J Wood Sci (2008) 54:174–178
DOI 10.1007/s10086-007-0913-9

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Characterization and antioxidant activity of Amazonian woods

Received: March 22, 2007 / Accepted: July 26, 2007 / Published online: November 7, 2007

Abstract The heartwood and sapwood characteristics of 11 Amazonian trees were investigated. Whereas 7 of the specimens had densities greater than 0.7 g/cm^3, the heartwood density of ipê amarelo (*Tabebuia serratifolia*), maçaranduba (*Manilkara huberi*), cumaru-ferro (*Dipteryx odorata*), and guarita (*Astronium lecointei*) exceeded 1.0 g/cm^3. Jatobá contained small amounts of Klason lignin and α-cellulose, and large amounts of holocellulose and alkali extract, suggesting that it has a high polysaccharide content that can be dissolved in an alkaline medium. The difference in the syringyl/guaiacyl (S/G) ratios of the samples before and after alkali extraction suggests that alkali extracts contain syringyl-type polyphenols. In all of the samples, the heartwood methanol extracts were larger in volume than the sapwood methanol extracts, and the sapwood alkali extracts were larger in volume than the heartwood alkali extracts. The antioxidant activities of the methanol and alkali extracts were assayed by measuring the levels of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity and super oxide dismutase (SOD)-like activity, respectively. The heartwood methanol extract of jatobá (*Hymenaea courbaril*) exhibited the highest level of activity (EC_{50} = 44 mg/l), which exceeded that of α-tocopherol (EC_{50} = 48 mg/l), and the heartwood alkali extracts of jatobá and ipê amarelo had high SOD-like activity comparable with red wine.

Key words Antioxidant activity · Amazonian wood · Extracts · Radical scavenging · SOD-like activity

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Introduction

The burning of forested areas is a quick and easy method of clearing land for cultivation, and enormous patches of Amazonian forest have been lost in recent decades through conversion to mainly soybean fields, cotton plantations, and pastures. In fact, between 2003 and 2004, 27.4 × 10^3 km^2 of forest was destroyed by burning in the Brazilian Amazon.[1] Further loss must be prevented to avoid global weather extremes, as well as threats to regional biodiversity and genetic resources. The fundamental cause of deforestation is that Amazonian woods are generally believed to possess no commercial value, but many valuable woods are found in Amazonian forests. As the potential for profit from Amazonian woods becomes more widely known, it is likely that deforestation will cease and sustainable forest management will be introduced. To preserve the Amazon Forest, we must find ways to use its valuable woods and give administrative guidance for reforestation efforts.

Several studies have shown the bioactivity of tropical woods. For example, Mihara et al.[2] reported that extracts of *Acacia mangium* and *Acacia auriculiformis* had high levels of antifungal activity, antioxidant activity, and laccase inhibitory activity. Similarly, Shimizu et al.[3,4] showed that the leaves of *Artocarpus altilis* possessed potent 5α-reductase inhibitory activity and that the extract of an *Amoora* species had strong antioxidant activity. Extracts from the bark and heartwood of *Acacia confusa* also had strong antioxidant effects, and were shown to reduce the amount of intracellular hydrogen peroxide.[5,6] Moreover, a methanol extract of the stem bark of *Lafoensia pacari* showed free-radical scavenging activity and inhibited xanthine oxidase.[7] Volatiles of *Tabebuia impetiginosa* also exhibited antioxidant activity.[8] Furthermore, Kawamura et al.[9] and Kawamura and Ohara[10] reported the antifungal activity of *Gmelina arborea* heartwood.

Currently, synthetic antioxidants such as sodium erythorbate, butylated hydroxytoluene (BHT), propyl gallate, butylated hydroxyanisole (BHA), and tertiary butylhydroquinone are used industrially to control lipid oxidation in

foods. The addition of BHA and BHT to food, however, is restricted in several countries due to undesirable effects on the enzymes of the liver and lungs.[11] As many tropical wood species are widely used as traditional medicines, it may be possible to use Amazonian woods as natural sources of antioxidants.

