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Phenolic constituents of woody residues of *Bagassa guianensis* Aubl. and their relationship to the resistance of wood to decay

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ABSTRACT
Many woody species occurring in the Amazon Rainforest lack studies on their metabolism. Bagassa guianensis Aubl. (Moraceae family), the only representative of this genus is an endemic species to the Amazon. This species represents an important resource of interest for the lumber sector due to the properties of its wood, such as its good mechanical strength, high density. This wood has been classified as being resistant to fungal and termite attack. In the present study, we evaluated the secondary metabolites of B. guianensis and discussed the relationship between these metabolites and the natural durability of its wood. The methanolic extract fractionation wood residues using different chromatographic techniques yielded give a resorcinol derivative (2,4-dihydroxybenzaldehyde), stilbenes (6-O-methyl-moracin N, moracin M, trans-oxyresveratrol, moracin P, moracin R-α-xylopyranose identified for the first time in addition, the flavonoids kaempferol and steppogenin. The antifungal nature of stilbenes makes them constitutive defense compounds in preventing wood decay and justifies the resistance of this Amazonian wood.
Keywords: *Moraceae; stilbenes; flavonoids; moracins*

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I. INTRODUCTION

Wood has a cell wall consisting of organic compounds (cellulose and hemicellulose), and phenols (lignin), and these macromolecules are the primary metabolites that in hardwood species contain 38–51% cellulose, 17–38% hemicellulose, and 21–31% lignin [1]. Organic extracts and secondary metabolites (around 3%) are smaller molecules marked by genetic factors that can be modified by physiological, ecological and evolutionary processes [2]. Many woody species occurring in the Amazon Rainforest lack studies on their metabolism. *Bagassa guianensis* Aubl. (known as "tatajuba") belongs to the Moraceae family, and is the only representative of this genus. It is an endemic species to the Amazon and its geographic distribution covers an area from the Amazon basin to the forests of the Guyanas and Suriname [3]. This species represents an important resource of interest for the lumber sector due to the properties of its wood, such as its good mechanical strength, high density, and since it can be used in structural parts in civil construction [4]. This wood has been classified as being resistant to fungal [5] and termite attack [4]. The species shows excellent development in tropical climates with high rainfall and temperatures, and is fast growing and can be planted in different spacings without influencing the basic density [6].

One study of a specimen of *B. guianensis* heartwood from French Guiana identified stilbenes, flavonoids and other compounds [7]. The stilbenes of the Moraceae family have bisbenzyl, (*E*)- and (*Z*)-stilbenes skeletons, in addition to the 2-arylbenzofuran derivatives known as moracins. This class of phenolic compounds occurs in some plant families and is structurally characterized by the presence of a 1,2-diphenylethylene nucleus and is reported to have antimicrobial, deterrent, or repellent roles in plants, thus protecting them from attacks by fungi, bacteria, nematodes, or herbivores [8-11].

In the present study, we evaluated the chemical constituents in wood residues of *B. guianensis* from the Amazon and discussed the relationship between these metabolites and the natural durability of its wood.

II. MATERIAL AND METHODS

2.1 General experimental procedures

Nuclear magnetic resonance spectra were measured using Bruker Avance III 500 spectrometers, and chemical shifts (δ) were expressed in ppm, and coupling constants (*J*) in Hertz; TMS was used as internal standard. Fractioning by medium pressure liquid chromatography (CLMP) was performed on a chromatograph (CLMP–Sepacore® Buchi) equipped with a control unit (C-620), fraction collector (C-660), binary pump (C-605) and UV (C-640). Low-resolution ESI-MS was recorded in an electrospray ionization mass spectrometer (Bruker Daltonics, Amazon Speed). 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), which were visualized using UV light (254 and 365 nm), vanillin-sulfuric acid and NP/PEG reagent spray.

Obtaining wood residues, identification and extraction

Wood rejects were provided by the Wood Technology Laboratory at the Instituto Nacional de Pesquisas da Amazônia (INPA) in which the largest residues were previously evaluated for their technological properties, the smallest resulting from these procedures became available for phytochemical studies. The identification of the wood samples was done through macroscopic comparisons with standard samples from the xylotheque at INPA (N^0 INPA-X-1089). The residues were chopped and ground (269 g) and then submitted to maceration for 7 days with hexane and methanol, which provided yields of 0.03 and 3.71%, respectively.

