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Steroids and phenolic derivatives from *Tabebuia aurea* (Bignoniaceae)

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Abstract: *Tabebuia aurea* (Bignoniaceae) is a medicinal plant traditionally used to treat various diseases, including infections and inflammatory conditions. In this study, the chemical composition of the root and stem bark extracts was investigated, leading to the isolation of steroids and phenolic compounds. The extraction process involved cold maceration with methanol, followed by liquid-liquid partitioning and chromatographic fractionation. The main metabolites identified were *p*-hydroxybenzoic acid, p-coumaric acid, cholestanone, β-sitosterol, stigmasterol, campesterol, (*E*)-4-methoxycinnamic acid, and (*Z*)-4-hydroxycinnamic acid**.** Structural elucidation was performed usingNMR and mass spectrometry**.**
Keywords: *Tabebuia aurea*, Bignoniaceae, Phytochemical analysis

Introduction

The Bignoniaceae family is extensive, comprising approximately 79genera and 790 to 860species, primarily distributed in tropical and subtropical regions (Castillo & Rossini, 2010; Lohmann & Ulloa Ulloa 2021). However, only a few species have been chemically analyzed (Olmstead*et al*., 2009). The Brazilian territory is a major center of diversification for this family, with 36 genera and 422 species occurring from the Cerrado to tropical rainforests (Bignoniaceae *in* Flora e Funga do Brasil, 2025).

Within Bignoniaceae, the *Tabebuia* genus stands out as one of the most significant in South and Central America, commonly referred to as "ipês" (Lemos *et al*., 2012; Ospina *et al*., 2013). Approximately 292 secondary metabolites have been identified from *Tabebuia,* including naphthoquinones, tannins, flavonoids, alkaloids, and iridoids, which exhibit relevant pharmacological properties, such as antibacterial, antifungal, anti-inflammatory, antioxidant, and antiparasitic activities (Lemos *et al*., 2012; Park *et al*., 2006; Ospina *et al*., 2013).

The species *Tabebuia aurea* (Silva Manso) Benth & Hook, known as "ipê-amarelo" or "paratudo", is traditionally used by the Pantanal population as a "holy remedy" to treat yellow fever, diabetes, malaria, hepatitis, bacterial and fungal infections, and even cancer (Pott & Pott, 1994; Oliveira *et al*., 2006). The bark is typically consumed as tea due to its diuretic properties, while the roots are infused in alcoholic beverages such as "cachaça" or wine to treat colds and flu (Byeon*et al*., 2008). Other popular uses include infusions, chewing, or maceration of the bark in alcohol for treating inflammation and snake bites (Reis*et al*., 2014).

Phytochemical investigations on *T. aurea* have previously reported the presence of β-sitosterol, betulinic acid, lapachol, and flavonoids (Barbosa-Filho*et al*., 2004). Considering the chemical diversity of *Tabebuia* and the pharmacological potential of *T. aurea*, this study aims to isolate and identify secondary metabolites from its stem bark and roots, expanding the phytochemical knowledge and relevance of the species.

Material and Methods

Plant material

Tabebuia aurea was collected in Santo Antônio de Leverger, Mato Grosso, Brazil (15°50'50"S, 56°04'00"W). A voucher specimen (no. 41.032) was stored at the Central Herbarium of the Universidade Federal de Mato Grosso, Cuiabá, Brazil.

