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Article

In silico analysis of the ADMET profile of the chemical constituents present in a hydroethanolic extract of the leaves of Embaúba (*Cecropia pachystachya* Trécul)

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Abstract: Embaúba (*Cecropia pachystachya* Trécul) is a tree native to Brazil, whose leaves have traditionally been used to treat respiratory, inflammatory, and gastrointestinal disorders. These biological activities are attributed to phenolic acids and flavonoids, including chlorogenic acid, epicatechin, isoorientin/orientin, and rutin. This study aimed to identify these compounds in the hydroethanolic extract (HE) of Embaúba and perform an *in silico* analysis of their pharmacokinetic parameters using the PreADMET® (Prediction of Absorption, Distribution, Metabolization, Excretion, and Toxicity) software. The chromatographic analysis confirmed the presence of the five compounds, as evidenced by co-elution with reference substances. The compounds exhibited weak interactions with plasma proteins and limited penetration across the blood-brain barrier, except for epicatechin. They demonstrated good permeability through the skin, facilitating their passage from the stratum corneum to deeper tissues. Additionally, these compounds showed low to moderate absorption and permeability in CaCo-2 cells, indicating their potential for human intestinal absorption. Although they did not inhibit P-glycoprotein, they were found to inhibit the isoenzymes CYP2C19 and CYP2C9. Additionally, chlorogenic acid, epicatechin, and isoorientin exhibited mutagenic effects in the Ames test. In conclusion, given the significant therapeutic potential of Embaúba, *in silico* assays should be employed to predict the pharmacokinetic profiles of its compounds, thereby facilitating and expediting the development of new drugs and reducing costs, time, and failures in the process.

Keywords: *Cecropia* plant; ADMET; computer simulation; high-performance liquid chromatography; phenolic compounds; flavonoids; chlorogenic acid; epicatechin; rutin.

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1. Introduction

Cecropia pachystachya Trécul (Urticaceae) is a tree native to Brazil, also found in other Latin American countries such as Paraguay and Argentina [1]. The popular name Embaúba originates from the indigenous term "ambaíba," meaning hollow trunk [2]. Like other species of the genus, Embaúba is a pioneer species in the early stages of ecological succession, commonly used for the recovery of degraded forest areas [3].

Phytochemical investigations of its leaves have revealed a predominance of phenolic acids, proanthocyanidins, organic acids, flavan-3-ols, and flavonoids, including both *C*- and *O*-glycosylated flavonoids [4]. The main phenolic acids reported are the derivatives of hydroxycinnamic acids, such as chlorogenic acid isomers, represented by 5-*O*-caffeoylquinic acid (5-CQA) and 3-*O*-caffeoylquinic acid (3-CQA). Identified flavonoids

include *C*-glycosylated flavones, such as the orientin/isoorientin isomers, and flavonols, such as rutin [5]. Flavonoids with a flavan-3-ol structure, such as procyanidin dimers and trimers, and catechin/epicatechin isomers have also been reported [6].

Traditionally, Embaúba leaves are prepared as decoctions or infusions to treat respiratory, inflammatory, and gastrointestinal disorders [7]. This traditional use has motivated pharmacological studies investigating its medicinal properties. The plant extracts have demonstrated potential as hypotensive and cardiotonic agents [8], hypoglycemic agents [9], antimalarials [10], anti-inflammatory drugs [11], antimicrobials [12], antidepressants [13], wound healers [14], anti-aging agents [15], diuretics [5], depigmenting agents [16], and promoters of inflammatory angiogenesis [17].

This diversity of pharmacological effects highlights the vast therapeutic potential of Embaúba. However, using plant extracts for therapeutic purposes requires a rigorous and scientifically guided approach. In this context, *in silico* tests should be recommended to predict the physicochemical, pharmacokinetic, and toxicity properties of bioactive compounds. These tests facilitate and accelerate drug development by reducing costs, time, and failures [18]. These assays are fundamental for better understanding how a substance interacts with the body, according to its structural characteristics, about its Absorption, Distribution, Metabolization, Excretion, and Toxicity (ADMET) [19]. Evaluating the ADMET profile before clinical studies in humans aims to ensure the safety and therapeutic efficacy of these substances, which are synergistically present in extracts derived from Embaúba leaves.

