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Antifungal and Antiplasmodial Activity of Isolated Compounds from *Handroanthus serratifolius* (Vahl) S. Grose Sawdusts

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Abstract *— Handroanthus serratifolius (Vahl) S. Grose [Tabebuia serratifolia (Vahl) G. Nicholson] popularly known in Brazil "ipê-amarelo" is a woody species with potential of sustainable use belonging to a genus known for being as a promising naphthoquinones source which has aroused interest in search for new biological activities. In this paper, the methanolic extract fractionation from H. serratifolius sawdust using different chromatogramphic techniques yielded naphthoquinones dehydro-α-lapachone, dehydro-iso-α-lapachone and α-lapachone, along with lignans paulownin and cycloolivil. The three naphthoquinones presented antiplasmodial activity against Plasmodium falciparum where the dehydro-iso-α-lapachone was more active (IC50 of 7.53 µg/mL). This same compound also exhibited significant antifungal activity against Cryptococcus neoformans.*

Keywords *— **Bignoniaceae, Cryptococcus neoformans, lignans, naphthoquinones, Plasmodium falciparum.***

I. INTRODUCTION

Handroanthus serratifolius (Vahl) S. Grose [*Tabebuia serratifolia* (Vahl) G. Nicholson] (Bignoniaceae) is a woody species with potential of sustainable use, and with a large occurrence in South America[1]. The trunk medium diameter ranging from 20-90 cm in this species foments furniture confection beyond its medicinal importance, especially the heartwood is used for pharmacological substrate. Popularly known in Brazil as "ipê", "ipê-amarelo" and "pau-d'arco", it is commonly used in landscaping and urban arborisation due to its attractive yellow flowers[2]. Phytochemical studies with *H. serratifolius* (ipê-amarelo) are scarce, there are reports of two naphthoquinones derivative of lapachol from stem bark[3]. The naphthoquinones of Bignoniaceae have been much investigated in the last decades, especially lapachol and analogs α-lapachone and β-lapachone, which are isolated from the heartwood and exhibit a diversity of biological activities[4,5]. The interest in three natural naphthoquinones as well as their semi-synthetic derivatives has intensified the search for new antifungal of medical interest[6-8] and antimalarial agents[9-11].

Malaria remains an important infectious disease, which is most prevalent in the tropical and poor regions of African countries. In 2017, according to the latest estimates from WHO, there were 219 million new cases, with 435,000 deaths, especially in children under five years of age[12]. The appearance of *Plasmodium falciparum* resistance to many antimalarial medicines is a concern in the fight against malaria; all over the world including in Brazil[13,14]. In this context, it is important the discovery of new drugs with new targets. Thus, in this study, we investigated antifungal and antiplasmodial potential of naphthoquinones and lignans isolated from the heartwood residues of *H. serratifolius*. Spectroscopic characterization of the compounds is also presented.

II. MATERIAL AND METHODS

General experimental procedures

NMR spectra were measured in a Bruker DRX 400

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apparatus; chemical shifts (δ) were expressed in ppm, and coupling constants (*J*) in Hertz; TMS was used as internal standard. Deuterated solvens chloroform and methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA**).** LC-HRMS measurements were obtained using a MicroTOF-QII (Bruker Daltonics) mass spectrometry connected to a Prominence UFLC (Shimadzu) LC. Melting points were determined on a Fisatom 430D apparatus. Fractioning by medium pressure liquid chromatography (CLMP) was performed on CLMP–Sepacore® Buchi equipped with control unit (C-620), fractions collector (C-660), binary pump (C-605) and UV (C-640). Column chromatography (CC) was performed with silica gel 60 (Merck, 70-230 and 230-400 mesh), cellulose (Merck), Sephadex LH-20 (Sigma-Aldrich) and Amberlite XAD-2 (Supelco). Analytical TLC was performed with silica gel 60 F_{254} (0.25 mm) pre-coated alumina sheets (Merck) visualized using UV light (254 and 365 nm), vanillin-sulphuric acid and NP/PEG reagent spray.

Obtaining of wood residues and extraction

Samples of wood residues were obtained from the Experimental Station of Tropical Forestry located at km 50 of BR-174, the basis of forest management of the Instituto Nacional de Pesquisas da Amazônia (INPA). These residues were classified and identified through macroscopic comparisons with standard samples of *H. serratifolius* available in the Xylotheque of INPA. Larger residues were previously evaluated about their technological properties of wood and use for experiments in the production of artifacts in the Laboratory of Engineering of Wood Artifacts. The resulting minor residues (sawdusts) from these procedures were available for phytochemical studies. Thus, sample of sawdusts from *H. serratifolius* heartwood (295.56 g) was extracted with n-hexane and then methanol providing 0.84 of hexane extract and 19.14 g of methanolic extract.