Chromatographic fractionation of *B. guianensis* extract

The hexane extract (0.1g) showed a predominance of lupeol and β-sitosterol. The methanol extract was partitioned with hexane, CH_2Cl_2 and EtOAc. The CH_2Cl_2 phase was fractionated over silica gel in the column (70-230 mesh; h X Φ = 30.0 X 4.0 cm), eluted with CH_2Cl_2, CH_2Cl_2-EtOAc (20-50%), EtOAc and EtOAc:MeOH (10-20%) to yield thirty one fractions. Fractions 11, 15 and 24 were submitted to new chromatographic fractions. Fr. 11 was fractionated in a silica gel column (230-400 mesh; h X Φ = 30.0 X 3.0 cm), eluted with hexane, hex:EtOAc (10-50%) and gave compound **1** (47 mg); fr 15 was fractionated on the Sepacore® system using a column (15/230 mm) with silica gel (230-400 mesh) and as the eluent DCM:Methanol (1:1) over a period of 20 min and compound **2** was obtained (5 mg). Fr 24 was subjected to a microcrystalline cellulose column eluted with hexane, hexane:EtOAc (2-10%) and provided compound **3** (1 mg).

The EtOAc phase was fractionated over silica gel in the column (70-230 mesh; h X Φ = 38.0 X 3.5 cm), eluted with hexane, Hex:EtOAc (20-50%) and generated eighteen fractions. Fractions 5 and 6 were subjected to new fractioning; fraction 5 was subjected to a Sephadex LH-20 column eluted with methanol followed by a gel column (230-400 mesh) eluted with CH_2Cl_2:MeOH (8:2) to give compound **4** (31 mg). Fraction 6 was subjected to a sephadex column eluted with methanol, and provided 21 fractions of which fractions 12 and 20 were submitted to new fractioning. Fractionation of Fr.12 in a silica gel column (230-400 mesh; h X Φ = 30.0 X 3.0 cm), and eluted with CH_2Cl_2-MeOH (2-10%) provided compounds **4** (5 mg) and **5** (4 mg). Fractionation of fr 20 in a silica gel column (230-400 mesh; h X Φ = 30.0 X 3.0 cm), eluted with Hex:EtOAc (1:1) and gave compounds **6** (4 mg), **7** (5 mg) and **8** (6 mg).

Spectroscopic data of phenolic compounds

2,4-Dihydroxybenzaldehyde (**1**). ESIMS m/z 139.15 $[M+H]^+$. ^{1}H NMR (300 MHz, MeOD, J/Hz): Text. ^{13}C NMR (75 MHz, MeOD): 194.2 (C-1'), 165.9 (C-4), 164.1 (C-2), 135.6 (C-6), 114.5 (C-1), 108.5 (C-5), 101.8 (C-3).

6-O-methyl-moracin N (**2**). 6-O-methyl-moracin N (**2**). ^{1}H NMR (500 MHz, $(CD_3)_2CO$, J/Hz): 7.25 (s, H-4), 7.09 (s, H-7), 6.90 (d, J = 0.8), H-3), 6.76 (d, J = 2.2, H-2', H-6'), 6.34 (t, J = 2.2, H-4'), 3.88 (s. OMe). Prenyl: 5.32 (m, H-2"), 3.34 (m, H-1"), 1.74 (d, J = 1.0, H-5"), 1.73 (d, 1.0, H-4"). ^{13}C NMR (125 MHz, $(CD_3)_2CO$): Table 1.