Accordingly, the present study sought to identify the main compounds in a hydroethanolic extract (HE) of Embaúba leaves and to perform an *in silico* analysis of the ADMET profile of each component. It should be noted that this is the first time that compounds from HE are analyzed together in relation to ADMET properties, focusing on the main phenolic acids and flavonoids found in Embaúba leaves. Therefore, the results found in this work are justified to contribute to other studies with the species [5, 16-17], detailing the best routes of absorption of these substances in the body, in addition to predicting toxicity in different biological models.

2. Results and discussion

2.1 *Chemical characterization*

The chromatographic profile of HE and its HPLC-UV/DAD spectrum are shown in Figure 1.

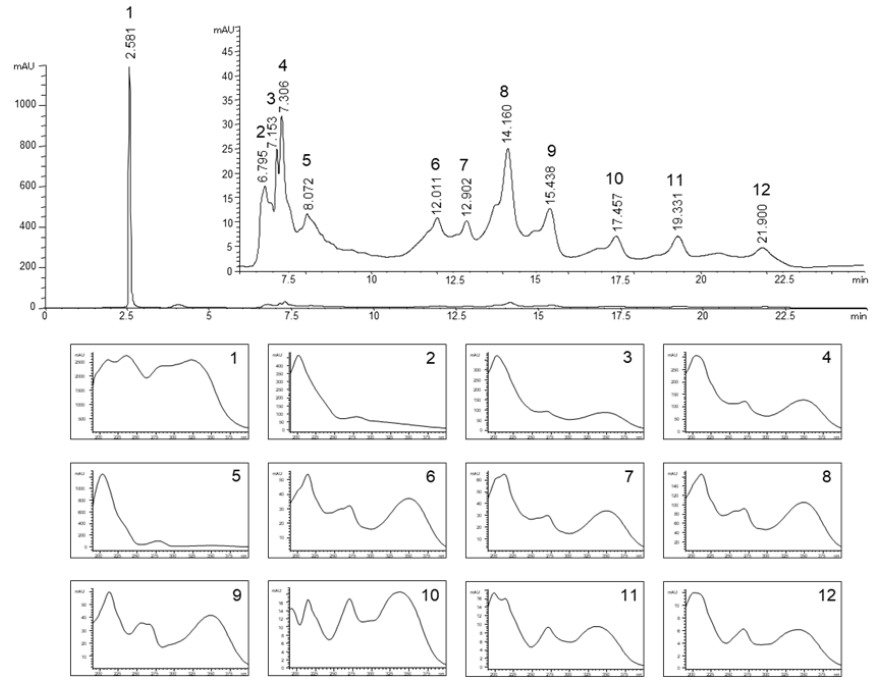


Figure 1. Chromatographic profile and ultraviolet spectra of the chemical constituents found in the hydroethanolic extract of *Cecropia pachystachya***.** The analyses were performed under the following conditions: Kromasil Eclipse C18 HPLC column (4.6 x 150 mm, 5 μm); Mobile phase A: 5 % acetonitrile and 95 % water (0-3.0 min.); Mobile phase B: 15 % acetonitrile and 85 % water (3.1-30.0 min.); Selected time range: 0-25.0 min.; Flow rate: 0.8 mL/min.; Injection volume: 20 μL; Detection length: 330 nm.

HE signal 1 has spectral characteristics of phenolic acid, probably chlorogenic acid, with two maximum absorbance points: the first at 217 nm with a shoulder at 240 nm and the second at 325 nm with one shoulder at 296 nm [20-21]. This last absorption point can be justified by the electron density observed in the benzene ring of the carbon chain of chlorogenic acid, as demonstrated in the chemical structure of the substance, schematized in Figure 2 [22].

