

Identification of phenolic compounds and their relationship to the natural resistance of wood from *Dipteryx polyphylla* Huber and *Acacia mangium* Willd

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Abstract

Flavonoids are the phenolic compounds that are predominant in the Fabaceae family, and isoflavonoids are especially recognized for their contribution to the natural resistance of wood from species of this family. Herein, we investigated the phenolic compounds from extracts of wood residues from the Fabaceae species *Dipteryx polyphylla* Huber and *Acacia mangium* Willd. A phytochemical study of *D. polyphylla* led to isolation and identification of isoflavans such as 3',7-dihydroxy-4'-methoxy-isoflavan (**1**), 2',8-dihydroxy-4',7-dimethoxy-isoflavan (**2**), 2',7-dihydroxy-4'-methoxyisoflavan (**3**) and 3',8-dihydroxy-4',7-dimethoxy isoflavan (**4**). Compounds **1** and **4** are new findings. *A. mangium* gave monocyclic phenolics, such as ferulic acid (**6**), methylparaben (**7**) and 4-hydroxybenzaldehyde (**8**); flavonol melatoxetin (**9**) as well as fatty acid esters of spinasterol (**5**). The phenolic compounds that were identified contribute to the knowledge regarding the natural resistance of its woods, thus aggregating value for solid residues and plantation species recommended for reforestation

Keywords: Isoflavonoids; monocyclic phenolics; Fabaceae; spectroscopic techniques.

1. Introduction

Phenolic compounds constitute a broad group of aromatic compounds that range from simple phenols to highly polymerized compounds that are of interest to researchers due to their extensive natural occurrence in plants, the variety in chemical structures and potential biological properties. According to their chemical structure, phenolic compounds are classified into phenolic acids (hydroxybenzoic and hydroxycinnamic acids), flavonoids, stilbenes, lignans, coumarins, and tannins, among other subgroups.

Flavonoids that are widely found in plant vascular tissues perform several functions, such as protection against ultraviolet B radiation, as constitutive antifungal agents (phytoalexins), and they also

play a role in plant-herbivory interactions (Harbone and Willians, 2000). These compounds are predominant in the Fabaceae (Leguminosae) family, and are distributed in its three subfamilies (Faboideae = Papilionoideae), Caesalpinioideae and Mimosoideae. Isoflavonoids are a distinctive subclass of flavonoids with a characteristic structure in which a phenyl group is attached to C-3 of the pyrone ring (skeleton II) instead of C-2, as in other flavonoids (skeleton I), and these are restricted almost entirely to Papilionoideae, the largest of its three subfamilies (Veitch, 2013). There are many reports about the important role of isoflavonoids as precursors for the development of phytoalexins during plant microbe interactions (Harbone and Willians, 2000).

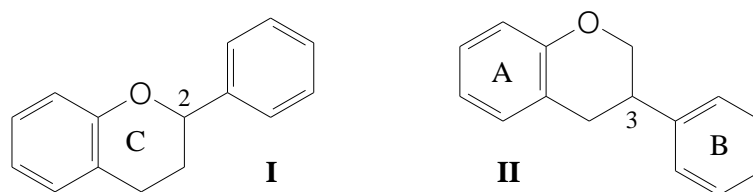


Figure 1. Basic skeletons of flavonoids and isoflavonoids

Natural resistance to decay is one of the most important properties of wood, and this is affected by wood density and the content and composition of extractives. Schultz and Nicholas (2000) proposed that extractives can protect the wood against colonization and subsequent degradation by a double mechanism, in other words, the extracts have some type of fungicidal activity and are also free radical scavengers (antioxidants). Studies have evidenced that flavonoids from Fabaceae present an important effect on the natural durability of its wood (Reves-Chilpa *et al.*, 1998; Barry *et al.*, 2005; Morimoto *et al.*, 2006; Pizzo *et al.*, 2011; Martínez-Sotres *et al.*, 2012). The correlation between density and extractive content of wood with the biological resistance of Amazonian woods was verified by Costa *et al.* (2019) and Abreu and Silva (2000), and including some species of Fabaceae.