Chromatographic fractionation of methanolic extract

The methanolic extract (16 g) was fractionated over silica gel column (70-230 mesh) eluted with hexane, hex:CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2:MeOH (10-50%) to yield twenty-one fractions. The grouped fractions 7-11 (TSM-7, 1.86 g) and 14-15 (TSM-14, 6.52 g) were subjected to further chromatographic fractionation. TSM-7 fraction was subjected to a microcrystalline cellulose column using hexane, hex:AcOEt (95:5) resulting in purification of compound **1** (10 mg) and mixture (368 mg) of two compounds that were purified on MPLC system using silica gel (230-400 mesh), eluting with hex:EtOAc (9:1) and hex:EtOAc (8:2) to give **2** (4.0 mg) and **3** (13.0 mg). TSM-14 fraction fractionated over silica gel column (70-230 mesh) eluted with hexane, hex:CH_2Cl_2 (20-50%), CH_2Cl_2 and CH_2Cl_2:MeOH (10-50 %), resulted in twenty-one fractions of which frs. 16-19 (2.35 g) were submitted to further silica gel columns chromatography followed by Sephadex LH-20 to give compounds **4** (9.0 mg) and **5** (14 mg).

Microorganisms reference strain and antifungal susceptibility testing

Strains of *Cryptococcus neoformans* (VN1 PCN6) and *Candida albicans* (ATCC 36232) of the culture collection from Instituto Nacional de Pesquisas da Amazônia-INPA, Manaus-AM, were used. These cultures were preserved through lyophilization process, later they were reactivated in Sabouraud Agar Dextrose. An inoculum was removed from the culture and suspended in 5.0 ml of sterile saline 0.085% and placed in vortex for 15 seconds. The cell density was adjusted equivalent to a standard solution of 0.5 McFarland. The suspension for testing was obtained by making a 1/100 dilution followed by a 1/20 dilution in RPMI 1640 (Sigma-Aldrich) buffered with 4-Morpholinepropanesulfonic acid to the required concentrations for the assays according to CLSI[15].

Minimum inhibitory concentration (MIC) assays were performed with the broth microdilution method[15]. Briefly, 100 μL of each evaluated compound diluted in RPMI 1640 broth was added to 96-well microplates, with the initial concentrations of 320 μg/mL. Amphotericin B was used as the antifungal standard. Next, 100 μL of an inoculum containing 2.5 ×103 cells/mL of the reference microorganism was added to 96-well microplates. The microdilution plates were incubated at room temperature (35°C) for 24 h. The amount of growth in the tubes containing the tested compound was visually compared with the amount of growth in the growth-control tubes (no antifungal agent) used in each set of tests.

Plasmodium falciparum cultures and Antiplasmodial tests

To assess the schizonticide activity of the compounds against *P. falciparum in vitro* were used trophozoite stages in sorbitol-synchronized with approximately 1–2% parasitemia and 2.5% haematocrit[16]. All parasites were maintained in continuous culture on human erythrocytes (blood groups A + or O +) using RPMI medium supplemented with 10% human serum as described by Trager & Jensen[17]. Then, erythrocytes were distributed in a 96-well plate. The drug stock solutions were prepared in 1% DMSO and further serially diluted (50–0.78 ug/mL) in complete culture medium. Blood smears were collected after 48 hours, methanol fixed and Giemsa stained, codified and read blindly. The antiplasmodial effect of the isolated compounds was measured by the inhibition of *P.*

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falciparum blood parasite growth relative to the control cultures in complete medium without drugs. The parasitemia was observed by optical microscopy. Negative controls were cultures free of treatment, culture in 1% DMSO and culture with addition of distilled water. The parasitemia in control groups without treatment was considered 100%.

The activity of the drug was expressed as the percentage of parasitemia inhibition compared to controls without drugs. Compounds that inhibited between 80 and 100% growth of the parasites were considered active; partially active when the inhibition was between 50 and 79%, and when the inhibition was lower than 50%, the drug was considered inactive[18]. The half-maximal inhibitory (IC_{50}) concentration responses were estimated by linear interpolation, as compared to the drug-free controls. A chloroquine control (as a reference antimalarial drug) was used in each experiment. Diluted at a ratio of 1:3 starting from 2500 ng/mL to 3.42 ng/mL in complete culture medium. All experiments were performed in duplicate or triplicate, and the slides were coded for quantification of parasitemia.