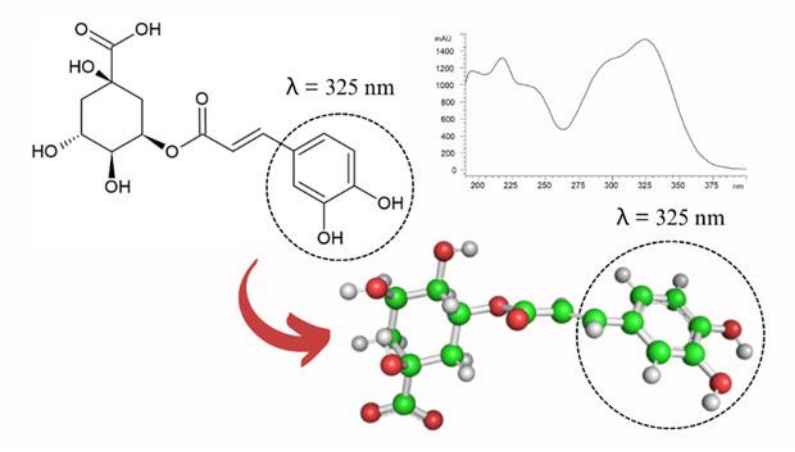


Figure 2. Chemical structure and molecular geometry of chlorogenic acid.

Other studies with the leaves of *C. pachystachya* have also pointed out and identified by CLAE-UV/DAD the presence of phenolic acids, with emphasis on chlorogenic acid, in plant extracts, as is the case of Pacheco *et al*. [11] who reported the presence of this class of secondary metabolites in the methanolic extract, as well as Fernandes *et al*. [15] in the ethanolic/hydroethanolic extract and Freitas *et al*. [16] in the glycolic extracts of this species.

Signals 2 and 5 present in the HE showed similar spectral characteristics, with maximum absorbance between 270 and 290 nm (region II corresponding to ring “A") and with no absorbance between 300 and 400 nm (region I corresponding to benzene ring "B") [23]. This fact can be justified by the lack of conjugation between the "B" ring and the rest of the molecule (i.e., without a double bond with the "C" ring), as occurs with flavan-3-ols flavonoids, favoring the occurrence of a spectrum characteristic of catechins [24].

Signals 3-4 and 6-12 showed similar spectra with absorbance maximums at 200, 265, and 350 nm. These data suggest that these compounds belong to the same class of secondary metabolites classified as flavonoids [25]. According to Merken and Beecher [24], these findings are due to the presence of two maximum absorption points that are characteristic of flavonoids: in the range of 240 to 285 nm (region II), absorbance is justified by the "A" ring; while in the range between 300 and 400 nm (region I), absorbance presumably arises due to the presence of ring "B".

In addition, it should be noted that flavones and flavonols, subclasses of flavonoids, have absorption points around 240 to 280 nm (region II corresponding to benzene ring "A") and 300 to 380 nm (region I corresponding to benzene ring "B") [24]. Thus, flavones already reported in the species, such as luteolin and apigenin derivatives (Figure 3), as well as flavonols, such as quercetin derivatives, have the same characteristics found in the spectra reported above, suggesting that they are also present in the HE.

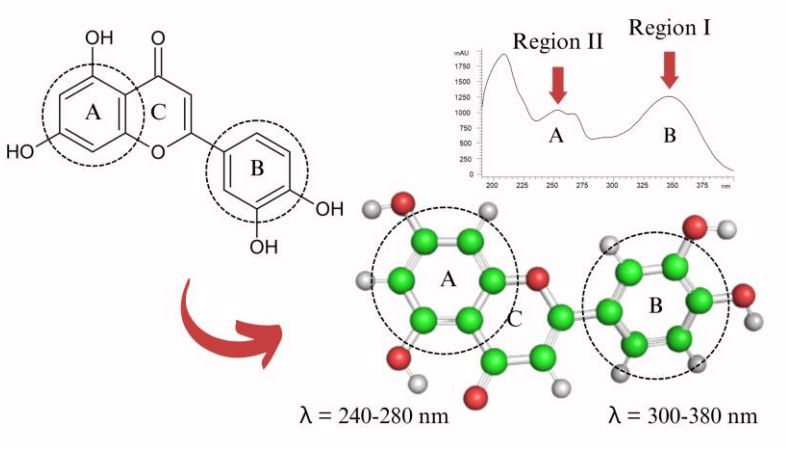


Figure 3. Chemical structure and molecular geometry of luteolin.