The Fabaceae species, *Dipteryx polyphylla* (Papilionoideae subfamily) and *Acacia mangium* (Mimosoideae subfamily), occur in the Amazon region. *Dipteryx polyphylla* Huber [synonym *Coumarouna polyphylla* (Huber) Ducke] (Tropicos, 2020) is popularly known in the Amazon region as *cumarurana*. Its wood has a high density (0.74 g/cm³) (Castro *et al.*, 2015), and the tests of susceptibility to xylophagous organisms carried out with this species has evidenced its natural resistance (Jesus *et al.*, 1998), however, there are no reports on its secondary metabolites. *Acacia mangium* Willd. [syn. *Racosperma mangium* (Willd.) Pedley] (Tropicos, 2020) originates from Australia and Malaysia, and has easily adapted to the tropical Brazilian soil and climatic conditions (Luz *et al.*, 2010). The commercial plantations of this species in Brazil extract tannins obtained from bark, which are used in the tannery industries, and the wood is used by the cellulose industry, as well as for generating energy and the production of wood panels (Attias *et al.*, 2013). The literature reports the potential of *A. mangium* for reforestation and recovery programs of areas with poor or degraded soils (Attias *et al.*, 2013; Schiavo and Martins, 2003; Tulod *et al.*, 2017). The wood of a specimen from the Amazon showed a basic density of 0.58 g/cm³ (Barros *et al.* 2012) and studies report the existence of three flavonoids (Barry *et al.*, 2005; Pietarine *et al.*, 2005).

In this study, we investigated the secondary metabolites from extractives of wood residues obtained from *Dipteryx polyphylla* and *Acacia mangium* and we discussed their relationship with the natural resistance of their wood.

2. Materials and Methods

2.1 General

Nuclear Magnetic Resonance (NMR) spectra were measured using spectrometers (Bruker Fourier-300, DRX 400 and Avance III, 14.1 Tesla/600 MHz) with tetramethylsilane (TMS) as the internal standard. LC-HRMS measurements were obtained using mass spectrometer (MicroTOF-QII, Bruker Daltonics) connected to a chromatograph (Prominence UFLC, Shimadzu). Mass spectra were acquired using an ion trap spectrometer (LCQ Fleet™, Thermo Scientific) equipped with an electrospray source, operating in positive mode. Melting points were determined on a melting-point apparatus (Fisatom 430D). Chromatographic fractionations by medium pressure liquid chromatography were performed on Sepacore® Buchi. Column chromatography was performed with silica gel 60 (Merck), microcrystalline cellulose (Merck), Sephadex LH-20 (Sigma) and Amberlite XAD-2 (Supelco). Analytical TLC was performed with silica gel 60 F254 (0.25 mm) pre-coated alumina sheets (Merck), and visualized using UV light (254 and 365 nm), vanillin-sulphuric acid and NP/PEG reagent spray.

2.2 Wood Residues and Extraction

Samples of wood residues from *Dipteryx polyphylla* were obtained from the *Estação Experimental de Silvicultura Tropical* (53 km north of the city of Manaus, Amazonas State), at the Instituto Nacional de Pesquisas da Amazônia (INPA) and *Acacia mangium* was obtained from a plantation located in the city of Iranduba (29 Km from the city of Manaus). The identification of the wood samples was done through macroscopic comparisons with standard samples from the xylotheque at INPA. The largest residues were previously evaluated for their technological properties, the smallest resulting from these procedures became available for phytochemical studies. Extraction was performed by macerating the samples with hexane followed by methanol at room temperature.

