Chemical investigation and anatomical aspects of wood residues from *Hymenaea courbaril* L, *Platymiscium ulei* Harms, *Hymenolobium petraeum* Ducke

Jennifer Araújo de Oliveira Lima[1], Willian Hayasida[1], Mauro Galucio Garcia[1], Jorge Alves de Freitas[2], Claudete Catanhede do Nascimento[2], Luiz Henrique Keng Queiroz Junior[3], Maria da Paz Lima[1]*

[1]Laboratório de Química de Produtos Naturais, Instituto Nacional de Pesquisas da Amazônia, 69011-970, Manaus, AM, Brazil

[2]Laboratório de Tecnologia da Madeira, Instituto Nacional de Pesquisas da Amazônia, 69011-970, Manaus, AM, Brazil

[3]Instituto de Química, Universidade Federal de Goiás, 74690-900 Goiânia, GO, Brazil

Abstract

*In recent years, the interest in the propagation of native forest species has intensified with the aim of recovering degraded areas and the restoration of the landscape. As such, some species of Fabaceae are considered promising woody species for planting in reforestation programs and agroforestry systems. In the present paper, the authors complement the need for studies related to secondary metabolites of the woody species Hymenaea courbaril L. Platymiscium ulei Harms and Hymenolobium petraeum Ducke. Thus, the phytochemical study of H. courbaril led to the isolation and identification of diterpenes eperuic acid (**1**) and methyl eperuate (**2**); triterpenes oleanolic acid (**3**) and hederagenin (**8**); flavonoides quercetin (**4**), fisetinediol (**5**), liquiritigenin (**6**) and 3-methoxy-5,7,3',5'-tetrahydroxyflavanonol (**7**). P. ulei gave pterocarpane homopterocarpin (**9**) and coumarin scoparone (**10**); H. petraeum gave isoflavan sativan (**11**), in addition to the amino acid tyrosine (**12**). Flavonoids were the predominant constituents in the three species of Fabaceae; however, the presence of isoflavonoids in Platymiscium ulei and Hymenolobium petraeum is probably associated with the resistance of this high density wood to pathogenic fungi.*

Keywords: Fabaceae; diterpenes; triterpenes; flavonoids.

1. Introduction

The Fabaceae family is divided into the subfamilies Faboideae, Mimosoideae, and Caesalpinioideae. The genus *Hymenaea* belongs to the subfamily Caesalpinioideae. *Hymenaea courbaril* L, known as "jatobá" in Brazil, is a plant species whose seed pods are mostly sold in northern Brazil for medicinal purposes. Chemical studies of the various vegetative parts have identified flavonoids, coumarins, procyanidin and terpenoids (sesquiterpenes and diterpenes) (Boniface et al., 2017). The woody species *Platymiscium ulei*

Harms, which belongs to the Papilonoideae subfamily, and is known in Brazil as “macacauba”, occurs throughout the Amazon region, has high economic value in the furniture making sector and lacks studies regarding its chemical constituents. *Hymenolobium petraeum* Ducke is a tree that belongs to the subfamily Papilonoideae. It is popularly known in Brazil as “angelim-pedra” and possesses high commercial value due to demand in the construction and carpentry industries. *Hymenaea courbaril* (Tonini et al., 2005) and *Platymiscium ulei* (Miranda et al., 2013) are promising woody species for planting in reforestation programs and agroforestry systems. In recent years, the interest in the propagation of native forest species has intensified with the aim of recovering degraded areas and the restoration of the landscape. Despite the importance of knowledge about the secondary metabolism from forest species, there is a lack of scientific studies of their wood.

The studies carried out using wood residues are an opportunity to obtain knowledge on the secondary metabolism of woody species of the Fabaceae family, and studies with three species of the Papilionoideae subfamily showed that the isoflavonoids (phenyl group is attached to C-3 of the pyrone ring) identified probably act as phytoalexins in response to pathogenic organism and that this is related to the natural resistance of the species to xylophagous fungi (Garcia et al., 2018; 2021). In studies of species of Mimosoideae, other flavonoids (phenyl group is attached to C-2 of the pyrone ring) were identified (Garcia et al., 2021; Melo et al., 2020).

In the present paper, the authors complement the need for studies related to secondary metabolites of wood residues with the study of *Hymenaea courbaril* L. (Caesalpinoideae), *Platymiscium ulei* Harms and *Hymenolobium petraeum* Ducke (Papilonoideae).

2. Materials and Methods

2.1. General

NMR spectra were measured using spectrometers (Bruker Fourier-300, DRX- 400). The chemical shifts (δ) were expressed in ppm, and coupling constants (*J*) in Hertz; TMS was used as the internal standard. Solid-state ^{13}C NMR spectra were acquired using a Bruker Avance III 400 spectrometer, operating at 400.15 MHz for ^{1}H and 100.62 MHz for ^{13}C, equipped with a CP/MAS (Cross-Polarization/Magic Angle Spinning) 4 mm probe. Column chromatography (CC) was performed with silica gel 60 (Merck 70-230 and 230-400 mesh), Sephadex LH-20 (Sigma) and microcrystalline cellulose (Merck). Analytical TLC was performed with silica gel 60 F_{254} (0.25 mm) pre-coated alumina sheets (Merck) that were visualized using UV light (254 and 365 nm), vanillin-sulphuric acid and NP/PEG reagent spray.

2.2. Obtaining wood residues, identification and basic density

Samples of wood residues were obtained from the National Institute for Amazonian Research’s (INPA) Tropical Forestry Experimental Station (53 km north of the city of Manaus, Amazonas State, Brazil) and these were subjected to analyses of their technological properties by the Wood Technology Laboratory at INPA. The leftovers from these studies were used in the phytochemical studies.