We analyzed several woods that are found in the Amazonian region to reveal their beneficial characteristics. The density and chemical properties of the woods were examined, and methanol and alkali extracts were prepared to measure the level of antioxidant activity in the heartwood and sapwood of each sample.

Experimental

Samples

Wood samples were collected by cutting 11 different species at Cotriguaçu in Mato Grosso State, Brazil, including angelim pedra (*Dinizia excelsa*), ipê amarelo (*Tabebuia serratifolia*), maçaranduba (*Manilkara huberi*), cedro rosa (*Cedrela odorata*), cumaru-ferro (*Dipteryx odorata*), garapa (*Apuleia molaris*), guarita (*Astronium lecointei*), jatobá (*Hymenaea courbaril*), ipê roxo (*Tabebuia heptaphylla*), marupá (*Simaruba amara*), and cachimbeiro (*Couratari oblongifolia*).

Analysis of density and chemical properties

The sample densities were determined by measuring the buoyancy of each specimen in mercury.[12] The levels of holocellulose, α-cellulose, and lignin were quantified according to standard methods.[13] In this study, acid-soluble lignin was not determined. Thioacidolysis was conducted according to Roland's method. The syringyl/guaiacyl (S/G) ratio was calculated from the yields of the respective triethylthioether derivatives.[14]

Extraction with methanol and alkali

Each species was separated into heartwood and sapwood except for marupá and cachimbeiro, which had nearly indistinguishable heartwood and sapwood. Briefly, the samples were prepared using a grinding mill (20–30 mesh) and were then subjected to Soxhlet extraction for 48 h with methanol, followed by vacuum drying. The residual wood meal (4 g) was extracted with 100 ml of 1% NaOH solution in darkness at room temperature for 1 week. The contents of the extracts were calculated from their differences before and after extraction.

Evaluation of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The level of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was measured as described by Inatani et al.[11] Briefly, each sample (0.5 ml of ethanol solution) was combined with 3 ml of 0.1 mM DPPH in ethanol, and the mixtures were shaken vigorously. After 20 min at room temperature, the absorbance of the mixtures was measured at 517 nm using a spectrophotometer (Jasco V-530; Jasco, Tokyo, Japan). Five concentrations were measured for each sample (0.01, 0.05, 0.1, 0.5, and 1.0 mg/ml). Japanese cedar (*Cryptomeria japonica*) methanol extracts and α-tocopherol were included as controls.

Super oxide dismutase-like activity

The level of super oxide dismutase (SOD)-like activity in the alkali extracts was evaluated using a SOD Assay Kit-WST (Dojindo, Kumamoto, Japan) as per Ukeda et al.[15] In short, superoxide anion, which is generated enzymatically in the assay medium, is scavenged by the sample and residual superoxide anion is detected by a probe {4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt; WST-1}.

The alkali extracts were neutralized with an ion exchange resin (Amberlite IRC50; Rohm and Haas, Saitama, Japan) that had been rinsed with distilled water a few times, and the mixture was concentrated in vacuo. Before the experiment, each sample solution was passed through a syringe filter (Minisart 0.20–0.45 μm; Sartorius, Tokyo, Japan).

Each sample solution (20 μl) was mixed with 200 μl of the working solution (xanthine + WST-1) and 20 μl of the enzyme working solution [xanthine + xanthine oxidase (XO)]. The mixture was then incubated for 20 min at 37°C, and the absorbance at 450 nm was measured using a microplate spectrophotometer (M-Spmax 250; Wako, Osaka, Japan). Three different concentrations were measured for each sample. Japanese cedar alkali extracts, commercial red wine (Carlo Rosse California Red; Gallo Japan, Tokyo, Japan), and sodium alginate were included as controls.

Total organic carbon analysis

The total organic carbon (TOC) concentration was determined using a total organic carbon analyzer (TOC-5000A; Shimadzu, Kyoto, Japan).