Moracin R-3'-α-D-xylopyranoside (**3**). ESIMS m/z 477.23 $[M+H]^+$. ^{1}H NMR (500 MHz, MeOD, J/Hz): 7.26 (s H-4), 7.00 (dd, J = 2.0, 1.5, H-2'), 6.96 (d J = 1.0, H-3), 6.93 (dd, J = 2.0, 1.5, H-6'), 6.89 (s, H-7), 6.51 (t, J = 2.2, H-4'). Xyl. 4.86 (H-1'"), 3.96 (dd, 12.0, 5.0, H-5'"), 3.63 (m, H-4'"), 3.45 (m, H-2'"), 3.43 (sl, H-3'"), 3.40 (dd, 10.0, 5.0, H-5'"). Prenyl: 3.81 (dd, 7.5, 5.5, H-2"), 3.15 (dd, J = 17.5, 5.5, H-1"b), 2.86 (dd, 17.5, 7.5, H-1'a), 1.38 (s, H-5"), 1.28 (s, H-4"). ^{13}C NMR (125 MHz MeOD): Table 1.

Moracin M **(4)**. ESIMS m/z 243 $[M+H]^+$. ^{1}H NMR (500 MHz, MeOD, J/Hz): 7.36 (dd, J = 8.4, 0.4, H-4), 6.90 (d J = 0.9, H-3), 6.74 (dd, J = 8.4, 2.1, H-5), 6.89 (ddd, J = 2.1, 0.9, 0.4, H-7), 6.75 (d, 2.2, H-2', H-6',), 6.24 (t, 2.2, H-4'). ^{13}C NMR (125 MHz MeOD): Table 1.

Kaempferol **(5)**. ^{1}H NMR (500 MHz, MeOD, J/Hz): 8.09 (ABX system, H-2', H-6'), 6.91 (ABX system, H-3', H-5'), 6.40 (d, J = 2.1, H-8), 6.18 (d, J = 2.1, H-6). ^{13}C NMR (125 MHz MeOD): Table 1.

Steppogenin **(6)**. ESIMS m/z 289.11 $[M+H]^{+}$. ^{1}H NMR (500 MHz, MeOD, J/Hz): 7.24 (d, J = 8.2, H-6'), 6.35 (dd, J = 8.2, 2.4, H-5'), 6.32 (d, J = 2.4, H-3'), 5.91 (d, J =2.2, H-6), 5.62 (dd, J = 13.1, 2.9, H-2), 5.88 (d, J = 2.2, H-8), 3.10 (dd, J =17.5, 13.1, H-3a), 2.72 (dd, J =17.5, 2.9, H-3b).

trans-Oxyresveratrol (**7**). ESIMS at m/z m/z 245.13 $[M+H]^{+}$. ^{1}H NMR (500 MHz, MeOD, J/Hz): 7.33 (d, J = 9.3, H-6), 7.27 (d, J = 16.4, H-7), 6.82 (d, J = 16.4, H-8), 6.44 (d, J = 2.2, H-2', H-4'), 6.31 (dd, J = 9.3, 2.4, H-5), 6.30 (d, J = 2.4, H-3), 6.13 (t, J = 2.2, H-6'). ^{13}C NMR (125 MHz MeOD): Table 1

Moracin P (**8**). ESIMS at m/z 327.16 $[M+H]^{+}$. ^{1}H NMR (500 MHz, MeOD, J/Hz): 7.24 (d, J = 0.5, H-4), 6.88 (d, J = 1.0, H-3), 6.85 (d, J = 0.5, H-7), 6.75 (d, J = 2.0, H-2', H-6'), 6.24 (t, J = 2.0, H-4'). Prenyl: 3.80 (dd, 7.5, 5.5, H-2"), 3.12 (ddd, 16.5, 5.0, 1.0, H-1a"), 2.83 (ddd, 16.5, 7.5, 1.0, H-1b"), 1.38 (s, H-5"), 1.27 (s, H-4").

III. RESULT AND DISCUSSION

From the wood residues of *B. guianensis*, the 0.03% extractive content of the hexane extract, which had a predominance of the triterpene lupeol and the steroid β-sitosterol, was obtained. The methanol extract showed excellent yield (3.74%) and its identified secondary metabolites were the following phenolic compounds: resorcinol derivative, stilbenes (restricted occurrence in some botanical families) and flavonoids (Figure 1).