The identification of the wood samples of *Hymenolobium petraeum and Platymiscium ulei* was done through macroscopic comparisons with standard samples from the xylotheque. The species *Hymenaea courbaril* was identified via macroscopic analysis based on sensory and macroscopic anatomical characteristics following the guidelines in the technical standards (Coradin and Muniz, 1992). The image of the sample was captured by using a Stereoscopic Zoom Microscope Nikon SMZ745T/Camera Moticam 2300 AXIOMAT Carl Zeiss. The determination of the basic density (ratio between the oven-dried wood sample mass and volume of the saturated state) was conducted using 2 x 2 x 3 cm wood blocks, in quadruplicate.

2.3. Preparation of organic extracts

Extractions of *Hymenaea courbaril* and *Hymenolobium petraeum* were performed by macerating the samples with hexane followed by methanol for a period of 7 days for each solvent, at room temperature. To obtain extracts of *Platymiscium ulei,* the macerations were carried out in hexane, dichloromethane and methanol under the same conditions.

2.4. Chromatographic fractionation of extracts from Hymenaea courbaril

The hexane extract (830 mg) was fractionated over silica gel in the column (230-400 mesh; h X Φ = 41.1 X 2.8 cm), eluted with hexane, hex-EtOAc (10-60%), to yield fifty six fractions. The grouped fractions 43-56 (333 mg) were subjected to a Sephadex LH-20 column eluted with MeOH to yield compound **1** (9.2 mg). The methanolic extract (8.9 g) was fractionated over silica gel in a column (70-230 mesh; h X Φ =26.5 X 4.1 cm), eluted with hexane, hex-CH_2Cl_2 (20-100%), CH_2Cl_2: EtOAc (20-100%) and EtOAc:MeOH (10-30%), and yielded twenty-seven fractions. The fractions 8-10 (Fr.8, 662 mg), 11 (Fr.11, 928 mg) and 12 (Fr.12, 1 g) were subjected to complementary chromatographic procedures. Fr. 8 was subjected to a Sephadex LH-20 column eluted with methanol followed by a silica gel column (230-400 mesh; h X Φ = 46.8 X 2.8 cm) eluted with hexane, hex-EtOAc (2-49%) to give compounds **1** (40 mg), **2** (11.0 mg) and **3** (3.0 mg). Fr. 11 was subjected to a Sephadex LH-20 column eluted with methanol and provided subfractions 6 and 11. The compounds **4** (9.2 mg) and **5** (8.5 mg), and subfraction 7 was fractionated over silica gel in a column (70-230 mesh; h X Φ =21.0 X 0.8 cm) eluted with CH_2Cl_2, CH_2Cl_2-MeOH (3-10%) to give compounds **6** (4.3 mg) and **7** (27.4 mg). Fr. 12 was subjected to a Sephadex LH-20 column eluted with methanol and provided compound **8** (6.4 mg).

2.5. Chromatographic fractionation of extracts from Platymiscium ulei

The hexane and dichloromethane extracts were grouped and subjected to chromatographic fractionation in a silica gel column (70-230 mesh; h X Φ = 46.5 X 3.2 cm) and eluted with hexane, hex-EtOAc (5-50%) and AcOEt, which provided 25 fractions. The grouped fractions 9-11 provided precipitate that was purified by recrystallization with hot hexane and drops of acetone (compound **9**, 126 mg); fractions 18-19 provided precipitate that was treated with MeOH (compound **10**, 38 mg).

2.6. Chromatographic fractionation of extracts from Hymenolobium petraeum

The hexane extract showed a predominance of β-sitosterol (40 mg). The methanol extract was suspended in MeOH:H_2O (7:3) and then partitioned with hexane followed by CH_2Cl_2 and EtOAc. The hexane phase provided β-sitosterol (26 mg), after drying, the CH_2Cl_2 phase was filtered and subjected to fractionation in a cellulose column eluted with hexane, hex-EtOAc (5-50%), which resulted in the isolation of compounds **11** (8 mg) and **12** (157 g).

2.7. Spectroscopic data of compounds

Eperuic acid (**1**). ^{1}H NMR (300 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): δ 4.80 (sl, H-17a), 4.47 (sl, H-17b), 2.40* (H-7a), 2.39 (dd, 14.9 and 5.6 Hz, H-14a), 1.97* (H-7b), 2.12 (dd, 14.9 and 8.5 Hz, H-14b), 1.93* (H-13), 1.76* (H-1a), 1.74* (H-6), 1.54 (m, H-2b, H-11a), 1.53* (H-9), 1.51* (H-2a, H-11a, H-12a), 1.36* (H-3a), 1.18* (H-3b), 1.08 (dd, 12.4 and 2.8 Hz, H-5), 1.03* (H-1b), 1.02* (H-12b), 0.97 (d, 6.6, Me-16), 0.87 (s, Me-18), 0.80 (s, Me-19), 0.67 (s, Me-20),*multiplete. ^{13}C NMR (75 MHz, $CDCl_3$): δ 179.1 (C-15), 148.7 (C-8), 106.2 (C-17), 57 .1 (C-9), 55.5 (C-5), 42.2 (C-3), 41.2 (C-14), 39.7 (C-10), 39.1 (C-1), 38.5 (C-7), 35.8 (C-12), 33.6 (C-4), 33.6 (C-18), 30.9 (C-13), 24.4 (C-6), 21.7 (C-19), 20.9 (C-11), 19.9 (C-16), 19.4 (C-2), 14.4 C-20). DEPT -135º (75 MHz, $CDCl_3$): text. HMBC (75 MHz, $CDCl_3$) Text.