Analytical and spectral data of isolated compounds

Dehydro-α-lapachone (**1**): crystallin, orange-colored solid, mp 139–140ºC. HRMS m/z 241.0902 $[M+H]^+$. ^1H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 8.10-8.09 (2H, m, H-5 and H-8), 7.72-7.68 (2H, m, H-6 and H-7), 6.66 (1H, d, J = 10.0, H-11), 5.73 (1H, d, J = 10.0, H-12), 1.56 (6H, s, H-14 and H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 181.9 (C-4), 179.9 (C-1), 152.5 (C-2), 134.0 (C-6), 133.2 (C-7), 131.6 (C-9), 131.5 (C-10), 130.9 (C-12), 126.2 (C-5 and C-8), 117.9 (C-3), 115.5 (C-11), 80.5 (C-13), 28.4 (C-14 and C-15).

Dehydro-iso-α-lapachone (**2**): crystallin, yellow-colored solid, mp 109–110^{0}C. HRMS m/z 241.0848 $[M+H]^+$. ^1H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 8.10-8.07 (2H, m, H-5 and H-8), 7.74-7.66 (2H, m, H-6 and H-7), 5.42 (2H, dd, J = 10.9 and 8.6, H-12), 5.13 (1H, s br, H-14), 5.01 (1H, sl, H-14), 3.36 (1H, dd, J = 17.2 and 10.9, H-11), 3.04 (1H, dd, J = 17.2 and 8.6, H-11), 1.81 (3H, s, H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 182.3 (C-4), 177.7 (C-1), 160.1 (C-2), 141.7 (C-3), 134.2 (C-6), 133.0 (C-7), 131.6 (C-10), 126.4(C-8), 126.1 (C-5), 124.0 (C-9), 114.0 (C-14), 88.5 (C-12), 32.0 (C-11), 16.9 (C-15).

α-Lapachone (**3**): crystalline, yellow greenish solid, mp 119–120ºC. HRMS m/z 243.1029 $[M+H]^+$. ^1H NMR (400 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 8.10-8.06 (2H, m, H-5 and H-8), 7.70-7.67 (2H, m, H-6 and H-7), 2.62 (H-11, t, J = 6.6, H-11), 1.82 (2H, t, J = 6.6, H-12), 1.44 (6H, s, H-14 and H-15). ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 184.4 (C-4), 180.0 (C-1), 154.7 (C-2), 133.9 (C-6), 132.9 (C-7), 131.2 (C-9), 132.1 (C-10), 126.3 (C-8), 126.0 (C-5), 120.2 (C-3), 78.2 (C-13), 31.5 (C-12), 26.5 (C-14 and C15), 16.7 (C-11).

Paulownin (**4**): crystalline, colourless solid, mp 78.3–81.0^{0}C. HRMS m/z 371.1116 $[M-H_2O\ H]^+$ and *m/z* 353.1014 $M+H]^+$. ^1H NMR (500 MHz, TMS, $CDCl_3$, δ, ppm, J/Hz): 6.95 (1H, d, J= 1.7 Hz, H-2), 6.92 (1H, d, J = 1.6, H-2'), 6.88 (1H, d, J = 8.0, H-6'), 6.87 (1H, dd, J = 8.0 and 1.7, H- 6), 6.85 (1H, d, J = 8.0, H-5), 6.80 (1H, d, J = 8.0, H-5'), 5.99 (2H, s, H-10'), 5.96 (2H, s, H-10), 4.83 (1H, s, H-7'), 4.86 (1H, d, J = 5.0, H-7), 4.53 (1H, dd, J = 9.2 and 8.1, H-9ax), 4.06 (1H, d, J = 9.4, H-9'ax), 3.92 (1H, d, J = 9.4, H-9'eq), 3.85 (1H, dd, J = 9.2 and 6.1, H-9eq), 3.07 (1H, ddd, J = 8.0, 6.1 and 5.0, H-8). ^{13}C NMR (75 MHz, $CDCl_3$, δ, ppm): 148,2 (C-4'), 148.0 (C-4), 147.9 (C-3'), 147.3 (C-3), 134.6 (C-1'), 129.1 (C-1), 119.8 (C-5), 120.1(C-6'), 108.6 (C-6), 108.2 (C-5'), 107.4 (C-2'), 106.9 (C-2), 101.3 (C-10'), 101.1(C-10), 91.6 (C-8'), 87.5 (C-7'), 85.8 (C-7), 74.8 (C-9'), 71.6 (C-9), 60.4 (C-8). HMBC (300 x 75 MHz, $CDCl_3$): 6.95 → 147.3, 85.8; 6.92 → 87.5; 6.88 →148.2, 107.4, 87.5; 6.87 → 148.0, 129.1, 106.9, 85.8; 6.85 →148.0, 129.1; 6.80 → 147.9, 134.6, 120.1; 5.99 →148.2; 5.96 →148.0; 4.86 →129.1, 91.6, 71.6, 60.4; 4.83 →134.6, 107.4', 120.1; 4.53 → 85.8, 60.4; 4.06 → 91.6, 85.8, 60.4; 3.92 → 91.6, 85.8, 60.4; 3.85 → 85.8, 60.4; 3.07 → 29.1, 91.6.