2.3 Chromatographic Fractionation of *Dipteryx polyphylla* Extract

The hexane extract of *D. polyphylla* showed a predominance of a triterpene known as lupeol. The methanolic extract (8.35 g) was fractionated on a silica gel chromatographic column (70-230 mesh; h X Φ = 28.5 X 4.7 cm), eluted with hexane, hex-EtOAc (8:2 and 1:1), EtOAc and EtOAc-MeOH (9:1) to yield twenty-two fractions and then fractions 5 and 7 were subjected to new chromatographic fractionations. The fractionation of fr. 5 on a silica gel column (230-400 mesh; h X Φ = 29.3 X 1.0 cm), when eluted with hex-EtOAc (8:2-100%), gave compounds **1** (3.0 mg), **2** (1.0 mg) and **3** (2.0 mg). Fr. 7 was fractionated on a silica gel column (70-230 mesh; h X Φ = 27.5 X 3.6 cm), eluted with CH₂Cl₂ and CH₂Cl₂-EtOAc (9:1-1:1), followed by a Sephadex LH-20 column eluted with MeOH to yield compound **4** (7.0 mg).

2.4 Chromatographic Fractionation of *Acacia mangium* Extract

Fractionation of the hexane extract (1.15 g) over silica gel in the column (230-400 mesh; h X Φ = 29.0 X 3.8 cm), eluted with hexane, hex-EtOAc (5-50%), EtOAc and EtOAc-MeOH (1-10%), yielded twenty-four fractions. The grouped fractions 8-24 were subjected to a new chromatographic fractionation in a silica gel

column (230-400 mesh, h X Φ = 24.5 X 2.0 cm) eluted with hex-EtOAc (5-20%) to give compound **5** (25 mg). Methanolic extract (13.0 g) fractionated over silica gel in a column (230-400 mesh; h X Φ = 24.0 X 4.7 cm), eluted with hexane, hex-EtOAc (10-50%), EtOAc and EtOAc-MeOH (10-50%), yielded twenty-nine fractions. The grouped fractions 7-11 (fr. ACM-7; 468 mg) and 14-16 (fr. ACM-14; 2.79 g) were submitted to the new chromatographic procedures.

ACM-7 was fractionated over silica gel in the column (230-400 mesh; h X Φ = 29.0 X = 2.8 cm), eluted with hexane, hex-EtOAc (3-50%) and EtOAc, and provided twenty-nine subfractions, compounds were purified after the following chromatographic procedures: compound **6** (3 mg) was purified from subfractions 13-17 by medium pressure liquid chromatograph over silica gel (230-400 mesh) eluted with hex-EtOAc (98:2-95:5); a mixture (20 mg) of β -sitosterol and stigmaterol was obtained from subfr. 19; compounds **7** (1.0 mg) and **8** (3.0 mg) were obtained from subfr. 20 and 21, respectively, using a microcrystalline cellulose column eluted with hexane and hex-EtOAc (9:1). ACM-14 (3.7 g) over Amberlite XAD-2 in a column (h X Φ = 25.0 X 3.6 cm), eluted with H₂O, MeOH-H₂O (1:1), MeOH and MeOH-EtOAc (1:1) gave compound **9** (3.0 mg).

2.5 Spectroscopic Data of Phenolic Compounds

3',7-Dihydroxy-4'-methoxy-isoflavan (**1**). Yellow solid, mp 87-89°C. HRMS m/z 273.1133 [M+H]⁺. ¹H NMR (600 MHz, Acetone-d₆, J/Hz) and ¹³C NMR (150 MHz, Acetone-d₆): Table 1. HMBC (Acetone-d₆): H-2 → C-3, 4, 9, 1'; H-3 → C-2, 4, 1', 2', 6'; H-4 → C-2, 3, 5, 9, 10, 1'; H-5 → C-4, 9; H-6 → C-7, 8, 10; H-8 → C-6, 8, 10; H-2' → C-3, 4', 6'; H-5' → C-1', 3'; OMe → C-4.