Extraction and isolation

Cold extraction with methanol yielded 553 g and 202 g of crude extract from the roots (3.5 kg) and stem bark (1.7 kg), respectively. The roots crude extract was submitted to liquid-liquid partitioning with ethyl acetate (EtOAc), producing 30.0 g of EtOAc residue. This residue was fractionated on a silica gel column resulting a mixture of *p*-hydroxybenzoic acid (**1**) and *p*-coumaric acid (**2**) (60.0 mg). The stem bark crude extract was partitioned with hexane, $CHCl_3$, and EtOAc, obtaining the hexane (5.3 g), $CHCl_3$ (13.8 g), and EtOAc (45.0 g) residues. These residues were chromatographed on a silica gel column, separately. The hexane residue (4.0 g) resulted in 52 fractions. Fractions 22/30 (35.7 mg) contained a mixture of steroids: cholestanone (**3**), β-sitosterol (**4**), stigmasterol (**5**), and campesterol (**6**) (12 mg). The 77 fractions collected from the $CHCl_3$ residue (13.6 g) resulted in a colorless crystal identified as *(E)*-4-methoxycinnamic acid (**7**, 32.6 mg) after purification on a Sephadex LH-20 column. The fractionation of the EtOAc residue (40.0 g) resulted in 95 fractions, with fractions 39/76 yielding *(Z)*-4-hydroxycinnamicacid (**8**, 13.8 mg) after purification on preparative TLC in a CH_2Cl_2:EtOAc (8:2) system.

p-hydroxybenzoic (**1**), brown amorphous powder, $C_7H_6O_3$, EIMS *m/z* 94 $[M\text{-}CO_2]$,^{1}NMR [500 MHz, CD_3OD, δ (ppm), *J* (Hz)]: 7.89 (2H, *d*, *J*=8.7, H-2/6), 6.82 (2H, d, J=8.6, H-3/5). NMR ^{13}C [125 MHz, CD_3OD, δ (ppm)]: 161.7 (C-4),131.5 (C-2/6),121.9 (C-1), 114.5 (C-3/5).

P-coumaric acid (**2**), pale yellow crystal, $C_9H_8O_3$, EIMS *m/z*120 $[M\text{-}CO_2]^{\cdot}$, ^{1}NMR [500 MHz, CD_3OD, δ (ppm), *J* (Hz)]: 7.58 (1H, *d*, *J*=15.9, H-7), 7.46 (2H, *d*, *J*=8.6, H-2/6), 6.82 (2H, *d*, *J*=8.6, H-3/5), 6.31 (1H, *d*, *J*= 15.9, H-8), NMR ^{13}C [125 MHz, CD_3OD, δ (ppm)]: 170.3 (C-9), 159.5 (C-4), 144.4 (C-7), 129.5 (C-2/6), 126.0 (C-1), 115.3 (C-3/5), 115.2 (C-8).

Cholestanone (**3**), white amorphous powder, ^{1}H NMR [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 2.33-2.13 (*m*, H-2/6), 2.06-1.81 (*m*, H-1/12), 1.89-1.81 (*m*, H-7), 1.62-1.45 (*m*, H-15), 1.02 (*s*, H-19), 0.93 (*d*, *J*= 6.5, H-21), 0.69 (*s*, H-18), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 207.1 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-5), 42.3 (C-16), 42.2 (C-13), 39.7 (C-24), 37.2 (C-1), 36.1 (C-20), 36.5 (C-10), 33.9 (C-22), 31.9 (C-7), 31.8 (C-8), 31.6 (C-2), 29.7 (C-6), 29.1 (C-25), 28.2 (C-12), 24.3 (C-15), 26.0 (C-23), 21.0 (C-11), 19.8 (C-27) 19.0 (C-26), 18.7 (C-21), 11.9 (C-18), 11.8 (C-19).

β-sitosterol (4), white amorphous powder, $C_{29}H_{50}O$, EIMS *m/z* 414 $[M]^{+\cdot}$, ^{1}H NMR [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 5.37 (*dd*, *J*= 2.7, H-6), 3.54 (*m*, H-3), 0.69 (*sl*, H-18), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-4), 42.2 (C-13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-7), 31.8 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 26.0 (C-23), 24.31 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-27), 19.4 (C-19), 19.0 (C-26), 18.7 (C-21), 11.9 (C-18), 11.8 (C-29).

Stigmasterol (**5**), white amorphous powder, $C_{29}H_{48}O$, EIMS *m/z* 412 $[M]^{+\cdot}$. ^{1}H NMR [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 5.37 *dd*, *J*= 2.7, H-6), 5.17 (*dd*, *J*= 15.0 and 8.8, H-23), 5.03 (*dd*, *J*= 14.8 and 9.3, H-22), 3.54 (*m*, H-3), 0.69 (*sl*, H-18), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-4), 42.2 (C-13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 31.9 (C-7), 31.8 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 24.31 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-27), 18.7 (C-21), 19.8 (C-27), 19.4 (C-19), 19.0 (C-26), 11.9 (C-18), 11.8 (C-29).

