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ORIGINAL PAPER

Chemical composition and antioxidant capacity of the aqueous extract of *Phellodendron amurense*

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Abstract The aqueous extract of *Phellodendron amurense* Rupr. (Amur Cork Tree) provides a rich source of antioxidants and chemical compounds, and can be used for food and wood preservative materials. In this study, we characterized the chemical composition of this extract by GC and GC/MS. The antioxidant capacity was evaluated using a variety of antioxidant assays (superoxide radical, hydroxyl radical, nitric oxide radical, and DPPH radical scavenging activity). Additionally, total polyphenolic content was determined. Phenolic acids and acetone derivatives were major compounds of the extract capable of scavenging the DPPH free radical and reducing ferric ions. DPPH and ferric ion reduction results were strongly correlated with total phenolic content of the extract which also exhibited strong nitric oxide, hydroxyl radical scavenging and superoxide anion radical scavenging activities.

Keywords *Phellodendron amurense* · Aqueous extract · Chemical characterization · Antioxidant activity

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Introduction

Wood, as a natural organic composite material, is widely used for construction material and home furnishings, but some types, for example, softwoods such as *Alstonia*, *Picea*, *Carya*, *Pycnanthus*, *Grewia*, and *Pinus*, are susceptible to wood rotting fungi (Yen and Chang 2008; http://www.tis-gdv.de/tis_e/misc/holzart.htm), resulting in a reduction of mechanical strength due to the degradation of wood. In order to prevent fungal decay and insect attack, many methods have been employed such as the impregnation of the wood with biocide solutions and various modification methods (Barnes and Murphy 1995; Vetter et al. 2009). Different modification methods using non-biocidal, thermal, chemical or resin have gained substantial support as potential alternatives to biocide solutions (Vetter et al. 2009). Some of the chemical preservatives are environmentally hazardous and have been restricted for use in recent years (Hsu et al. 2009; Yen and Chang 2008). These include CCA (copper-chromiun-arsenate), creosote oil and polychlorophenols (Vahaoja et al. 2005; Schultz and Nicholas 2000a, b). Importantly, one widely used wood preservative, pentachlorophenol, is a major pollutant and extremely toxic to human health from acute (short-term) ingestion and inhalation exposure (Krogh and Tooke 1944; USEPA 1999). Neurological, blood and liver effects and eye irritations were due to acute inhalation of pentachlorophenol and subsequently its usage was restricted

(USEPA 1999). It is also of concern in agricultural practices where the use of traditional agrochemicals has caused serious environmental pollution. Recently, a wood extractive-based formulation, a combination of two different wood extractives, has been developed for the protection of wood and bamboo products and is currently available in Indian markets (http://www.neist.res.in/Active%20And%20Mature%20Technologies%20CSIR-NEIST.pdf). Commercialization of this type of eco-friendly product validates the development of eco-friendly fungicides.

The initial phase of fungal-mediated wood degradation involves a major role of hydroxyl radicals. A report by Backa et al. (1993) suggested that fungal growth in wood is accompanied by the generation of hydroxyl radicals. A study by Tanaka et al. (1999) also reported that hydroxyl radicals are generated in redox reactions and they increase during wood degradation. Thus, the prevention of hydroxyl radical formation may lead to the inhibition of fungal wood decay. Hydroxyl radical formation can be blocked by plant extractives because of their antioxidant properties. Consequently, antioxidants and metal chelators may be promising additives for the development of novel and more environmentally benign preservative systems (Suttie et al. 1996; Baya et al. 2001). Recently, Kaur (2016) proposed using 0.3% copper naphthenate as metal chelator and 25% neem oil in kerosene as a new, efficient, eco-friendly preservative called Copperised Neem Oil for long-term outdoor applications to bamboo. A study by Schultz and Nicholas (2002) reported that a combination of an organic biocide with metal chelating and/or antioxidant additives gave enhanced protection to wood against fungi compared to the biocide alone. Consequently, the authors claimed that this approach can result in the development of better eco-friendly wood preservative systems.