Methyl eperuate (**2**). ^{1}H NMR (300 MHz, TMS, $(CD_3)_2CO$, δ, ppm, J/Hz): δ 4.82 (sl, H-17a), 4.52 (sl, H-17b), 3.60 (s, OC**H$_3$**), 2.36* (H-7a), 2.35 (dd, 14.9 and 5.6 Hz, H-14a), 2.13 (dd, 14.9 and 8.5 Hz, H-14b), 1.98* (H-7b), 1.95* (H-13), 1.70* (H-6), 1.68* (H-1a), 1.54* (H-2a, H-9, H-11a), 1.51* (H-2, H-11b H-12a), 1.30* (H-3a), 1.18 (dd, 12.8 and 2.7 Hz, H-5), 1.17* (H-3b), 1.02* (H-1b), 0.93 (d, 6.6 Hz, Me-16), 0.88 (s, Me-18), 0.82 (s, Me19), 0.70 (s, Me-20).*multiplete. ^{13}C NMR (75 MHz, $(CD_3)_2CO$): 172.7 (C-15), 148.5 (C-8), 105.9 (C-17), 57.1 (C-9), 55.3 (C-5), 50.5 (O**C**H$_3$), 41.9 (C-3), 40.8 (C-14), 39.5 (C-10), 38.8 (C-1), 38.1 (C-7), 35.7 (C-12), 33.2 (C-4), 33.0 (C-18), 30.9 (C-13), 24.3 (C-6), 21.1 (C-19), 20.8 (C-11), 19.4 (C-16), 19.1 (C-2), 13.9 (C-20).

Oleanolic acid (**3**). ^{1}H NMR (300 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): δ 5.30 (t, 3.7 Hz, H-12), 3.25 (dd, 10.2 and 5.1 Hz, H-3), 2.85 (dd, 14.2 and 4.0 Hz, H-18), 1.99* (H-11a), 1.77* (H-15a), 1.69* (H-2a, H-19a), 1.63* (H-2b, H-11b, H-19a), 1.60* (H-22a), 1.59* (H-1a), 1.56* (H-7a), 1.45* (H-16), 1.33* (H-21a), 1.29* (H-7b, H-6a, H-22), 1.24* (H-6b), 1.15* (H-5), 1.14 (s, Me-27), 1.10* (H-21b), 1.07* (H-15b), 1.05* (H-1b), 0.99 (s, Me-23), 0.93 (s, Me-30), 0.92 (s, H-24), 0.91 (s, Me-29), 0.78 (s, Me-25), 0.76 (s, Me-26),*multiplete. ^{13}C NMR (75 MHz, $CDCl_3$): δ 182.2 (C-28), 143.6 (C-13), 122.6 (C-12), 79.0 (C-3), 55.2 (C-5), 47.6 (C-9), 46.5 (C-17), 45.8 (C-19), 41.6 (C-14), 41.0 (C-18), 38.7 (C-4), 39.2 (C-8), 38.3 (C-1), 37.0 (C-10), 33.8 (C-21), 33.1 (C-29), 32.6 (C-7), 32.4 (C-22), 30.6 (C-20), 28.1 (C-23), 27.7 (C-15), 27.1 (C-2), 25.9 (C-27), 23.6 (C-30), 23.4 (C-16), 22.9 (C-11), 18.3 (C-6), 17.1 (C-26), 15.5 (C-24), 15.3 (C-25).

Quercetin (**4**): ^{1}H NMR (300 MHz, TMS, CD_3OD, δ, ppm, J/Hz): δ 12.19 (1H, s, OH), 7.82 (1H, d, 2.2 Hz, H-2'), 7.70 (1H, dd, 8.5 and 2.2 Hz, H-6'), 6.99 (1H, d, 8.5 Hz, H-5'), 6.52 (1H, d, 2.0 Hz, H-8), 6.26 (1H, d, 2.0 Hz, H-6). ^{13}C NMR (75 MHz, CD_3OD): Table 2.

Fisetinediol (**5**): ^{1}H NMR (300 MHz, TMS, CD_3OD, δ, ppm, J/Hz): δ 6.90 (1H, d, 8.2 Hz, H-5), 6.89 (1H, d, 2.0 Hz, H-6'), 6.81 (1H, d, 8.1 Hz, H-3'), 6.76 (1H, dd, 8.1 and 2.0 Hz, H-2'), 6.40 (1H, dd, 8.2 and 2.5 Hz, H-6), 6.30 (1H, d, 2.5 Hz, H-8), 4.63 *(1H, d, 7.3 Hz, H-2),* 4.04 (1H, m, H-3), 2.93 (1H, dd, 15.8 and 5.0 Hz, H-4a), 2.74 (1H, dd, 15.8 and 8.6 Hz, H-4b). ^{13}C NMR (75 MHz, CD_3OD): Table 2.

Liquiritigenin (**6**): ^{1}H NMR (300 MHz, TMS, CD_3OD, δ, ppm, J/Hz): δ 7.73 (1H, d, 8.6 Hz, H-5), 6.58 (1H, dd, 8.6 and 2.3 Hz, H-6), 7.41 (2H, dd, 6.6 and 1.8 Hz, H-2', H-6'), 6.90 (2H, dd, 6.6 and 1.8 Hz, H-3', H-5'), 6.41 (1H, d, 2.3 Hz, H-8), 5.47 (1H, dd, 13.0 and 2.8 Hz, H-2), 3.10 (1H, dd, 16.7 and 13.0 Hz, H-3a), 2.69 (1H, dd, 16.7 and 2.8 Hz, H-3b), ^{13}C NMR (75 MHz, CD_3OD): Table 2.