Results and discussion

Density and chemical properties

The densities and chemical properties of the samples are shown in Tables 1 and 2. All of the samples except cedro rosa, marupá, and cachimbeiro had densities greater than 0.7 g/cm^3. In fact, the heartwood of ipê amarelo, maçaranduba, cumaru-ferro, and guarita exhibited densities greater than 1.0 g/cm^3. In general, heartwood was denser than sapwood for all of the samples except angelim pedra.

The volume of the methanol extract from heartwood was greater than that from sapwood in all of the samples, while

the volume of the sapwood alkali extracts was greater than that of the heartwood alkali extracts. Jatobá contained a small amount of Klason lignin and α-cellulose, and a large amount of holocellulose and alkali extract, which suggests it has a large amount of polysaccharides that can be dissolved in an alkaline medium. The sapwood of angelim pedra also produced a very large amount of alkali extract. Alkali extracts are expected to contain lignin-like material, phenolic compounds, and polysaccharides. Watanabe et al.[16] reported that the vestures of vessel elements and wood fibers in *Eucalyptus* consist mainly of alkali-soluble polyphenols and polysaccharides. In our study, a large amount of starch was detected in the sapwood alkali extract from angelim pedra using the iodine coloring test.

The S/G ratios of the samples varied over a wide range, from less than 0.4 to more than 2.2 (Fig. 1). Watanabe et al.[17] reported that the syringyl-type polyphenols found in *Eucalyptus* could be removed by alkali extraction. Accordingly, we observed a reduction in the S/G ratio following alkali extraction, suggesting the presence of syringyl-type polyphenols in our extracts.

Radical scavenging activity of the methanol extracts

The level of DPPH radical scavenging activity in our samples, defined as the oxidant required for 50% consumption of the

Table 1. Density and quantity of chemical components of 11 Amazonian woods

Wood	Density (g/cm^3)	Klason lignin (%)	Holocellulose (%)	α-Cellulose (%)
Angelim-pedra				
S	0.89	22.3	71.9	51.4
H	0.79	27.1	71.3	55.4
Ipê amarelo				
S	0.96	32.0	70.7	57.8
H	1.10	28.1	69.7	59.9
Maçaranduba				
S	0.94	27.7	70.6	57.5
H	1.03	22.5	69.5	56.8
Cedro rosa				
S	0.44	20.9	70.9	56.2
H	0.46	30.0	65.8	51.6
Cumaru-ferro				
S	1.00	22.5	70.7	57.6
H	1.04	26.4	72.1	61.6
Garapa				
S	0.78	22.4	69.3	56.5
H	0.88	22.6	73.7	62.0
Guarita				
S	0.93	24.8	70.8	50.3
H	1.00	21.8	69.1	51.9
Jatobá				
S	0.79	14.6	72.6	56.1
H	0.84	17.4	72.4	57.8
Ipê roxo				
S	0.88	21.4	72.1	60.7
H	0.89	19.5	69.3	59.0
Marupá	0.37	28.4	69.9	54.5
Cachimbeiro	0.59	28.8	71.2	53.6

S, Sapwood; H, heartwood

Table 2. Extractives of 11 Amazonian woods

Wood	Methanol extracts (%)	Alkali extracts (%)
Angelim-pedra		
S	2.7	23.2
H	13.3	6.4
Ipê amarelo		
S	2.5	5.1
H	15.8	4.3
Maçaranduba		
S	9.2	5.7
H	11.6	5.9
Cedro rosa		
S	7.2	6.1
H	8.8	5.8
Cumaru-ferro		
S	3.6	13.0
H	7.5	3.5
Garapa		
S	3.2	10.3
H	13.7	7.5
Guarita		
S	1.8	6.4
H	8.4	7.2
Jatobá		
S	4.3	17.4
H	17.7	13.8
Ipê roxo		
S	4.5	9.9
H	10.0	7.1
Marupá	3.8	8.2
Cachimbeiro	4.1	4.7