Many researchers have investigated the antioxidant and antimicrobial properties of various plant extracts (Yang and Clausen 2007; Tepe et al. 2005; Wang et al. 2009; Bortolomeazzi et al. 2007). *Phellodendron* is a genus of deciduous trees in the family Rutaceae. *Phellodendron amurense* Rupr. is a timber species native to the Amur Valley in Manchuria, and widely distributed in Southeast Asia (Lis et al. 2004; Ma et al. 2015). It is a category II protected plant in China (Ma et al. 2015) and is cultivated in USA and Europe for landscape and environmental planting, and in botanical gardens and parks. (Lis et al. 2004). *P. amurense* or Amur Cork Tree is one of the 50 fundamental herbs used in Chinese traditional medicine (Ma et al. 2015; Xu and Ventura 2010). The species is well-known for its anti-inflammatory, antineuralgic, antipyretic, antidiarrhetic and disinfectant properties (Lis et al. 2004). Extracts of *P. amurense* have been reported for antimicrobial activity against a wide range of microbes including fungi, bacteria, protozoans, viruses, helminthes and chlamydia (Li et al. 2006). It has also attracted attention because of the wide range of phytochemicals it produces, such as, alkaloids, flavonoids, phenolic compounds, limonoids, polysaccharides, phytosterols, fatty acids and essential oils (Lis et al. 2004). One of the phytochemicals, berberine, has strong antifungal and antibacterial properties (Ma et al. 2015). A study by Wang et al. (2009) examined antimicrobial activity of aqueous and ethanolic extracts of *P. amurense* and found that both extracts possessed antimicrobial activity against *Escherichia coli*, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus aureus* and *S. pyogenes* in high concentrations. *P. amurense* has gained economic value as it is been used in traditional medicine and for its timber (Ma et al. 2015).

The objective of this study was to evaluate the chemical composition, free radical scavenging activities, and ferric-reducing antioxidant power of the aqueous extract of *P. amurense*. In addition, total polyphenolic content was also determined.

Materials and methods

Extracts

The 80 g of dried bark of *P. amurense* obtained from China were extracted with 1.2 L double distilled water for 3 h at 100 °C and afterwards filtered through an advanced No. 1 filter paper. The collected filtrate was dried using a rotary evaporator, stored at 4 °C until tested and analyzed. The yield of the water extract was 5.8%.

Characterization of extracts

Extracts were prepared for GC–MS (gas chromatography/mass spectrometry) analysis by loading five mg in solvent in a silica gel glass column, and eluted by dichloromethane, ethyl ether, acetone, and methanol. Different solvent extracts were separately evaporated by rotary evaporator at 40 °C, and the final fraction was dissolved in 2 mL of acetone. The samples were injected into a gas chromatogram (GCMS-QP2010 plus, Shimadzu). Separation and identification of compounds were achieved using SHIM-50MS column (30 m × 250 μm × 0.25 μm).

Total polyphenol content determination

Total phenolic constituents were determined using the Folin–Ciocalteu reagent (Merck, Germany) and gallic acid monohydrate (Samchun, Korea) used as a standard (Slinkard and Singleton 1977; Chandler and Dodds 1983). The 100 μL solution containing 1000 μg of the extract was

placed in a volumetric flask, 46 mL of distilled water added, followed by the addition of 1 mL of the Folin–Ciocalteu reagent. The flask was shaken thoroughly and incubated at room temperature. After 3 min., 3 mL of 2% sodium carbonate solution was added and the mixture allowed to stand for 2 h with intermittent shaking. The same procedure was repeated to all standard gallic acid monohydrate treatments (0–600 μg/0.1 mL). Absorbance was measured at 760 nm and a standard curve obtained.

DPPH radical scavenging activity

The free radical scavenging activity of the extract was measured by the 2-2-diphenyl-1-picryl hydrazyl (Wako, Japan) method (Williams et al. 1995). A 0.1 mM solution of DPPH in methanol was prepared and 1 mL added to 500 μL samples in different concentrations. After 20 min of incubation at room temperature, absorbance was measured at 525 nm and the percentage of DPPH scavenging activity calculated. L-ascorbic acid (Junsei, Japan) was used as a positive control and all tests were carried out in triplicate.