Cycloolivil (**5**): Amorphous, white solid, mp 164–66ºC. HRMS m/z 375.1567 $[M-H]^+$. ^1H NMR (400 MHz, TMS, MeOD, δ, ppm, J/Hz): 6.75 (1H, d, J = 8.0, H-5'), 6.70 (1H, d, J = 1.9, H-2'), 6.66 (1H, dd, J = 8.0 and 1.9, H-6'), 6.62 (1H, s, H-2), 6.18 (1H, d, J = 0.8, H-5), 4.02 (1H, d, J = 11.7, H-7'), 3.81 (1H, dd, J = 11.1 and 2.6, H-9'a), 3.78 (1H, d, J = 11.1, H-9a), 3.77 (3H, s, OMe-3'), 3.59 (1H, d, J = 11.1, H-9b), 3.58 (1H, dd, J =11.1 and 4.2, H-9'b), 3.21 (1H, d, 16.7, H-7eq), 2.61 (1H, d, J = 16.7, H-7ax), 2.03 (1H, ddd, J = 11.7; 4.2 and 2.6, H-8'). ^{13}C NMR (100 MHz, MeOD, δ, ppm): 147.7 (C-31), 146.1 (C-3), 144.7 (C-4'), 143.9 (C-4'), 137.2 (C-1'), 132.1 (C-1), 125.0 (C-6), 122.2 (C-6'), 115.9 (C-5), 114.6 (C-5'), 112.5 (C-2'), 111.6 (C-2), 73.6 (C-8), 68.0 (C-9), 59.4 (C-9'), 54.9 (OMe-3'), 46.2 (C-8'), 43.5 (C-7'), 38.5 (C-7). HMBC (400 x 100 MHz, MeOD): 6.75 → 137.2, 147.7, 144.7; 6.70 → 147.7, 144.7, 122.2, 43.5; 6.66 → 144.7, 112.5; 6.62 →146.1, 143.9, 132.1, 38.5; 6.18 → 146.1, 125.0, 43.5; 4.02 → 137.2, 132.1, 122.2, 112.5, 59.4, 46.2; 3.81 →73.6, 46.2; 3.58 → 73.6, 46.2; 3.21 → 132.1, 125.0, 111.6, 73.6, 68.0, 46.2; 3.78 → 73.6, 46.2, 38.5; 3.77 → 147.7; 3.59 → 73.6, 46.2, 38.5; 2.61 →

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132.1, 125.0, 111.6, 73.6, 68.0, 46.2; 2.03 → 137.2, 59.4, 43.5.

III. RESULTS AND DISCUSSION

Phytochemical studies with heartwood sawdusts from *H. serratifolius* using different chromatographic techniques resulted in the isolation of compounds identified as dehydro-α-lapachone (**1**), dehydro-iso-α-lapachone (**2**), α-lapachone (**3**), paulownin (**4**) and cycloolivil (**5**) (Figure 1). Compounds **1** and **3** were identified on basis of ^{1}H and ^{13}C NMR spectral data and comparison with literature[19] as pyranonaphthoquinone dehydro-α-lapachone and α-lapachone, respectively. In previous studies with heartwood from *H. serratifolius*, dehydro-α-lapachone was detectados by GC/MS as predominant compound[20]. The ^{1}H and ^{13}C NMR spectral data allowed identification of **2** as dehydro-iso-α-lapachone whose data were similar to those published by Ribeiro et al[21]. Identification of lignans **4** and **5** was based on experiments ^{1}H and ^{13}C NMR, and HMBC. ^{1}H and ^{13}C NMR data are in accordance with those reported in the literature as paulownin[22,23] and cycloolivil[24], respectively. In this study furanonaphthoquinone **2** and lignans **4** and **5** are being reported for the first time from ipê-amarelo wood.

