Yáñez, T., Theoduloz, C., and Schmeda-Hirschmann, G. (2005). "Gastroprotective and cytotoxic effect of dehydroabietic acid derivatives," *Pharmacol. Res.* 52(5), 429-437. DOI: 10.1016/j.phrs.2005.06.004

Si, C. L., Jiang, J. Z., Liu, S. C., Hu, H. Y., Ren, X. D., Yu, G. J., and Xu, G. H. (2012). "A new lignan glycoside and phenolics from the branch wood of *Pinus banksiana* Lambert," *Holzforschung* 67(4), 357-363. DOI: 10.1515/hf-2012-0137

Sipponen, A., and Laitinen, K. (2011). "Antimicrobial properties of natural coniferous rosin in the European Pharmacopoeia challenge test," *APMIS* 119(10), 720-724. DOI: 10.1111/j.1600-0463.2011.02791.x

Steynberg, J. P., Ferreira, D., and Roux, D. G. (1983). "The first condensed tannins based on a stilbene," *Tetrahedron Lett.* 24(38), 4147-4150. DOI: 10.1016/S0040-4039(00)88284-2

Tumen, I., Hafizoglu, H., Kilic, A., Dönmez, I. E., Sivrikaya, H., and Reunanen, M. (2010). "Yields and constituents of essential oil from cones of *Pinaceae* spp. natively grown in Turkey," *Molecules* 15(8), 5797-5806. DOI: 10.3390/molecules15085797

Ulusu, N. N., Ercil, D., Sakar, M. K., and Tezcan, E. F. (2002). "Abietic acid inhibits lipoxygenase activity," *Phytother. Res.* 16(1), 88-90. DOI: 10.1002/ptr.983

Viiri, H., Annila, E., Kitunen, V., and Niemelä, P. (2001). "Induced responses in stilbenes and terpenes in fertilized Norway spruce after inoculation with blue-stain fungus, *Ceratocystis polonica*," *Trees* 15(2), 112-122. DOI: 10.1007/s004680000082

von Bruchhausen F., Ebel S., and Hackenthal E., *et al.* (eds) (1993). *Hagers Handbuch der Pharmazeutischen Praxis*, Springer Berlin Heidelberg, Berlin, Heidelberg.

Willför, S. M., Ahotupa, M. O., Hemming, J. E., Reunanen, M. H., Eklund, P. C., Sjöholm, R. E., Eckerman, C. S., Pohjamo, S. P., and Holmbom, B. R. (2003). "Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species," *J. Agric. Food Chem.* 51(26), 7600-7606. DOI: 10.1021/jf030445h

Yessoufou, K., Elansary, H. O., Mahmoud, E. A., and Skalicka-Woźniak, K. (2015). "Antifungal, antibacterial, and anticancer activities of *Ficus drupacea* L. stem bark extract and biologically active isolated compounds," *Ind. Crops Prod.* 74(November), 752-758. DOI: 10.1016/j.indcrop.2015.06.011

Zeneli, G., Krokene, P., Christiansen, E., Krekling, T., and Gershenzon, J. (2006). "Methyl jasmonate treatment of mature Norway spruce (*Picea abies*) trees increases the accumulation of terpenoid resin components and protects against infection by *Ceratocystis polonica*, a bark beetle-associated fungus," *Tree Physiol.* 26(8), 977-988. DOI: 10.1093/treephys/26.8.977

Zhou, Y. (2013). "Mathematical modelling of protein precipitation based on the phase equilibrium for an antibody fragment from *E. coli* lysis," *J. Bioprocess. Biotech.* 3(2), 129. DOI: 10.4172/2155-9821.1000129

Article submitted: June 28, 2016; Peer review completed: September 4, 2016; Revised version received and accepted: September 7, 2016; Published: September 21, 2016.
DOI: 10.15376/biores.11.4.9421-9437.