Campesterol (**6**), white amorphous powder, $C_{28}H_{48}O$, EIMS *m/z* 400 $[M]^{+\cdot}$, ^{1}H NMR [500 MHz, $CDCl_3$, δ (ppm), *J* (Hz)]: 5.37 (*dd*, *J*= 2.7, H-6), 3.54 (*m*, H-3), 0.69 (*sl*, H-18), NMR ^{13}C [125 MHz, $CDCl_3$, δ (ppm)]: 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24),42.3 (C-4), 42.2 (C-13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 31.9 (C-7), 31.8 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 26.0 (C-23), 24.31 (C-15), 23.0 (C-28) 21.0 (C-11), 19.8 (C-27), 19.4 (C-19), 19.0 (C-26), 18.7 (C-21), 11.9 (C-18).

(*E*)-4-methoxycinnamic acid (**7**), colorless crystal, $C_{10}H_{10}O_3$, EIMS *m/z* 178 $[M]^{+\cdot}$.^{1}NMR [500 MHz, CD_3OD, δ (ppm), *J* (Hz)]: 7.63 (1H, *d*, *J*= 16, H-7),7,47 (2H, *d*, *J*= 8.6, H-2/6), 6.82 (2H, *d*, *J*=8.6, H-3/5), 6.35 (1H, *d*, *J*=15.9, H-8), 3.78 (3H, *s*, H-10).

(*Z*)-4-hydroxycinnamic acid (**8**), colorless crystal, $C_9H_8O_3$, EIMS *m/z*120 $[M\text{-}CO_2]^{\cdot}$, ^{1}NMR [500 MHz, CD_3OD, δ (ppm), *J* (Hz)]: 7.89 (2H, *d*, *J*= 8.6, H-2/6), 7.04 (1H, *d*, *J*=8.3, H-7), 6.79 (2H, *d*, *J*=8.5, H-5/6), 6.72 (1H, *d*, *J*=8.4, H-8), NMR ^{13}C [125 MHz, CD_3OD, δ (ppm)]: 172.8 (C-9), 159.0 (C-4), 129.4 (C-7), 126.5 (C-1), 131.3 (C-2/6), 114.7 (C-8), 114.3 (C-3/5).

Structural elucidation of the compounds

^{1}H (500 MHz), DEPT-Q (125 MHz), HSQC, and HMBC spectra were acquired at room temperature on a Bruker AscendTM 500 NMR spectrometer with $CDCl_3$ and CD_3OD from Sigma-Aldrich as the solvent. Spectra were referenced to residual solvent signals and tetramethylsilane as the internal standards. Low-resolution mass spectra were recorded on a chromatograph gaseous (CG) coupled to a mass spectrometer with electron impact ionization at 70 e.V, model Shimadzu GCMS-QP2010 Plus.

Results and discussion

Chemical investigation of the crude methanolextract from the roots and stem bark of *T. aurea* led to the isolation and identification of *p*-hydroxybenzoic acid (**1**), *p*-coumaric acid (**2**), cholestanone (**3**), β-sitosterol (**4**), stigmasterol (**5**), campesterol (**6**), (*E*)-4-methoxycinnamic acid (**7**) and

(*Z*)-4-hydroxycinnamic acid (**8**). These compounds, reported for the first time in *T. aurea*, were characterized using one- and two-dimensional (1D and 2D) NMR spectroscopy and mass spectrometry, with structural confirmation based on comparisons with literature data.