Nitric oxide radical scavenging assay

The interaction of the aqueous extract with nitric oxide was assessed by the nitrite detection method of Sreejayan and Rao (1997). Two fifty μL of 10 mM sodium nitroprusside (Alfa Aesar, USA) in phosphate-buffered saline were mixed with 250 μL of different concentrations (100–500 μg mL^{-1}) of extract and incubated at 30 °C for 3 h in the dark. After incubation, 0.25 mL of Griess reagent A containing 1% sulphanilamide (Daejung, Korea) in 5% phosphoric acid was added and further incubated at 30 °C for 10 min. Then, 250 μL of Griess reagent B containing 0.1% *N*-1-naphthylethylene diamine dihydrochloride (Yakuri, Japan) was added and incubated for 20 min at 30 °C. The absorbance of the chromophore formed with sulphanilamide and subsequent coupling with napthylethylenediamine was read at 546 nm. The same reaction mixture without the extract served as control. L-ascorbic acid was used as positive control and the analysis was performed in triplicate.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of the extract was measured using the deoxyribose method of Halliwell et al. (1987). One mL of the final reaction solution contained various concentrations (100–500 μg) of the extract: 1 mM $FeCl_3$ (Sigma, USA), 1 mM EDTA (Sigma USA), 20 mM H_2O_2, 1 mM L-Ascorbic acid and 30 mM deoxyribose (Acros, Belgium) in potassium phosphate buffer at pH of 7.4. The tubes were incubated at 37 °C for 1 h and then heated in boiling water for 15 min. followed by the addition of 1 mL of 2.8% (w/v) trichloroacetic acid and 1 mL of 1% (w/w) 2-thiobarbituric acid (Alfa Aesar, USA). The color developed was measured at 532 nm against a blank containing phosphate buffer. The control without the extract and the L-ascorbic acid, and the positive control were treated similarly and all reactions were done in triplicate.

Superoxide anion radical scavenging activity

This activity was measured by the reduction of NBT by the extract (Fontana et al. 2001). The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals which reduce nitro blue tetrazolium (NBT) to a purple formazan. One mL of the reaction mixture contained 20 mM phosphate buffer at pH of 7.4, and 73 μM NADH (Sigma, USA), 50 μM NBT (Sigma, USA), 15 μM PMS (ICN, Ohio) and various concentrations (100–500 μg mL^{-1}) of the sample solution. After 5 min. incubation at ambient temperature, the absorbance at 562 nm was measured against an appropriate blank to determine the quantity of formazan generated. All tests were performed three times. L-ascorbic acid was used as a positive control.

FRAP assay

Total antioxidant activity is measured by the ferric reducing antioxidant power (FRAP) assay of Benzie et al. (1999). At low pH, the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to a ferrous form (which has an intense blue color) can be monitored by measuring the change in absorption at 593 nm. The 100 μL of the sample was mixed with 3 mL of working FRAP reagent containing 300 mM acetate buffer at pH of 3.6, TPTZ (2,4,6-tripyridyl-*s*-triazine, Dojindo, USA), 10 mM in 40 mM HCl and $FeCl_3$. $6H_2O$ (Junsei, Japan), and 20 mM in a ratio of 10:1:1. The absorbance (593 nm) was measured at 0 min after vortexing. Samples were then placed at 37 °C in water bath and absorption again measured after 4 min. Ascorbic acid standard (1000 μM) were processed in the same way.

Results

Chemical composition of the extract

A total of nine compounds were identified in GC–MS analysis of the aqueous extract. Two, 3, 2, and 2 spectral peaks for methanol, acetone, ethyl ether, and dichloromethane fractions, respectively, were obtained (Table 1).