3-Methoxy-5,7,3',5'-tetrahydroxyflavanonol (**7**): ^{1}H NMR (300 MHz, TMS, CD_3OD, δ, ppm, J/Hz): δ 7.03 (1H, d, 1.5 Hz, H-4'), 6.87 (2 H, d, 1.5 Hz, H-2', H-6'), 5.97 (1H, d, 2.1 Hz, H-6), 5.94 (1H, d, 2.1 Hz, H-8), 5.17 (1H, d, 9.8 Hz, H-2), 4.30 (1H, d, 9.8 Hz, H-3), 3.41 (3H, s, OC**H$_3$**). ^{13}C NMR (75 MHz, CD_3OD): Table 2.

Hederagenin (**8**): ^{1}H NMR (300 MHz, TMS, CD_3OD, δ, ppm, J/Hz): δ 5.25 (t, 3.3 Hz, H-12), 3.64 (dd, 11.1 and 4.9 Hz, H-3), 3.73* (H-23a), 3.55 (d, 10.9 Hz, H-23b), 2.88 (dd, 13.8 and 4.1 Hz, H-18), 2.17* (H-7a), 1.93*(H-11a), 1.90* (H-11b), 1.77* (H-15a), 1.74* (H-21a), 1.69* (H-2a), 1.65* (H-1a), 1.64* (H-19a), 1.60* (H-2b), 1.45* (H-16a), 1.44* (H-22a), 1.40* (H-16b, H-22b), 1.29* (H-6a, H-7b), 1.24* (H-6b), 1.17 (s, Me-27), 1.16* (H-19b), 1.15* (H-5), 1.10* (H-15b, H-21b), 1.05* (H-1b). 098 (s, Me-25), 0.94 (s, Me-30), 0.91 (s, Me-29), 0.82 (s, Me-26), 0.70 (s, Me-24), *multiplete. ^{13}C NMR (75 MHz, CD_3OD): δ 180.5 (C-28), 143.9 (C-13), 122.1 (C-12), 72.5 (C-3), 65.9 (C-23), 47.3 (C-5), 46.9 (C-17), 46.2 (C-19), 45.8 (C-21), 41.5 (C-14), 41.8 (C-4), 41.3 (C-18), 38.1 (C-1), 39.9 (C-9), 36.5 (C-10), 33.4 (C-22), 32.2 (C-29), 32.0 (C-6), 30.2 (C-20), 29.4 (C-7), 27.4 (C-15), 26.0 (C-2), 25.1 (C-27), 23.1 (C-11), 22.6 (C-30), 17.7 (C-16), 16.4 (C-26), 14.9 (C-25), 11.3 (C-24).

Homopterocarpin (**9**): ^{1}H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): δ 7.43 (1H, d, J = 8.6 Hz, H-5), 7.14 (1H, dd, J = 8.6 and 2.8 Hz, H-6), 6.65 (1H, dd, 8.4 and 2.4 Hz, H-3'), 6.46 (1H, d, 2.8 Hz, H-8), 6.47-644 (2H, m, H-2' and H-5'), 5.52 (1H, d, 6.8 Hz, H-4), 4.27 (1H, dd, 10.8 and 4.8 Hz, H-2a), 3.79 (3H, s, MeO-4'), 3.76 (3H, s, MeO-7), 3.66 (1H, t, 10.8 Hz, H-2b), 3.53 (1H, ddd, 10.8, 5.7 and 5.0 Hz, H-3). RMN ^{13}C (100 MHz, $CDCl_3$, δ, ppm): Table 2.

Scoparone (**10**): RMN ^{1}H (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): δ 7.68 (1 H, d, 9.6 Hz, H-4), 6.80 (1H, s, H-8), 6.79 (1H, s, H-5), 6.30 (1 H, d, 9.6 Hz, H-3), 3.80 (3H, s, MeO-6), 3.85 (3H, s, MeO-7). RMN ^{13}C (100 MHz, $CDCl_3$, δ, ppm): δ 161.38 (C-2), 146.39 (C-6), 152.90 (C-7), 150.07(C-8a), 143.29 (C-4), 113.58 (C-3), 111.47 (C-4a), 108.06 (C-5), 100.05 (C-8), 56.40 (MeO-6 and MeO-7).

Sativan (**11**): RMN ^{1}H (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): δ 7.02 (1H, d, 8.0 Hz, H-6'), 6.94 (1H, d, 8.4 Hz, H-5), 6.48 (1H, d, 2.5 Hz, H-3'), 6.47 (1H, dd, 8.3 and 2.5 Hz, H-5'), 6.39 (1H, dd, 8.1 and 2.5 Hz, H-6), 6.35 (1H, d, 2.5 Hz, H-8), 4.28 (1H, dd, 10.0 and 2.0 Hz, H-2b), 3.99 (1H, t, 10.0 Hz, H-2a), 3.81

(MeO-4'), 3.80 (MeO-2'), 3.53 (1H, m, H-3), 2.81 (1H, t, 7.5 Hz, H-4a), 2.78 (1H, dd, 7.5 and 2.0 Hz, H-4b). RMN ^{13}C (100 MHz, $CDCl_3$, δ, ppm): Table 2.

Tyrosine (**12**): HR/MAS (400 MHz): δ 7.18 (2H, AA'BB, H-2 and H-6), 6.89 (2H, AA'BB, H-3 and H-5), 3.78 (1H, t, 4.9 Hz, H-2'), 3.15 (2H, d Hz, 4.9, H-1'), 2.67 (2H, s, NH_2). CP/MAS (100 MHz): δ 173.1 (C-3'), 156.8 (C-4), 132.3 (C-2 and C-6), 125.2 (C-1), 115.6 (C-3, C-5), 63.8 (C-2'), 32.7 (C-1').