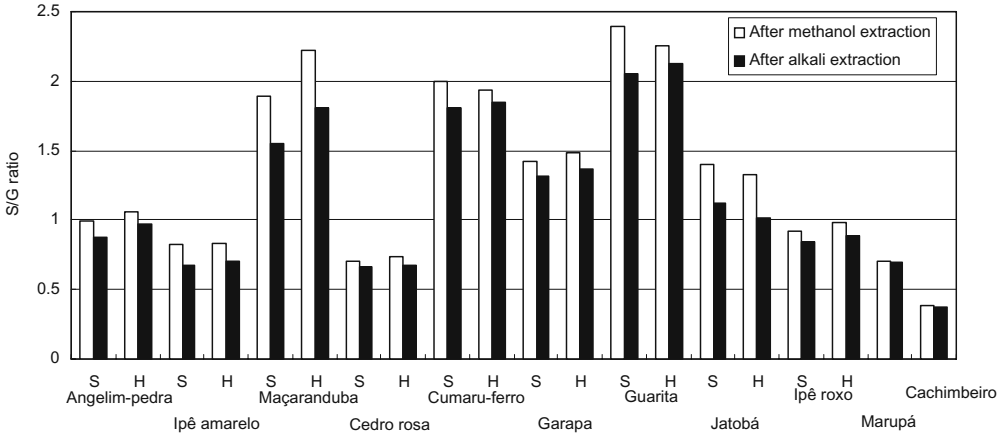

Fig. 1. Syringyl/guaiacyl (*S/G*) ratio of lignins from Amazonian woods. *H*, Heartwood; *S*, sapwood

Table 3. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the methanol extracts from the Amazonian woods, Japanese cedar, and α-tocopherol

Wood	EC_{50} (mg/l)	
	Sapwood	Heartwood
Angelim-pedra	>1000	430
Ipê amarelo	424	278
Maçaranduba	182	170
Cedro rosa	281	233
Cumaru-ferro	>1000	91
Garapa	349	219
Guarita	66	50
Jatobá	220	44
Ipê roxo	606	425
Marupá	>1000[a]	
Cachimbeiro	>1000[a]	
Japanese cedar	671	194
α-Tocopherol	48	

EC_{50}, Oxidant concentration required for 50% consumption of the initial DPPH radical concentration
[a] Heartwood and sapwood nearly indistinguishable

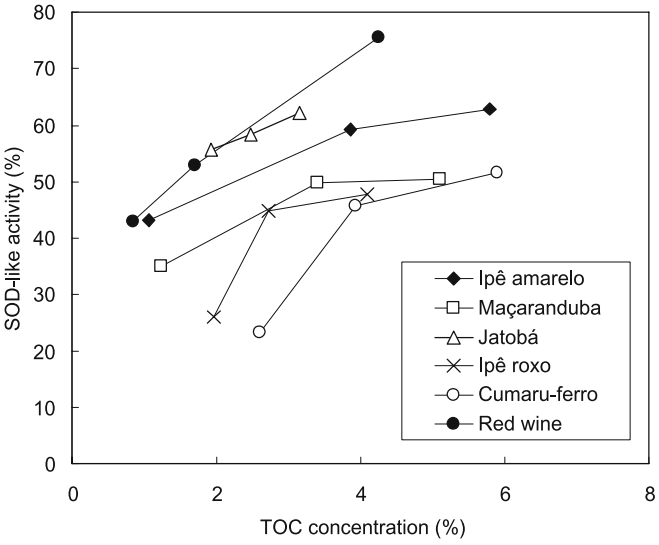

Fig. 2. Super oxide dismutase (*SOD*)-like activities of alkali extracts from heartwood of Amazonian woods and red wine. *TOC*, Total organic content

initial DPPH radical concentration (EC_{50}), is summarized in Table 3. The methanol extracts from three of the Amazonian woods, jatobá (heartwood), guarita (heartwood and sapwood), and cumaru (heartwood), exhibited high levels of activity that were similar to that in α-tocopherol, a well-known antioxidant. The EC_{50} values for jatobá (heartwood), guarita (heartwood), guarita (sapwood), cumaru (heartwood), and α-tocopherol were 44, 50, 66, 91, and 48 mg/l, respectively.