The ^{1}H NMR spectrum of *p*-hydroxybenzoic acid displayed signals consistent with a 1,4-disubstituted aromatic system, with duplets at δ 7.89 (H-2/6) and δ 6.82 (H-3/5), confirming its structure. The DEPTQ spectrum showed signals at δ 131.5 (C-2/6) and 114.5 (C-3/5), along with δ 161.7 (C-4), which corresponds to the hydroxyl-substituted carbon, and δ 170.3, confirming the presence of a carboxyl group. For *p*-coumaric acid, the ^{1}H NMR spectrum revealed an AA'BB' aromatic system, with signals at δ 7.46 (H-2/6) and δ 6.82 (H-3/5), alongside two olefinic hydrogen signals at δ 7.58 (H-7) and δ 6.31 (H-8), indicating a trans α,β-unsaturated carboxyl system. The DEPTQ spectrum further confirmed these assignments, with characteristic carbon shifts at δ 129.5 (C-2/6), 115.3 (C-3/5), 144.4 (C-7), 115.2 (C-8), and 170.3 (C-9, COOH). GC-MS analysis of the fraction containing both acids revealed two distinct retention times, t_R 8.76 min for the major component and t_R 5.47 min for the minor one, with molecular ion peaks at *m/z* 120 and *m/z* 94, respectively. The observed mass fragments suggested decarboxylation within the injection chamber, resulting in fragment ions at *m/z* 91, 72, and 66, supporting the structural identification of *p*-hydroxybenzoic acid and *p*-coumaric acid. These combined spectroscopic and spectrometric analyses confirmed the structural elucidation of these two phenolic acids (Huang *et al*., 2019; Wu *et al*., 2023). The characterization of cholestanone (3) was confirmed by NMR analyses. The ^{1}H NMR spectrum displayed signals between δ 0.69–2.33, characteristic of steroidal methine, methyl, and methylene groups, with no hydroxyl-associated deshielded hydrogens, indicating the presence of a carbonyl group at C-3. The ^{13}C NMR (DEPTQ) spectrum confirmed this assignment with a carbonyl signal at δ_C 207.14.Comparison with literature data led to the identification of the compound as cholestanone (Souza *et al*., 2001).The steroids β-sitosterol (**4**), stigmasterol (**5**), and campesterol (**6**) was performed using NMR (^{1}H, ^{13}C, DEPTQ) and GC-MS analyses. The ^{1}H NMR spectra revealed characteristic signals for a steroidal skeleton, including a double doublet at δ 5.37 (H-6), attributed to an olefinic hydrogen, and a multiplet at δ 3.54 (H-3), indicating an oxymethine hydrogen in a cyclic system. The ^{13}C NMR (DEPTQ) spectra confirmed structural assignments with olefinic carbons at δ 140.77 (C-5) and 121.73 (C-6), and a carbinolic carbon at δ 71.80 (C-3). GC-MS analysis identified three major peaks at t_R 26.06, 27.53, and 25.63 min, corresponding to β-sitosterol (**4**), stigmasterol (**5**), and campesterol (**6**), respectively. Mass spectra revealed molecular ions at *m/z* 414 (β-sitosterol), *m/z* 412 (stigmasterol), and *m/z* 400 (campesterol), with fragmentation patterns confirming their identities. The characteristic loss of H_2O, CH_3, and side-chain fragments supported the sterol structures. These combined analyses confirmed the presence of β-sitosterol, stigmasterol, and campesterol (Ragasa *et al*., 2014; Noor*et al*., 2014).