4. Results and Discussion

Table 1 shows that there was a relationship between the yield of extracts and the basic density of the three species, i.e., the sample of *Platymiscium ulei* with the highest percentage of extracts (secondary metabolites) obtained by maceration in organic solvents also presented the highest basic density (0.94 (g/cm^3).

Table 1. Yield of extracts and basic density of three species of Fabaceae

Species	**Extracts (%)**	**Density** (g/cm^3)
Hymenaea courbaril	2.45	0.77 ± 0.0095
Platymiscium ulei	12.52	0.94 ± 0.0206
Hymenolobium petraeum	1.89	0.64 ± 0.0189

The heartwood has an accentuated red to reddish-brown color with dark spots, and the sapwood has a whitish-colored wavy grain with a medium texture. It has a characteristic taste and smell, and the surface has a moderate shine. The macroscopic description showed a simple aliform paratracheal axial parenchyma with confluences and in terminal bands. The radial parenchyma has an irregular arrangement (tangential plane). Pores are medium to large in size, predominantly solitary, multiples of 2-3, empty and some occluded (figure 1).

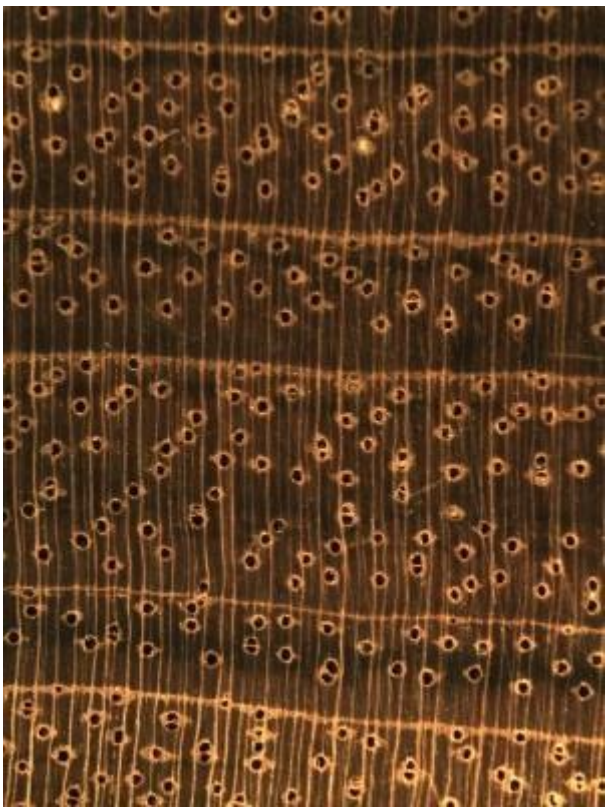

Figure 1. Macrography of transversal plane (10 X) from *Hymenaea courbaril*

Chromatographic fractionation of the hexane extract of *Hymenaea courbaril* resulted in the isolation of compound **1**, and from the methanolic extract, compounds **1**-**8** (figure 2), which were identified based on NMR in 1D (^{1}H, DEPT 135^{0} and ^{13}C) and confirmed by 2D (HSQC and HMBC).

The ^{1}H NMR spectrum of compound **1** showed characteristic signals of labdane-type diterpene due to the presence of methyl groups as singlets (δ 0.87, 0.80 and 0.67) and a doublet (δ 0.97, J = 6.6 Hz). Signals of an exocyclic double bond were verified with broad singlets at δ 4.80 and 4.47. The ^{13}C NMR spectrum showed acid carbonyl shift at δ 179.1 and double bond at δ 148.73 and 106.24. The HMBC experiment confirmed all assignments. The NMR data of compound **2** was similar to that of compound **1** and was identified as methyl eperuate. Both diterpenes were previously identified in resin from the stem of *Hymenaea stigonocarpa* Mar (Doménech-Carbó, 2009).

The ^{1}H NMR spectrum of compound **3** displayed signals assignable to seven angular methyl groups at δ 0.72, 0.76, 0.78, 0.91, 0.92, 0.93, 0.99 and 1.14 (3H s, each), one olefinic proton at δ 5.30 (t, 3.7 Hz, H-12) and one oxygen-bearing methine protons 3.25 (dd, 10.2 and 5.1 Hz, H-3). The characteristic chemical shifts of oleanane type triterpene were verified at δ 143.6 (C-13), 122.6 (C-12), and the deshielded chemical shift carbonyl group of carboxylic acid was observed at δ 182.2 (C-28). Thus, compound **3** was identified as oleanolic acid. The spectra of triterpene oleanane **8** showed in addition to signals similar to triterpene 3, one primary alcoholic function at δ_H 3.55 (d, J = 10.9 Hz) and 3.73 (m)/ δ_C 65.9 (CH_2-23), and this compound was identified as hederegenin. Oleanolic acid and its derivatives are triterpenoid compounds that frequently occur in nature in free acid form or as an aglycone precursor for triterpenoid saponins, which

possess several interesting biological activities (Ayeleso et al., 2017).