2',8-Dihydroxy-4',7-dimethoxy-isoflavan (**2**). White solid, mp 105°C. ¹H NMR (600 MHz, Acetone-d₆, J/Hz): 7.06 (d, J = 8.5, H-6'), 6.66 (d, J = 8.3, H-6), 6.51 (d, J = 2.5, H-3'), 6.42 (dd, J = 8.5, 2.5, H-5'), 6.40 (d, J = 8.3, H-5), 4.35 (ddd, J = 10.1, 3.5, 2.1, H-2eq), 4.03 (t, J = 10.1, H-2ax), 3.76 (s, 7-OCH₃), 3.72 (s, 4'-OCH₃), 3.49 (m, H-3), 2.99 (ddd, J = 15.5, 11.0, 0.7, H-4ax.), 2.81 (ddd, J = 15.5, 5.2, 2.1, H-4eq), ¹³C NMR (150 MHz, Acetone-d₆): 159.5 (C-4'), 156.1 (C-2'), 148.6 (C-8), 147.6 (C-9), 135.7 (C-7), 127.5 (C-6'), 123.6 (C-6), 119.8 (C-1'), 114.7 (C-10), 107.2 (C-5), 104.6 (C-5'), 101.3 (C-3'), 69.3 (C-2), 59.4 (7-OCH₃), 54.2 (4'-OCH₃), 31.5 (C-3), 30.3 (C-4). HMBC (Acetone-d₆): Text.

2',7-Dihydroxy-4'-methoxy-isoflavan (vestitol) (**3**). Yellow solid, mp 157-158°C. ¹H NMR (600 MHz, Acetone-d₆, J/Hz): 7.05 (d, J = 8.5, H-6'), 6.89 (d, J = 8.3, H-5), 6.50 (d, J = 2.5, H-3'), 6.42 (dd, J = 8.5, 2.5, H-5'), 6.36 (dd, J = 8.3, 2.5, H-6), 6.27 (d, J = 2.5, H-8), 4.23 (ddd, J = 10.2, 3.5, 2.1, H-2eq), 3.98 (t, J = 10.2, H-2ax), 3.72 (4'-OCH₃), 3.47 (m, H-3), 2.96 (dd, J = 15.5, 11.0, H-4ax), 2.80 (ddd, J = 15.5, 5.1, 2.1, H-4eq). ¹³C NMR (150 MHz, Acetone-d₆): 160.5 (C-4'), 157.6 (C-7), 156.8 (C-2'), 156.2 (C-9), 131.1 (C-5), 121.0 (C-1'), 114.3 (C-10), 108.8 (C-6), 105.7 (C-5'), 103.7 128.8 (C-6'), (C-8), 102.5 (C-3'), 70.6 (C-2), 55.4 (4'-OCH₃), 32.75 (C-3), 31.1 (C-4). HMBC (Acetone-d₆): text.

3',8-Dihydroxy-4',7-dimethoxy-isoflavan (**4**). Yellow solid, mp 143°C. HRMS m/z 303.1247 [M+H]⁺. ¹H NMR (400 MHz, Acetone-d₆, J/Hz) and ¹³C NMR (100 MHz, Acetone-d₆): see Table 1. HMBC (Acetone-d₆): H-2 → C-3, 4, 9, 1'; H-3 → C-2, 4, 10, 1', 2', 6'; H-4 → C-2, 3, 6, 9, 10, 1'; H-5 → C-7, 10; H-6 → C-

5, 7, 8; H-2' → C-3, 4', 6'; H- → C-1', 3', 6'; H-6' → C-3, 2', 4'; OMe → C-7; OMe → C-4'.

Ferulic acid (**6**). Amorphous solid. ^1H NMR (400 MHz, CDCl_3 , J/Hz): 7.61 (d, $J = 15.9$, H-7), 7.08 (dd, $J = 8.2$ and 1.9 , H-6), 7.03 (d, $J = 1.9$, H-2), 6.91 (d, $J = 8.2$, H-5), 6.29 (d, $J = 15.9$, H-8), 5.82 (s, OH), 3.93 (s, OMe). ^{13}C NMR (estimated by HMBC and HSQC): 166.7 (C-9), 146.2 (C-3), 146.1 (C-4), 143.9 (C-7), 126.5 (C-1), 122.4 (C-6), 115.1 (C-8), 114.0 (C-5), 108.7 (C-2), 55.43 (OMe-3).