The compounds 2-[(4-chlorophenyl)sulfanyl]-4,6-bis(trichloromethyl)-1,3,5-triazine (RT 20.331), and 1,2-dimethoxy-4-[(4-methoxyphenoxy)methyl]benzene (RT 28.676) were found in the methanol extract (Table 1). Beta,-D-galactopyranose pentaacetate (RT 22.413), Cyanophenphos (RT 30.919), and 5,7-Dichloro-3,4-dihydro-3-[4-[trifluoromethyl]phenyl]-1,9(2H,10H)-acridinedione (RT 20.353) compounds were found in the acetone fraction (Table 1). The compounds 2-[7-chloro-3,4,9,10-tetrahydro-10-hydroxy-9-oxo-3-[4-trifluoromethyl]-1(2H)-acridinelideneamino]-1-phenylguanidine (RT 22.405), and 3-(3-bromophenyl)propenoic acid, 2-(diethoxyphosphinyl)-, ethyl ester (RT 30.914) were found in the ethyl ether extract. The chemical constituents 2,4-diiodoimidazole-5-propionic acid, methyl ester (RT 22.404), and 3-ethoxyacetyl-3-demethylthiocolchicine (RT 30.912) were found in dichloromethane (Table 1). Complete GC–MS chromatograms of four fractions are provided in Supplementary Materials (Figs. S1-S4).

Determination of total polyphenol content

Phenolic compounds are the principal antioxidant constituents of natural products and are composed of phenolic acids and flavonoids. Phenolic compounds or polyphenols constitute one of the most numerous and widely distributed groups of substances in the plant kingdom with more than 8000 known structures. They are potent free radical terminators. The high potential of polyphenols to scavenge free radicals may be because of their many phenolic groups. Based on the absorbance values of the extract solution reacted with Folin–Ciocalteu reagent, a comparison was made with the standard solutions of gallic acid equivalents as described previously. A crude estimation of the total amount of phenolic compounds present was estimated using the Folin–Ciocalteu reagent. The redox reaction between the phenols in the extract and phosphotungstic and phosphomolybdic acids in the reagent was determined in the final phenolic content (Wong et al. 2006). The equation of the standard curve is $y = 0.0898x - 0.1097$ ($R^2 = 0.996$). The result was expressed in gallic acid equivalent (μg mg^{-1}). The amount of the total phenolics was 70 μg mg^{-1}.

DPPH radical scavenging activity

The stable radical DPPH has been widely used for the determination of primary antioxidant activity, i.e., the free radical scavenging activities of pure compounds, plant and fruit extracts and food materials. The assay is based on the reduction of DPPH radicals in methanol which causes an absorbance decrease at 525 nm. DPPH radical scavenging capacities of the aqueous extract of *P. amurense* were measured and the results are shown in Fig. 1. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability (Baumann et al. 1979). The aqueous extract of *P. amurense* possesses good DPPH scavenging activity. When compared to the positive control of L-ascorbic acid it has less activity but there is little difference at a concentration of 500 μg.

Nitric oxide radical scavenging activity

Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. Thus, the scavenging activity of the extract is based on their ability to prevent the production of nitrite ions. The nitric oxide radical scavenging of the extract and ascorbic acid are presented in Fig. 2. Results for both the extract and ascorbic acid are similar. Studies by Jagetia et al. (2004), Madson et al.

Table 1 Major chemical components in aqueous extract of *Phellodendron amurense* identified by GC–MS (See the Supplementary Materials for GC–MS chromatograms)

RT	MW	Formula	Name
20.331	455	C11H4Cl7N3S	2-[(4-chlorophenyl)sulfanyl]-4,6-bis(trichloromethyl)-1,3,5-triazine
28.676	274	C16H18O4	1,2-dimethoxy-4-[(4-methoxyphenoxy)methyl]benzene
22.413	390	C13H22O11	beta,-D-galactopyranose pentaacetate
30.919	303	C15H14NO2PS	Cyanophenphos
20.353	425	C20H12C12F3NO2	5,7-Dichloro-3,4-dihydro-3-[4-[trifluoromethyl]phenyl]-1,9(2H,10H)-acridinedione
22.405	539	C27H21CIF2N5O2	2-[7-chloro-3,4,9,10-tetrahydro-10-hydroxy-9-oxo-3-[4-trifluoromethyl]-1(2H)-acridinelideneamino]-1-phenylguanidine
30.914	390	C15H20BrO5P	3-(3-Bromophenyl)propenoic acid, 2-(diethoxyphosphinyl)-, ethyl ester
22.404	406	C7H8I2N2O2	2,4-Diiodoimidazole-5-propionic acid, methyl ester
30.912	487	C25H29NO7S	3-Ethoxyacetyl-3-demethylthiocolchicine