The ^{1}H and ^{13}C NMR (table 2) data of **4** were similar to those obtained by Kalegari et al. (2011) for the flavonol quercetin. The ^{1}H NMR spectrum of **5** showed characteristic signals of the heterocyclic ring C of flavan 3-ol at δ 4.63 (d, 7.3 Hz, H-2), 4.04 (m, H-3), 2.93 (dd, 15.8 and 5.0 Hz, H-4a) and 2.74 (dd, 15.8 and 8.6 Hz, H-4b). The HMBC experiment showed the correlations between the H at δ 4.63 and carbons at δ 67.4 (C-3), 32.6 (C-4), 131.2 (C-1'), 119.0 C-2') and 114.2 (C- 6'), thus compound **5** was identified as fisetinediol. The NMR spectra of **6** showed characteristic signals of the heterocyclic ring C of flavanone at δ_H 5.47 (1H, dd, 13.0 and 2.8, H-2), 3.10 (dd, 16.7 and 13.0, H-3a), 2.69 (dd, 16.7 and 2.8, H-3b), in addition to the shift of the carbonyl to δ_C 189.7. These data were in accordance with those reported previously for liquiritigenin (Zhao et al., 2011).

The ^{1}H NMR spectrum of **7** showed characteristic signals of the heterocyclic ring C of flavanonol at δ_H 5.17 (1H, d, 9.8, H-2) and 4.30 (1H, d, 9.8, H-3), in addition to the shift at δ_C 166.9 observed in the ^{13}CNMR. The HMBC experiment showed the correlations between the signals δ 5.17 (H-2) with the carbons C-3, C-4, C-9, C-1', C-4' and C-6'. The ^{1}H and ^{13}C NMR data were compared with flavononol containing the same oxygenation pattern in the aromatic rings (Elwekeel et al., 2012), and compound **7** differs from the literature due to the presence of methoxyl in C-3, named as 3 methoxy 5,7,3',5'-tetrahydroxyflavanonol.

In the genus *Hymenaea*, the flavonoid quercetin was previously reported in heartwood from *Hymenaea stigonocarpa* (Maranhão et al., 2013), fisetinediol isolated from heartwood (Imai et al., 2008) and xylem sap from *H. courbaril* (Costa et al., 2014). In this paper, liquiritigenin was isolated for the first time from *H. courbaril,* and compound 7 has not yet been reported.

Chromatographic fractionation of the grouped hexane and dichloromethane extracts from *Platymiscium ulei* gave compounds **9** and **10.** The ^{1}H NMR spectrum of **9** suggested a pterocarpan structure due to the splitting pattern of the hydrogens at δ 5.52 (d, 6.8 Hz, H-14), 4.27 (dd, 10.8 and 4.8 Hz, H-2α), 3.66 (t, 10.8 Hz, H-2b) and δ 3.53 ddd, 10.8, 5.7 and 5.0 Hz, H-3) related to the protons of the heterocyclic ring C. The compound was identified as homopterocarpin. The chemical shifts of the carbons are shown in Table 2 and are similar to those reported by Ndukwe et al. (2020). The ^{1}H NMR spectrum of **10** showed, in addition to aromatic hydrogen signals, a typical doublet pair of coumarin at δ 7.68 (d, 9.6 Hz) and 6.20 (d, 9.6 Hz), assigned to H-4 and H-3, respectively. The ^{13}C NMR spectrum showed signs of carbonyl at δ 161.38, olefin at δ 143.29 and 113.58, methoxyl at δ 56.40 and 6 aromatic carbons compatible for the coumarin, which are known as scoparone.

Chromatographic fractionation of methanolic extract from *Hymenolobium petraeum* gave compounds **11** and **12**. Compound **11** was found to be an isoflavan on the basis of its characteristic spectral data of ^{1}H NMR at δ 4.28 (dd, H-2b), 3.99 (t, H-2a), 3.53 (m, H-3), 2.81 (t, 7.5, H-4a) and 2.78 (dd, H-4b). The ^{13}C-NMR spectrum exhibited signals for 17 carbons (Table 2). Thus, compound **11** was identified as sativan whose data were compared with literature (Noviany et al., 2012). As Compound **14** was insoluble in organic

solvents, it was analyzed via solid-state NMR. ^{1}H HRMAS NMR spectra showed signals of a *p*-hydroxybenzene-type aromatic system at δ 7.18 (2H, dt) and 6.89 (2H, dt), methine at δ 3.78 (t), methylene at δ 3.15 (d), in addition to the signal at δ 2.78 (s) of the amine group hydrogens. ^{13}C CPMAS NMR showed the chemical shifts of the aromatic system and the side chain at δ 173.1, 63.8 and 32.7. The HSQC and HMBC/MAS confirmed the assignments for tyrosine (**14**). In research with *Inga* species (Fabaceae), it was noted that the anti-herbivore defense includes triterpene saponins, phenolic compounds, and the protein amino acid, tyrosine (Bixenmann et al., 2016).

Table 2. ^{13}C NMR of flavonoids **4-7** (300 MHz, acetone), and **9** (400 MHz, $CDCl_3$)

C	**4**	**5**	**6**	**7**	**9**	**11**
2	147.5	82.0	79.6	81.9	66.37	70.14
3	135.9	67.4	43.8	80.3	39.86	31.56
4	175.7	32.6	189.7	196.2	78.94	30.39
5	161.4	130.1	128.6	164.4	131.74	130.40
6	98.2	108.1	110.3	96.2	109.37	107.81
7	164.2	156.8	164.0	166.9	161.07	154.81
8	93.5	102.5	102.7	94.9	101.64	103.20
9	156.8	155.2	164.8	162.6	156.64	155.24
10	103.2	111.6	131.1	101.2	112.39	114.90
1’	122.8	131.2	130.3	128.5	119.15	121.86
2’	114.8	119.0	128.1	115.0	125.51	158.30
3’	146.1	114.8	115.2	145.6	106.37	98.74
4’	145.0	144.7	157.7	114.5	161.17	159.69
5’	115.3	144.8	115.2	145.0	96.92	104.5
6’	120.5	114.2	128.1	119.4	160.73	127.55
OMe-3				59.5		
OMe-2’						55.35
OMe-4’					55.50	55.35
OMe-8					55.37	