Methylparaben (**7**). Amorphous solid. HRMS m/z 153.0548 $[\text{M}+\text{H}]^+$. ^1H NMR (400 MHz, CDCl_3 , J/Hz): 7.94 (d, $J = 8.8$, H-2 and H-6), 6.84 (d, $J = 8.8$, H-3 and H-5), 3.87 (s, OCH_3). ^{13}C NMR (100 MHz, CDCl_3): 166.7 (C-1'), 159.5 (C-4), 131.7 (C-2 and C-6), 122.8 (C-1), 115.0 (C-3 and C-5), 51.7 (OCH_3).

4-Hydroxybenzaldehyde (**8**). Amorphous, yellow solid, strong sweetish flavor HRMS m/z 123,0450 $[\text{M}+\text{H}]^+$. ^1H NMR (400 MHz, CDCl_3 , J/Hz): 7.79 (d, $J = 8.8$, H-2 and H-6), 6.93 (d, $J = 8.8$, H-3 and H-5), 9.86 (s, CHO).

Melatoxetin (**9**). Crystalline, yellow solid, mp 279-280°C. HRMS m/z 285.0383 $[\text{M}+\text{H}]^+$. ^1H NMR (600 MHz, MeOD, J/Hz): 7.59 ($J = 8.8$, H-5), 6.99 (d, $J = 8.8$, H-6), 8.29 (d, $J = 8.9$, H-2' and H-6'), 6.97 (d, $J = 8.9$, H-3' and H-5'). ^{13}C NMR (150 MHz, MeOD): 174.9 (C-4), 160.5 (C-4'), 151.6 (C-7), 147.8 (C-9), 147.7 (C-2), 138.25 (C-3), 134.2 (C-8), 131.0 (C-2' and C-6'), 124.3 (C-1'), 116.6 (C-5), 116.3 (C-10, C-3' and C-5'), 115.1 (C-6), HMBC (MeOD): text.

3. Results and Discussion

3.1 Identification of Compounds 1-4 from *Dipteryx polyphylla*

The compounds **1-4**, which were isolated from the methanolic extract (Figure 1), were analyzed using ^1H , and ^{13}C NMR spectra (Table 1), which showed shifts that are indicative of isoflavans due to characteristic signals of a heterocyclic ring between δ 4.36-4.20 (ddd, H-2eq), 4.04-3.96 (t, H-2ax), 3.49-3.03 (m, H-3), 2.99-2.87 (dd, H-4ax) and 2.90-2.80 (ddd, H-4eq). The HSQC experiments showed the correlations of these hydrogens with C-2 (δ 71.5-69.3), C-3 (38.8-31.5) and C-4 (32.80-30.3), respectively. The oxygenation pattern of the A and B rings was based on the multiplicities, the coupling constants and the correlations observed in the HMBC.

The ^1H NMR spectra of compounds **1** and **3** showed the same oxygenation pattern of ring A. The nuclear overhauser effect spectroscopy (NOESY) of **1** presented a spatial interaction of the methoxyl hydrogens and the aromatic hydrogen H-5' (δ 6.91), which confirmed the position of the methoxyl of ring B at C-4'. These spectral data, combined with those of ^{13}C NMR, allowed us to identify compound **1** as a new 7,3'-dihydroxy-4'-methoxy-isoflavan and **3** as a known vestitol (8,2'-dihydroxy-7,4'-dimethoxy-isoflavan) for which the ^1H and ^{13}C NMR data were identical to those in the literature (Zhao *et al.*, 2011). The ^1H NMR spectra of **2** and **4** showed similarity in the signals attributed to ring A. NOESY of **4** indicated spatial proximity between the methoxy hydrogens (δ 3.77) and the aromatic hydrogen (δ 6.66) of ring A and δ 3.83

with 6.91 (ring B) that confirm the methoxyl groups at C-7 and C-4. Thus, compound **2** was identified as 2',8-dihydroxy-4',7-dimethoxy-isoflavan and **4** compound as 8,3'-dihydroxy-7,4'-dimethoxy-isoflavan, for which there are no previous reports. Thus, isoflavans **1** and **4** are new findings. Compounds **2** and **3** have been previously identified from the Papilionoideae subfamily, but are reported for the first time from *Dipteryx*. Vestitol (**3**) has been found this subfamily with clear evidence of its action as a phytoalexin (Masai *et al.*, 2013), and it is also indicated as a free radical scavenger (Kaducová, *et al.*, 2019).