Compound **1** had an ion peak at m/z 139.15 $[M+H]^{+}$, as determined by the ESIMS analysis. The ^{1}H NMR spectrum showed signals from three aromatic hydrogens at δ 7.50 (d, 8.5 Hz), 6.44 (dd, 8.5, 2.0 Hz) and 6.27 (ddd, 2.0, 0.5, 0.2 Hz), in addition to the signal of the aldehyde group at δ 9.70 (d, 0.5 Hz). The ^{13}C NMR showed the carbonyl signal of aldehyde at δ 194.2. This compound was identified as 2,4-dihydroxybenzaldehyde (characteristic odor) isolated for the first time from *Bagassa guianensis*. It is an important phenolic aldehyde that is used as a marker for the authenticity of the wood when used for barrels in the aging of wines [12].

The ^{1}H NMR spectra of compounds **2**, **3** and **8** showed characteristic signs of stilbenes of the 2-arylbenzofuran type due to the chemical shift of aromatic hydrogens (δ 7.26-6.26), in addition to the furan ring signal as a doublet at δ 6.90 (**2**), 6.96 (**3**) and 6.88 (**8**) that, in the HSQC, showed correlations with carbons at δ 100.8. The ^{13}C NMR data (Table 1) of compound **2** were similar to the literature for 6-O-methyl-moracin N [10]. For compound **3**, the glycoside substitution was verified by the correlation observed in the HMBC experiment of the anomeric hydrogen at δ 4.86 and aromatic hydrogen at δ 6.51 (H-4'), with the carbon signal at δ 158.7 (C-3'), thus, **3** was identified as moracin R-3'-α-D-xylopyranoside reported here for the first time in the literature. The NMR data of **8** are compatible for moracin P [10].

The ^{1}H NMR spectrum signals (Table 1) of **4** were indicative of 2-arylbenzofuran-type stilbenes with a trisubstituted A ring: δ 6.89 (ddd, 2.1, 0.9 and 0.4, H-7), 6.74 (dd, 8.4 and 2.1, H-5) and 7.36 (dd, 8.4 and 0.4, H-4). Based on these data, allied to chemical shifts of ^{13}C NMR (Table 1) and mass spectrum compound **4** was identified as moracin M. ^{1}H NMR spectrum of compound **7** was indicative of (*E*)-stilbene because, in addition to the aromatic hydrogen signals (δ 7.33-6.13), it showed olefinic hydrogen signals at δ 7.27 and 6.82 as a doublet (16.4 Hz). Thus, data from **7** were compatible for *trans*-oxyresveratrol. These two stilbenes have been previously identified in *B. guianensis* wood [10].

The ^{1}H and ^{13}C NMR data of **5** and **6** are characteristic of flavonoids. The ^{1}H NMR spectrum of **5** showed meta-coupled hydrogen aromatic signals (δ 6.40 and 6.18), assigned to the A ring, as well as aromatic system signals (δ 8.09 and 6.91) of AA'BB' type (C ring). Chemical shifts of the carbonyl at δ 174.1 (^{13}C NMR, Table 1) and the double bond at δ 146.7 and 155.1 characterized the C ring of the flavanol known as Kaempferol, isolated for the first time from *B. guianensis*. The ^{1}H and ^{13}C NMR spectra of **6** showed characteristic signals of the heterocyclic C ring of flavanone whose ^{13}C NMR data were similar to those obtained by Zhang et al. for steppogenin [13].

Flavonoids are widely distributed in all parts of plants, and are compounds that offer protection against ultraviolet radiation, pathogens and herbivores [14]. The antifungal nature of stilbenes makes them constitutive defense compounds in preventing wood decay [13,15] and, in this context, the stilbenes and flavonoids identified in this study with wood from *B. guianensis* have the role of phytoalexins in plant defence mechanisms.