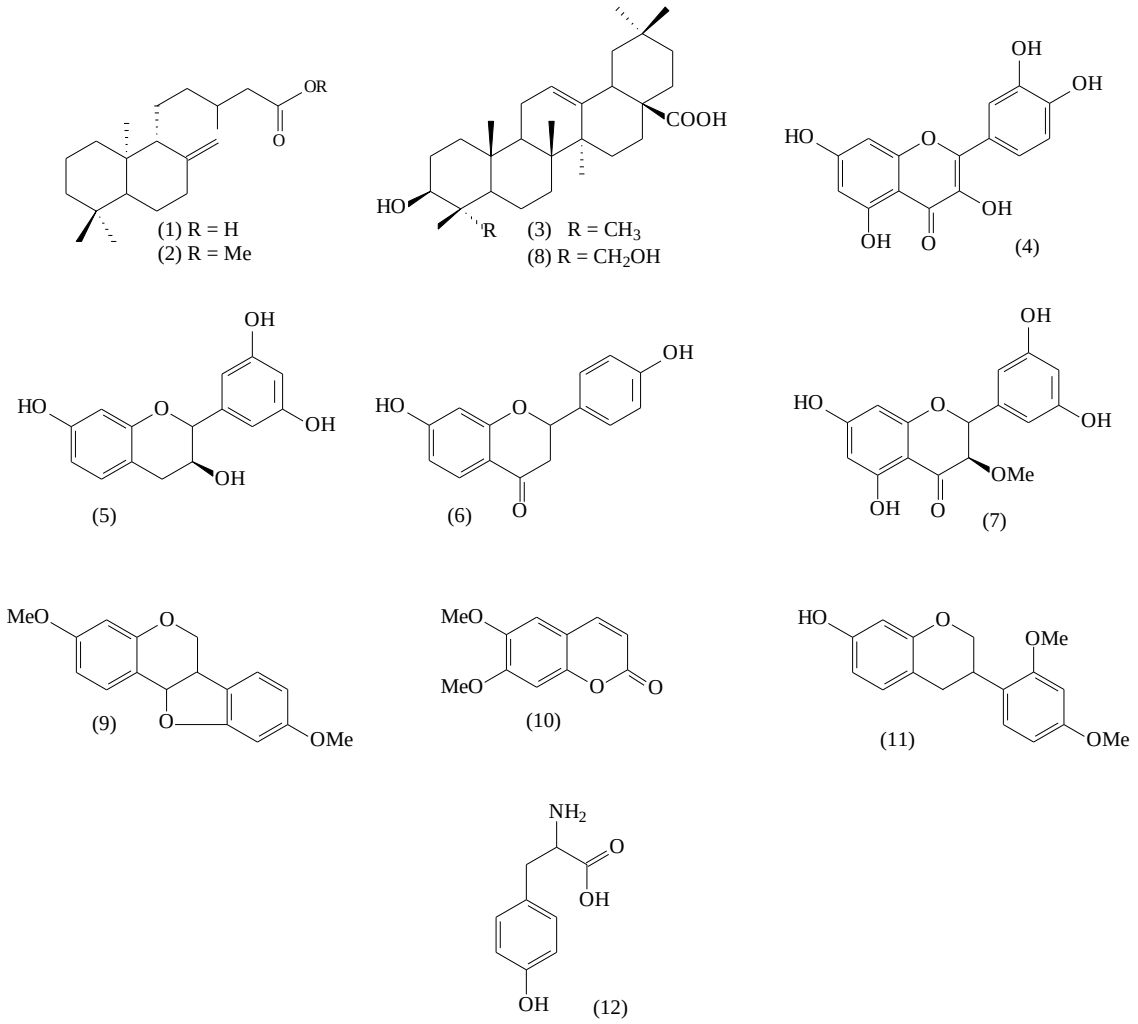

Figure 2. Compounds identified in wood residues of *H. courbaril, P. ulei* and *H. petraeum*

5. Conclusion

The phytochemical studies carried out with the wood residues were an opportunity to generate knowledge from the discarded material during the process of evaluation of wood properties and also to add value to the solid residues that are discarded in industry. Flavonoids were the predominant constituents in the three species of Fabaceae evaluated; however, the presence of isoflavonoids in the two species of the Papilonoidae subfamily (*Platymiscium ulei* and *Hymenolobium petraeum*) was probably associated with resistance this high density wood to pathogenic fungi.

6. Acknowledgments

The authors are grateful to the Fundacão de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) for financial support.

7. References

Ayeleso, T. B., Matumba, M. G. and Mukwevho, E. (2017). Oleanolic acid and its derivatives: biological activities and therapeutic potential in chronic diseases. *Molecules* **22**, 16p. https://doi.org/10.3390/ molecules22111915

Bixenmann, R. J., Coley, P. D., Weinhold, A. and Kursar, T. A. (2016). High herbivore pressure favors constitutive over induced defense. *Ecology and Evolution* **6**, 6037–6049. https://doi.org/10.1002/ece3.2208

Boniface, P. K., Ferreira, S. B., and Kaiser, C. R. (2017). Current state of knowledge on the traditional uses, phytochemistry, and pharmacology of the genus *Hymenaea*. *Journal of Ethnopharmacology* **206**, 193–223. https://doi.org/ 10.1016/j.jep.2017.05.024.