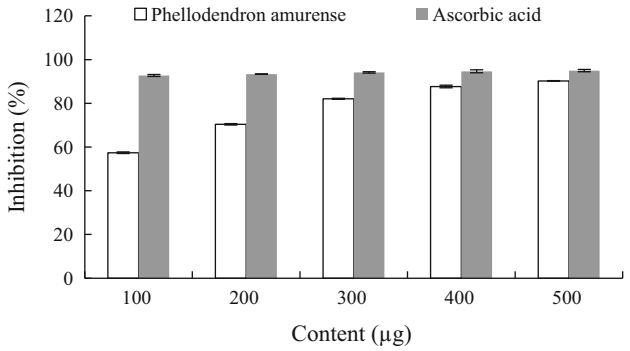

Fig. 1 DPPH radical scavenging activity and L-ascorbic acid at different concentrations

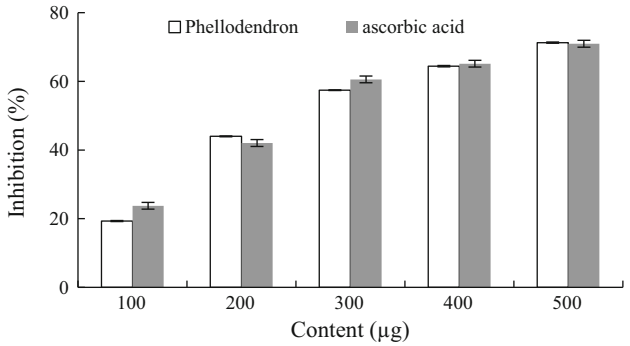

Fig. 2 Nitric oxide radical scavenging activity and L-ascorbic acid at different concentrations

(2000) and Crozier et al. (2000) showed that flavonoids and phenolic compounds possess nitric oxide radical scavenging activity. The nitric oxide radical scavenging activity of the extract may be due to the presence of such compounds.

Hydroxyl radical scavenging activity

In the deoxyribose assay, a mixture of Fe^{3+}-EDTA, H_2O_2 and ascorbic acid generates hydroxyl radicals which can be detected by their ability to degrade sugar deoxyribose into fragments (Li 2000). If the resulting complex mixture of products is heated under acidic conditions, malonaldehyde is formed and may be detected by its ability to react with thiobarbituric acid to form a pink chromogen (Halliwell et al. 1987). The aqueous extract of *P. amurense* possessed better hydroxyl scavenging activity. The results obtained for the hydroxyl radical scavenging and L-ascorbic acid is presented in Fig. 3. The aqueous extract scavenged the hydroxyl radical effectively when compared to the positive control. The involvement of hydroxyl radicals in cell wall degradation was reported by Backa et al. (1993) and Tanaka et al. (1999).

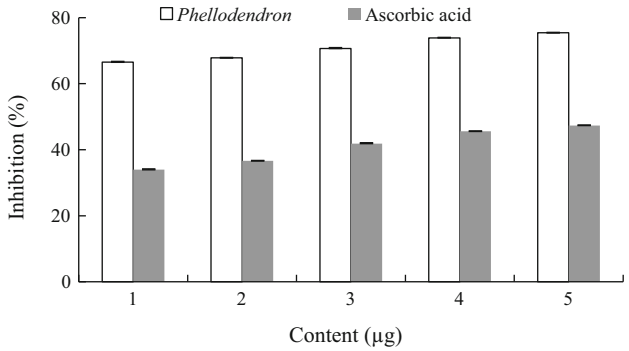

Fig. 3 Hydroxyl radical scavenging activity and L-ascorbic acid at different concentrations

Superoxide radical scavenging activity

The nonenzymatic phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH) system generates superoxide radicals, which reduce nitro blue tetrazolium (NBT) to a purple formazan. The superoxide radical scavenging activity of the extract is presented in Fig. 4.