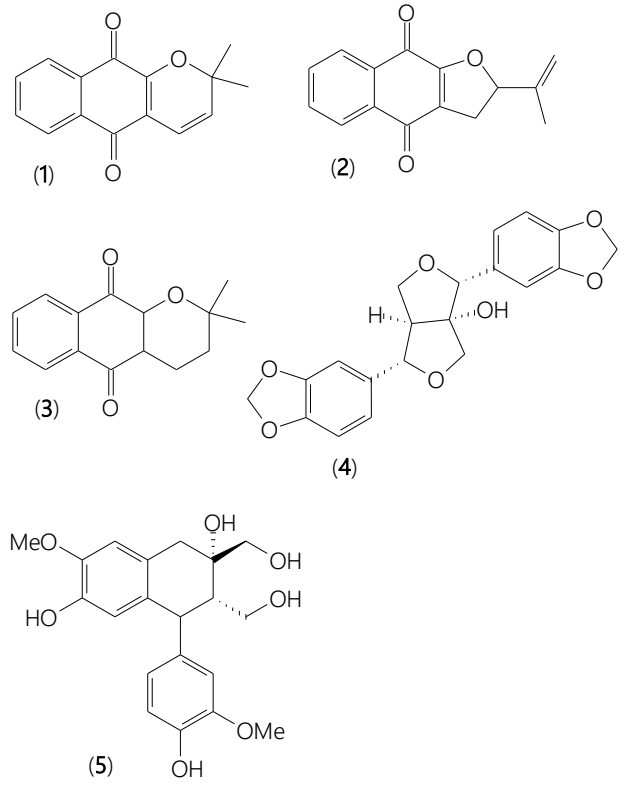

Fig. 1: Compounds isolated from heartwood sawdusts of H. serratifolius

Naphthoquinones and lignans were tested against pathogenic strains of *Cryptococcus neoformans* and *Candida albicans* (Table 1). Dehydro-iso-α-lapachone (**2**) showed a moderate effect (40 μg/mL) against *C. neoformans* strains. Interest in antifungal activity of synthetic and natural naphthoquinones have intensified in recent years. The importance of the search for natural prototypes for these pathogens is due to the small number of drugs available for treatment, the adverse effects associated with the use of certain compounds and the increasing incidence of antifungal resistance[25-27].

Table 1. MIC (μg/mL) of compounds from H. serratifolius against Cryptococcus neoformans and Candida albicans

Compounds	*C. neoformans* VNIPCN6	*C. albicans* ATCC 36232
1	80	> 320
2	40	160
3	160	160
4	80	> 320
5	> 320	> 320
Amphotericin B	0.125	0.25

The compounds were evaluted for their *in vitro* antimalarial activity against *Plasmodium falciparum* W-2 a chloroquine-resistant strain. Table 2 shows that naphthoquinones were considered active, the highest activity was observed for furanonaphthoquinone **2** (IC_{50} 7.53 μg/mL). The results suggest that the furan-type heterocyclic ring of **2** is important in bioactivity when compared to the pyran ring of naphthoquinones **1** and **3**. Lapachol derivatives are source of inspiration for structural modifications by synthetic transformations for developing new and more active products antiplasmodial against *P. falciparum*[9,10]. Lignans containing furofuran (**4**) and dibenzilbutane (**5**) skeleton were inactive suggesting chemical transformation for obtaining of their synthetic analogues more active because this class of compounds also has potential antimalarial.

Table 2. Antiplasmodial activity (IC_{50} μg/mL) of compounds 1-5 against Plasmodium falciparum (W-2)

Compounds	IC_{50}
1	21.12
2	7.53
3	14.00
4	≥ 50
5	≥ 50

IV. CONCLUSION

Phytochemical and biological research on wood residues from species with potential for sustainable use resulted in the identification of compounds with

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antimicrobial and antiplasmodial activity. The obtaining of residues from *H. serratifolius* heartwood from the area of management and studies of the technological properties of wood was an opportunity for the increase of knowledge of its secondary metabolism as well as to find active principles.

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AUTHOR CONTRIBUTIONS

LESM performed the extraction, isolation and identification of constituents. KSC, PILS and JVBS performed in the microbiological activities. CCN performed the identification and anatomical analysis of wood. BMMM and VFAN performed the antiplasmodial assay, AGF performed the NMR experiments. MPL designed the study and supervised the phytochemical laboratory. All authors have read and approved the final manuscript.

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