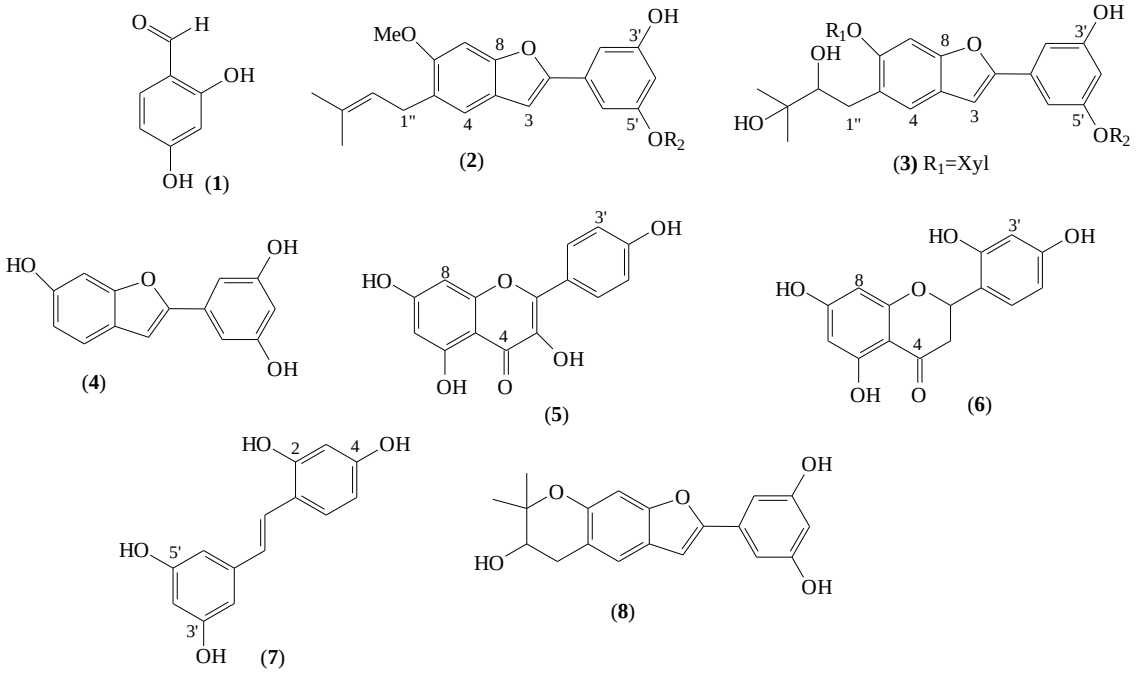

Figure 2: The compounds from woody residues of *B. guianensis*

Table 1. ^{13}C NMR of compounds 1, 3-7 (MeOD) and 2 $(CD_3)_2CO$

C	2	3	4	5	6	7
1						116.7
2	156.3	154.7	154.6	146.7	74.6	155.8
3	100.8	100.8	100.8	155.1	41.6	102.3
4	120.9	120.5	120.4	174.1	197.4	157.8
5	115.4	116.3	111.7	161.1	165.6	107.0
6	158.0	151.5	155.2	97.8	94.8	127.0
7	99.1	98.2	97.0	163.6	166.6	123.3
8	156.3	154.5	155.7	93.0	95.7	125.1
9	123.4	122.5	121.7	157.0	164.9	
10				103.0	102.2	
1’	133.2	132.3	132.4	122.4	116.2	140.7
2’	103.2	103.8	102.5	129.1	155.5	104.1
3’	158.9	158.7	158.4	114.9	102.1	157.5
4’	102.6	105.3	102.2	159.1	158.2	104.1
5’	158.9	158.7	158.4	114.9	106.1	157.5
6’	103.2	103.8	102.5	129.1	127.2	101.0
OMe	56.1					
Xylose (3)	101.5(C-1’’’), 76.5 (C-2’’’), 73.2 (C-3’’’), 70.2 (C-4’’’), 65.7 (C-5’’’).					

IV. CONCLUSION

The phytochemical study carried out with wood residues from *Bagassa guianensis* add value to discarded solid residues and provide knowledge about its secondary metabolites. The high extractive content, allied with the phenolic compounds identified, justify the resistance of this Amazonian wood.

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REFERENCES

[1]. Nhuchhen, D.R., Basu, P., Acharya, B.A. 2014. Compressive review on biomass torrefecation. *Intern J Renew Energy Biofuels* 2014, 506376, 56p. https://doi.org/10.5171/2014.506376

[2]. Gottlieb, G. 1992. Individual development and evolution. The genesis of novel behavior. Oxford University Press, New York, 231p.

[3]. Tropicos - Missouri Botanical Garden 2023. Available from: https://www.tropicos.org/name/Search?name=Bagassa%20guianensis. Accessed on 25/09/2023.