3.2 Identification of Compounds 5-9 from *Acacia mangium*

Chromatographic fractionation of the hexane extract from *A. mangium* gave **5** and the methanolic extract yielded compounds **6-9** (Figure 1). The ^1H and ^{13}C NMR data of **5** showed signs of olefins, which is compatible for spinasterol (Ragasa & Lim, 2005). The presence of the signal at δ 173.5 in the ^{13}C NMR spectrum and the correlation of hydrogen at δ 4.70 with the carbinolic at δ 73.2 (HSQC) suggested esterification in C-3, thus identifying them as fatty acid esters of spinasterol, which has not been previously reported from Fabaceae.

The ^1H NMR spectrum of **6** showed signals referring to three aromatic hydrogens, two olefinic hydrogens, methoxyl and hydroxyl groups. These data associated with those analyzed by HMBC and HSQC permits the identification of **6** as ferulic acid. The ^1H NMR spectra of **7** and **8** showed an aromatic region indicative of a type AA'BB' system, as well as a methoxy group at δ 3.87 for **7** and aldehyde hydrogen at δ 9.86 for **8**, thus identifying these benzoic acid derivatives as methyl *p*-hydroxybenzoate (methylparaben) and 4-hydroxybenzaldehyde, respectively. The ^1H and ^{13}C NMR data for **9** were similar to those published by Ponce *et al.* (2009) for melatoxetin. The HMBC experiment confirmed the oxygenation pattern of the aromatic rings.