The cinnamic acids (*E*)-4-methoxycinnamic acid (**7**) and (*Z*)-4-hydroxycinnamic acid (**8**) was performed using NMR and GC-MS analyses. The ^{1}H NMR spectrum of (*E*)-4-methoxycinnamic acid displayed signals for a p-substituted aromatic ring, with duplets at δ 7.47 (H-2/6) and δ 6.82 (H-3/5). Additional olefinic hydrogen signals at δ 7.63 (H-7) and δ 6.35 (H-8) confirmed the conjugation with the aromatic ring. A singlet at δ 3.78 was attributed to a methoxyl group at the para position. GC-MS analysis identified a molecular ion at *m/z* 178, consistent with the molecular formula $C_{10}H_{10}O_3$, and fragmentation patterns supported the proposed structure.(*Z*)-4-hydroxycinnamic acid was identified in a mixture with its trans isomer (*p*-coumaric acid). The ^{1}H NMR spectrum revealed duplets at δ 7.89 (H-2/6) and δ_H 6.79 (H-3/5), characteristic of an AA'BB' system, along with olefinic hydrogen signals at δ 7.04 (H-7) and δ 6.72 (H-8), confirming the cis configuration. The ^{13}C NMR (DEPTQ) spectrum showed signals at δ_C 159.0 (C-4), 126.5 (C-1), and 172.8 (C-9, COOH), along with shifts for the α,β-unsaturated system. Mass spectrometry revealed a molecular ion at *m/z*120, suggesting CO_2 loss during volatilization, consistent with the molecular formula $C_9H_8O_3$ (164 g/mol). These combined analyses confirmed the identification of (*E*)-4-methoxycinnamic acid and (*Z*)-4-hydroxycinnamic acid (Kim *et al*., 2012;Tori et al, 2000).

These findings contribute to the phytochemical characterization of *T. aurea* and expand knowledge on its secondary metabolite profile.

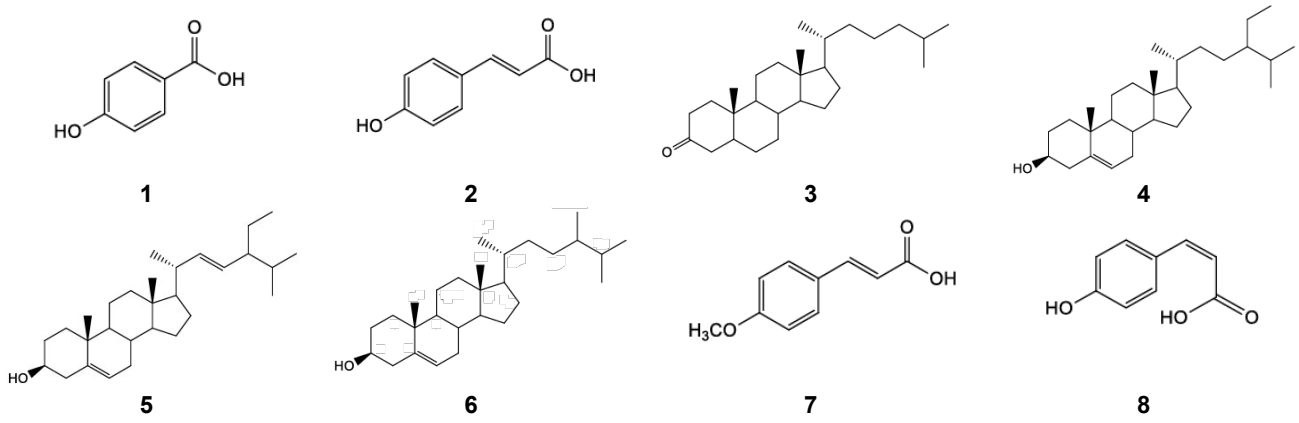

Figure 1. Structural formulas of *p*-hydroxybenzoic acid (**1**), *p*-coumaric acid (**2**), cholestanone (**3**), β-Sitosterol (**4**), stigmasterol (**5**), campesterol (**6**), (*E*)-4-methoxycinnamic acid (**7**) and (*Z*)-4-hydroxycinnamic acid (**8**).

Conclusion

The chemical investigation of *T. aurea* led to the isolation and identification of eight secondary metabolites, including steroids and phenolic derivatives. The structural elucidation of p-hydroxybenzoic acid, p-coumaric acid, cholestanone, β-sitosterol, stigmasterol, campesterol, (*E*)-4-methoxycinnamic acid, and (*Z*)-4-hydroxycinnamic acid was confirmed through NMR (^{1}H, ^{13}C, DEPTQ, HSQC, HMBC) and GC-MS analyses, revealing key structural features and fragmentation patterns consistent with literature data.These compounds, described for the first time in *T. aurea*, contribute to the phytochemical knowledge of this species.

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