[4]. Costa, F.N., Cardoso, R.P., Mendes, C.S., Rodrigues, P.R.G., Reis, A.R.S. 2019. Natural resistance of seven amazon woods to xylophagous termite *Nasutitermes octopilis* (Banks). *FLORAM*, 26 (3), e20170145, 7pg. https://doi.org/10.1590/2179-8087.014517.

[5]. Reis, A.R.S., Reis, L.P., Alves, M., Carvalho, J.C., Ribeiro, J.R.S. 2017. Natural resistance of four Amazon woods submitted to xylophagous fungal infection under laboratory conditions. *Madera Bosques*, 23(2), 155-162. https://doi.org/10.21829/ myb.2017.232968.

[6]. Carvalho, M.S.P., Carvalho, J.O.C. 2001. Influência do espaçamento na densidade da madeira de *Bagassa guianensis* Aubl. (Tatajuba) no planalto de Belterra, Pará. *Rev. Bras. Cienc. Agrar*., 35(1), 73-78.

[7]. Royer, M., Herbette, G., Eparvier, V., Beauchêne, J., Thibaut, B., Stien, D. 2010. Secondary metabolites of *Bagassa guianensis* Aubl. wood: A study of the chemotaxonomy of the Moraceae family. *Phytochemistry*, 71(14-15), 1708-1713. https://doi.org/10.1016/j. phytochem.2010.06.020.

[8]. Jeandet, P., Douillet, A.C., Debord, S., Sbaghi, M., Bessis, R., Adrian, M. 2002. Phytoalexins from the Vitaceae: biosynthesis, phytoalexin gene expression in transgenic plants, antifungal activity, and metabolism. *J. Agric. Food Chem*., 50(10), 2731-2741. https://doi.org/10.1021/jf011429s.

[9]. Chong, J., Poutaraud, A., Hugueney, P. 2009. Metabolism and roles of stilbenes in plants. *Plant Sci.* 117(3), 143-155. https://doi.org/10.1016/j.plantsci.2009.05.012.

[10]. Rivière, C., Pawlus, A.D., Mérillon, J.M. 2012. Natural stilbenoids: distribution in the plant kingdom and chemotaxonomic interest in Vitaceae. *Nat Prod Rep*., 29(11),1317-1333. https://doi.org/10.1039/c2np20049j.

[11]. Jeandet, P., Vannozzi, A., Sobarzo-Sánchez, E., Uddin, S., Roque, B. R., Martínez-Márquez, A., Clément, C., Cordeliera, S., Manayi, A., Nabavi, S.F., Rasekhian, M., Gaber, El-Saber B., GE-S, Khan. H., Belwal, I.M., Jiang, J., Koffas, M., Nabavi, S.M. (2021). Phytostilbenes as agrochemicals: biosynthesis, bioactivity, metabolic engineering and biotechnology. *Nat Prod Rep*., 38(7),1282-1329. https://doi.org/10.1039/d0np00030b.

[12]. Simón, B.F., Martínez, J., Sanz, M., Cadahía, E., Esteruelas, E., Muñoz, A.M. 2014. Volatile compounds and sensorial characterisation of red wine aged in cherry, chestnut, false acacia, ash and oak wood barrels. *Food Chem.*, 147(15), 346-356. https://doi.org/10.1016/j.foodchem.2013.09.158.

[13]. Zhang, L., Tao, G., Chen, J., Zong-Ping Zheng, Z-P. 2016. Characterization of a new flavone and tyrosinase inhibition constituents from the twigs of *Morus alba* L. *Molecules*, 21, 1130, 1-9. https://doi.org/10.3390/molecules21091130.

[14]. Harborne, J.B., Williams, C.A. 2000. Advances in flavonoid research since 1992. *Phytochemistry*, 55(6), 481-504. https://doi.org/10.1016/s0031-9422(00)00235-1.

[15]. Likhitwitayawuid, L. 2021. Oxyresveratrol: Sources, productions, biological activities, pharmacokinetics, and delivery systems. Molecules, 26(14), 4212, 1-30. https://doi.org/10.3390/molecules26144212.