Kukić et al.[18] reported a high correlation between the level of DPPH radical scavenging activity and the total phenolic content of methanol extracts from the aerial flowering parts of *Stachys* plants. In our samples, the heartwood methanol extracts showed higher levels of activity than the sapwood methanol extracts. Generally, more phenolic compounds were present in the methanol extracts of heartwood than those of sapwood, which may indicate a high level of radical scavenging activity. The heartwood of Japanese cedar, which is known to contain norlignans,[19–21] also exhibited radical scavenging activity.

We are currently searching for compounds with high levels of radical scavenging activity in the methanol extracts of jatobá, guarita, and cumaru.

SOD-like activity of the alkali extracts

Research on the bioactivities of tropical wood has focused on extracts made with organic solvents, and few studies have investigated the antioxidant activity of alkali extracts. In this study, we estimated the antioxidant activity present in alkali extracts of Amazonian woods by measuring the level of SOD-like activity (i.e., superoxide anion scavenging activity).

All of the alkali extracts from sapwood showed low levels of activity (14%–42% at 5% TOC concentration). In contrast, five extracts from heartwood exhibited high levels of SOD-like activity. In particular, the activity present in jatobá and ipê amarelo heartwood was equal to that in red wine (Fig. 2).

Sodium alginate was included as a control because the sapwood extracts were expected to contain water-soluble polysaccharides. Our results showed sodium alginate had no activity. Therefore, additional studies are needed to clarify the characteristics of alkali extracts of Amazonian woods.

There are some literature reports on the use of the SOD Assay Kit-WST.[22–24] However, in the system investigated in the present study, there is a possibility that the alkali extract exhibits the xanthine oxidase activity and leads to reduction of superoxide anion and overestimation of the SOD-like activity. Further detailed research of the SOD-like activity is required.

Conclusions

To prevent further losses of the Amazon Forest by burning, we must identify the commercial value of Amazonian wood. We investigated the characteristics of 11 Amazonian woods. Seven specimens had a density greater than 0.7 g/cm^3, and the heartwood of ipê amarelo, maçaranduba, cumaru-ferro, and guarita had densities that exceeded 1.0 g/cm^3. The quantity of the heartwood methanol extracts was greater than that of the sapwood extracts for all samples, while the yield of the alkali extracts was higher in sapwood than in heartwood. The difference in the S/G ratios before and after alkali extraction implies that syringyl-type polyphenols were present in the alkali extracts. In addition, jatobá had a small amount of Klason lignin and α-cellulose, a large amount of holocellulose, and a large alkali extract volume. This suggests that appreciable quantities of polysaccharides were present in the alkali extracts.

The antioxidant character of the methanol and alkali extracts was examined by testing for DPPH radical scaveng-

ing activity and SOD-like activity, respectively. The level of activity exhibited by the methanol extracts of jatobá heartwood (EC_{50} = 44 mg/l) was higher than that of α-tocopherol (EC_{50} = 48 mg/l). While the antioxidant activities of organic solvent extracts from plants have been reported in various disciplines, investigations of the antioxidant activity of alkali extracts from wood are rare. Our results indicate that the alkali extracts of jatobá heartwood and ipê amarelo heartwood have high SOD-like activity comparable with red wine.

Amazonian woods may one day be used as antioxidizing agents for foods and other industrial products. Indeed, both the methanol and alkali extracts of jatobá exhibited high levels of antioxidant activity, a factor that supports its potential as a commercially valuable wood. We are currently searching for compounds with high levels of radical scavenging activity in the methanol extracts of jatobá, guarita, and cumaru.

Acknowledgments The authors thank Mr. Artemio Richter (entrepreneur in Cotriguaçu in Mato Grosso State, Brazil), Prof. Norman Barros Logsdon, Prof. Adenauer Tarquínio Daltro and Prof. José Eduardo Penna (Federal University of Mato Grosso, Brazil) for supporting our sampling in the Amazon, and Prof. Keigo Aoi (Nagoya University, Japan) for advice on TOC analysis. This research was conducted with the support of Grants-in-Aid for Scientific Research (15255016 and 18208016) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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