Costa, M. P., Bozinis, M. C., Andrade, W. M., Costa, C. R., Da Silva, A. L., Alves, C. M. O., Kato, L., Fernandes, O. F., Souza, L. K. and Silva M. R. (2014). Antifungal and cytotoxicity activities of the fresh xylem sap of *Hymenaea courbaril* L. and its major constituent fisetin. *BMC Complementary Alternative Medicine* **14**, 1–7. https://doi.org/10.1186/1472-6882-14-245

Doménech-Carbó, M. T., Cruz-Canizares, J., Cortina, L. O., Doménech-Carbó, A. and David, H. (2009). Ageing behaviour and analytical characterization of the Jatobá resin collected from *Hymenaea stigonocarpa* Mart. *International Journal of Mass Spectrometry* **284,** 81–92. https://doi.org/ 10.1016/ j.ijms.2008.12.015

Elwekeel, A., Elfishway, A. and AbouZid, S. (2012). Enhanced accumulation of flavonolignans in *Silybum marianum* cultured roots by methyl jasmonate. *Phytochemistry Letters* **5**, 393–396. http://dx.doi.org/10. 1016/j.phytol.2012.03.010

Garcia, M. G., Nascimento, C. C., Ferreira, A. G. and Lima, M. P. (2018). Identification of isoflavonoids in wood residue from *Swartzia laevicarpa*, *Dipteryx odorata,* and *Andira parviflora*. *Chemistry of Natural Compounds* **54***,* 856–859. https://doi.org/10.1007/s10600-018-2498-5

Garcia, M. G., Gomes, R. F., Nascimento, C. C., Oliveira, L. M., Thomasi, S. S., Ferreira, A. G. and Lima, M. P. (2021). Isolation of new compounds from *Andira parviflora* and *Inga alba* wood residues using LC-DAD-SPE/NMR. *Chemistry of Natural Compounds* **57**, 300–305. https://doi.org/10.1007/s10600-021-03352-8

Coradin, V. T. R. and Muniz, G. I. (1992). Normas e procedimentos em estudos de anatomia da madeira: I–angiospermae II-gimnospermae. Brasília: IBAMA; DIRPED; LPF. 17 p. (Série técnica, 15).

Imai, T., Inoue, S., Matsushita, N. O. Y., Suzuki, R., Sakurai, M., Jesus, J. M. H., Kiyoka, S., Finger, O. Z.

and Fukushima, K. (2008). Heartwood extractives from the Amazonian trees *Dipteryx odorata*, *Hymenaea courbaril*, and *Astronium lecointei* and their antioxidant activities. *Journal of Wood Science* **54**, 470–475. https://doi.org/10.1007/s10086-008-0975-3.

Kalegari, M, Miguel, M. D., Dias, J. F. G., Lordello, A. L. L., Lima, C. P., Miyazaki, C. M. S., Zanin, S. M. W., Verdam, M. C. S. and Miguel, O. G. **(**2011). Phytochemical constituents and preliminary toxicity evaluation of leaves from *Rourea induta* Planch. (Connaraceae). *Brazilian Journal of Pharmaceutical Sciences* **47**, 635–642. https://doi.org/10.1590/S1984-82502011000300023

Maranhão, C. A., Pinheiro, I. O., Santana, A. L. B. D., Oliveira, L. S.,Nascimento, M. S. and Bieber, L.W. (2013). Antitermitic and antioxidant activities of heartwood extracts and main flavonoids of *Hymenaea stigonocarpa* Mart. *Internacional Biodeterioration & Biodegradation* **79**, 9–13. http://dx.doi.org/10. 1016/j.ibiod.2013. 01.005

Melo, L. E. S., Silva, J. B., Nascimento, C. C., Ferreira, A. G.and Lima, M. P. (2020). Identification of phenolic compounds and their relationship to the natural resistance of wood from *Dipteryx polyphylla* Huber and *Acacia mangium* Willd. *International Journal for Innovation Education and Research* **8**, 471–480. https://doi.org/10.31686/ijier.vol8. iss12.2860

Miranda, J. F., Batista, I. M. P., Tucci, C. A. F., Almeida, N. O. and Guimarães, M. A. (2013). Substrato para produção de mudas de macacaúba (P*latymiscium ulei* harms) no município de Autazes, AM. *Ciência Florestal* **3**, 555–562. https://doi.org/10.5902/1980509812339

Ndukwe, G. I., Oluah, A. and Fekarurhobo, G. K. (2020). Isolation of an isoflavonoid and a terpenoid from the heartwood of *Baphia nitida* Lodd. (camwood). *Ovidius University Annals of Chemistry* **31**, 5–8, https://doi.org /10.2478/auoc-2020-0002

Noviany, H O., Chong, W. K., Awang, K., and Manshoor, N. (2012). Isolation and characterisation of l,l'-binaphthalene-2,2'-diol, a new biaryl natural product from *Sesbania grandiflora* root. *Journal of Basic & Applied Sciences* **8***,* 253–256. http://dx.doi.org/10.6000/1927-5129.2012.08.01.39

Tonini, H., Arco-Verde, M. F. and SÁ, S. P. P. (2005). Dendrometria de espécies nativas em plantios homogêneos no Estado de Roraima - Andiroba (*Carapa guianensis* Aubl), Castanha-do-Brasil (*Bertholletia excelsa* Bonpl.), Ipê-roxo (*Tabebuia avellanedae* Lorentz ex Griseb) e Jatobá (*Hymenaea courbaril* L). *Acta Amazonica* **35**, 353 – 362. https://doi.org/10.1590/S0044-59672005000300008

Zhao, X., Mei, W., Gong, M., Zuo, W., Bai, H. and Dai, H. (2011). Antibacterial Activity of the flavonoids from *Dalbergia odorífera* on *Ralstonia solanacearum*. *Molecules* **16**, 9775–978. https://doi.org/10.3390/ molecules16129775.