FRAP assay

The FRAP assay measures the antioxidant effect of the substance in the reducing medium as its reducing ability. The FRAP value of the aqueous extract was 12.88 ± 0.02 μM/250 μg of the extract. This suggests that the extract converts ferric ions to ferrous ions and thus possesses ferric reducing power. The reduced form of the blue ferrous-TPTZ is generated due to the single electron donation of the ferric-TPTZ. This is responsible for the antioxidant capacity of the extract (Wong et al. 2006).

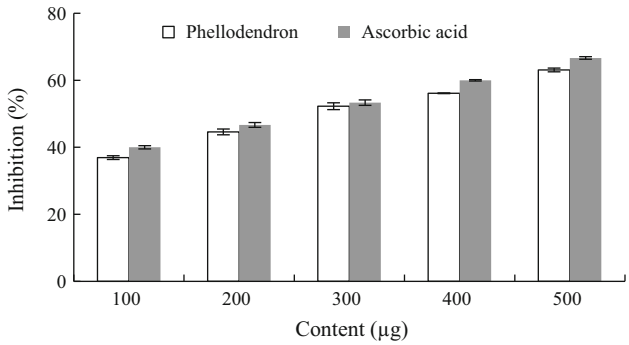

Fig. 4 Superoxide radical scavenging activity and L-ascorbic acid at different concentrations

Discussion

In our study, we used hot water to obtain the aqueous extract. It has been reported that temperature plays a vital role in dissolving bioactive compounds in water (Plaza and Turner 2015). The study by Srinivas et al. (2009) demonstrated that betulin, an antifungal and anti-inflammatory compound from birch bark, needed a temperature of 250–325 °C to dissolve in water. Triazine, methoxy and phenyl compounds are well known for their fungicidal activity (Aligiannis et al. 2001; Dorman and Deans 2000). Toxicity of the extract and potential for registration for use are dependent upon the specific chemical structures of the effective components in the crude extract. By knowing the chemical structures, commercially available or synthetic compounds may be used for application. Extraction from naturally available trees may not be ecologically. The importance of the phenolic hydroxyl groups for antimicrobial activity has previously been reported (Adam et al. 1998; Aligiannis et al. 2001; Dorman and Deans 2000; Nostro et al. 2004; Sivropoulou et al. 1996). Generally extracts that contain a high amount of polyphenols exhibit high antioxidant activity (Rice-Evans et al. 1996). Wong et al. (2006) reported on the total phenolic contents in aqueous extracts of various plants and noted the limitations of Folin reagent in the estimation of phenolic compounds. Any oxidizing substance present in the extract can react with the Folin reagent, not only the phenolics in the extract. This can change the specificity of the assay. The antifungal activity of phenolic compounds based on fungal enzyme inhibition by oxidized compounds through protein non-specific interaction or reaction with sulfhydryl groups depends on the degree of steric hindrance, lipid solubility and the location of hydroxyl group on the molecule (Raccach 1984; Voda et al. 2004). The antioxidant capacity of phenolic compounds depends on the chemical structure of the molecule and the ability of a compound to act as a reducing agent and thus to function as an antioxidant (Bortolomeazzi et al. 2007; Velmurugan et al. 2009). Phenolic extract of olive pomace inhibited the growth of the pathogenic fungi *Alternaria solani*, *Fusarium culmorum* and *Botrytis cinerea* (Winkelhansen et al. 2005). The phenolic compounds *p*-cresol, *p*-ethyl phenol, *p*-n propyl phenol, thymol, guaiacol, cresol, eugenol and isoeugenol possess antifungal activity against *Blastomyces dermatitidis, Histoplasms capsulatum, Trichophyton rubrum, Fonsecaea pedrosoi, Aspergillus nidulans, Penicillium frequentans* and *Penicillium cyclopium* (Kurita et al. 1981). Cowan (1999) reported that the antifungal ability of phenolic compounds depends on fungal enzyme inhibition which contains SH groups in their active sites. The antifungal activity of phenolic compounds might be influenced by the water soluble properties of phenolic compounds.