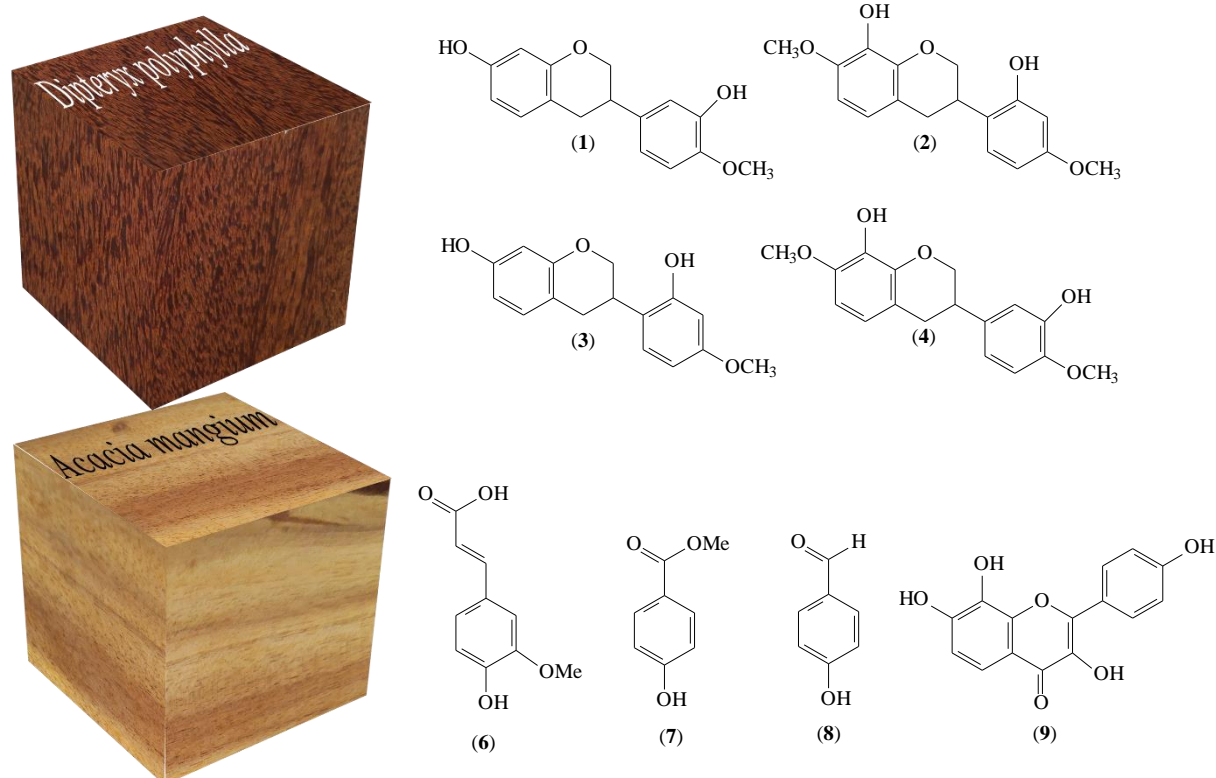


Figure 1. Phenolic compounds from *D. polyphylla* and *A. mangium*Table 1. ¹H and ¹³C NMR data for isoflavans **1** and **4** (δ ppm, J/Hz) in Acetone-d₆)

Position	δ H (1)	δ H (4)	δ C (1)	δ C (4)
2	3.94 t (10.5, H-ax.) 4.20 ddd (10.5, 3.6, 2.0 H-eq)	3.98 t (10.4, H-ax.) 4.31 ddd (10.4, 3.6, 1.8 H-eq)	70.6	70.5
3	3.04 m	3.10 m	38.1	37.8
4	2.89 m (H-ax) 2.87 m (H-eq)	2.93 m (H-ax) 2.90 m (H-eq)	31.7	31.8
5	6.90 d (8.2)	6.66 d (8.3)	130.1	124.8
6	6.36 dd (8.2, 2.5)	6.40 d (8.3)	107.9	108.8
7			156.7	136.6
8	6.28 d (2.5)		102.7	149.6
9			155.0	148.7
10			113.0	114.3
1'			134.8	134.6
2'	6.81 d (2.2)	6.82 d (2.2)	114.2	114.1
3'			146.7	147.6
4'			146.4	147.3
5'	6.91 d (8.2)	6.91 d (8.2)	111.8	111.7
6'	6.75 dd (8.2, 2.2)	6.77 dd (8.2, 2.2)	118.2	118.2
7-OMe		3.77 s		59.6
4'-OMe	3.83 s	3.83 s	55.4	55.3

3.3 Natural durability of wood from *D. polyphylla* and *A. mangium*

The presence of isoflavonoids from *D. polyphylla*, allied with the extractive content (secondary metabolites), and which was significantly higher than in the extract of *A. mangium* (Table 2), contribute to the greater biological resistance of the wood of this species, since these are recognized to be the most important factors in determining the natural durability of wood.

The basic density is a property of wood that also contributes to its natural resistance. In fact, previous studies on Amazonian species showed that *D. polyphylla* wood (0.74 g/cm³) (Castro *et al.*, 2015) has a higher density than *A. mangium* (0.58 g/cm³) (Barros *et al.*, 2012).

Table 2. Extractive content of wood residues

Species	Extractive content (%)	
	Hexane extract	Methanolic extract
<i>D. polyphylla</i>	0.35	3.26
<i>A. mangium</i>	0.14	1.51

4. Conclusion

In this study, the phenolic compounds identified are probably important as defense mechanisms of the two species evaluated. Isoflavonoids from *D. polyphylla* (Papilionoideae) must act as phytoalexins, which are well known for existing in this subfamily, while *A. mangium* (Mimosoideae) gives other phenolics that are also important for the defense of the timber species. Thus, this phytochemical study adds to knowledge in relation to the natural resistance of its woods and increases the value of woody residues and plantation species indicated for reforestation.

5. Acknowledgements

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