DPPH activity results reveal that the extract possesses hydrogen donating ability and, may act as primary antioxidants. Some authors report the antioxidant activities of major phenolic compounds such as 2-methoxyphenol (guaiacol), and 2,6-dimethoxyphenol (syringol) present in plant extracts (Ogata et al. 1997; Kajiyama and Ohkatsu 2001; Velmurugan et al. 2009). Bortolomeazzi et al. (2007) reported that guaiacol and its derivatives have higher radical scavenging capacity especially, 4-ethylguaiacol, 4-propylguaiacol, eugenol and 2-propiovanillone. The effectiveness of antioxidant activity depends on the inhibition reaction between a peroxyl radical and the antioxidant. The electron-attractive inductive effect of the alpha carbonyl group in the *para* position should in fact destabilize the phenoxyl radical and reduce the radical scavenging ability of compounds (Kajiyama and Ohaskatsu 2001). The following possible mechanism can be applied for DPPH radical scavenging activity. The dimer formation during the reaction between two phenoxyl radicals can influence the antioxidant property of the extract. These dimers may initially reduce DPPH radical scavenging (Bortolomeazzi et al. 2007).

Few reports exist in the literature regarding the nitric oxide radical scavenging activity of plant extracts. Usually, some plant (especially medicinal) extracts do not exhibit the nitric oxide radical scavenging activity. Saha et al. (2008) briefly explained the scavenging activity of nitric oxide in ethanol extracts of medicinal plants. Under aerobic conditions, the unstable form of nitric oxide can react with oxygen and produce stable forms of nitrate, nitrite and peroxynitrite. These products could directly affect the basic bioorganic macromolecules and lead to the death of the cell (Saha et al. 2008).

The aqueous extract of *P. amurense* actively scavenges the hydroxyl radical to prevent cell damage caused by wood degrading fungi. The allyl groups, which contain phenolic compounds, are effective antioxidants because of the O_2 and hydroxyl radical scavenging ability. However, phenolic compounds without allyl groups also play a role in the termination of free radical chain reactions (Velmurugan et al. 2009). The antifungal activity of some polyphenols in the extract, especially guaiacol, is enhanced by the presence of alkyl group(s) on their benzene ring and ether compounds are enhanced with a methyl group (Kurita et al. 1981).

The superoxide radical scavenging ability low when compared with the other free radical scavenging activity. Initial free radical superoxide is formed from the mitochondrial electron transport system. Superoxide anion is a reduced form of molecular oxygen generated by receiving

one electron. The formation of a major reactive oxygen component in the cells such as hydrogen peroxide, hydroxyl radical, or singlet oxygen, are medicated by superoxide anion. The activity of main catalytic enzyme catalases and glutathione peroxidases, having a potential to act against antioxidant molecules, can be decreased by superoxide anion molecules (Lee et al. 2004). This property of superoxide anion can enhance the scavenging activity of other antioxidant molecules in the extract.

In general, there was a strong correlation between DPPH scavenging and FRAPS activity in the plant extracts. Usually the DPPH values were higher than the FRAP reduction rate but in some circumstances the FRAP reduction rate can be high than DPPH values. This may be because of the initial reduction of ferric ions by polyphenols in the extract and may result in the inefficient scavenging of DPPH free radicals due to steric hindrance (Wong et al. 2006). Our results coincident with the general proposal that DPPH scavenging rates were higher than that of FRAP reducing capacity.

Finally, to conclude, as interest in the development of natural fungicide preservative with strong antioxidant activity is growing, the aqueous extract of *P. amurense* possessed the desirable characters. The different antioxidant methods carried out in this study clearly show the effect of substituents on the antioxidant capacity of phenolic compounds. The extract has also shown various radical scavenging activities such as DPPH, hydroxyl, nitric oxide and superoxide radical and the ability to reduce ferric ions and phenolic compounds found to be the major antioxidants. DPPH radical scavenging results were strongly correlated with ferric ion reduction results. In addition, long-term wood preservation may be achieved by combining the antioxidants with suitable carrier solvents, for example, using a combination of biocide and antioxidants (Schultz and Nicholas 2000a). Therefore, the antioxidant activity of the extract may find potential application in the area of food protection, mostly in the process of food storage. These results suggest that the aqueous extract of *P. amurense* may be used as a natural fungicidal preservative since the antioxidant properties of the extract protect the wood by blocking fungal attack.

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