2.1.1 Identification of chemical constituents

From the spectra obtained for HE signal 1 (Figure 1), characteristic of phenolic acid and, due to the prevalence of chlorogenic acid in extracts from Embaúba leaves, which is considered a chemical and biological marker of the species [4], it was decided to perform a chromatographic analysis under the same conditions as the samples with 5-CQA (PubChem Code: 5280633), as shown in Table 1.

Table 1. Compounds identified in the hydroethanolic extract of *Cecropia pachystachya*.

Position	Compounds	Rt (min) of the substance in the HE	Rt (min) of the reference substance	Rt (min) of co-elution	Quantification (mg/g)
1	Chlorogenic acid (5-CQA)	2.52	2.53	2.51	25.50
2	Epicatechin	6.79	6.73	6.71	n.c.
8	Isoorientin	14.16	13.64	13.86	n.c.
9	Orientin	15.43	15.00	15.17	n.c.
12	Rutin	21.90	22.23	22.19	n.c.

Legend: HE - Hydroethanolic extract of *C. pachystachya*; n.c. - not calculated; Rt - Retention time. Source: Prepared by the authors (2024).

Among the structural isomers of chlorogenic acid, 5-CQA is the most common, followed by 3-CQA and 4-CQA [26]. In the present study, we identified one of these chlorogenic acid isomers, as well as other studies with species of the genus *Cecropia,* that also reported the presence of these compounds.

In the study conducted by Alves *et al*. [27], 3-CQA was identified in the ethanolic extract of *Cecropia obtusa* leaves using chromatographic techniques, as previously described in *Cecropia* spp. [12]. Similarly, Ortmann *et al*. [28] reported the presence of 3-CQA in plant extracts, as did Mathias and Oliveira [6], who identified both 3-CQA and 5-CQA in methanolic extracts from the leaves of *C. pachystachya* and *Cecropia hololeuca*. The presence of chlorogenic acid was also confirmed in methanolic extracts of *C. pachystachya* leaves by Rivera-Mondragón *et al*. [4] and other studies [11]. Additionally, 5-CQA was identified and quantified in the glycolic extracts of this species [16].

Several studies have demonstrated biological activities related to chlorogenic acid, with emphasis on the hypoglycemic [9], antioxidant and anti-inflammatory [11] and healing [14] effects, evidencing the need to identify and quantify this compound in HE.

Based on the spectra obtained for signal 2 by HPLC-UV/DAD (Figure 1), which is characteristic of catechins, a chromatographic analysis was conducted under the same conditions as the samples with epicatechin (PubChem Code: 72276), as detailed in Table

1. Mourão *et al*. [29] identified 7.29 mg/g of epicatechin in the ethanolic extract obtained from the roots of *C. pachystachya*. Similarly, Cruz *et al*. [10] reported the presence of epicatechin in the ethanolic extract of *C. pachystachya* leaves.

From the spectra obtained for signal 8 by HPLC-UV/DAD (Figure 1), characteristic of flavones, and corroborated by previous studies [15], a chromatographic analysis was performed under the same conditions as the samples containing a glycosylated derivative of luteolin, luteolin-6-*C*-glucoside, also known as isoorientin (PubChem Code: 114776), as detailed in Table 1. In the literature, Fernandes *et al*. [15], Pacheco *et al*. [11], and Mathias and Oliveira [6] identified and quantified isoorientin in extracts from *C. pachystachya* leaves, with concentrations ranging from 28.68 to 118.8 mg/g. Other studies, including those by Campos *et al*. [30], Pereira *et al*. [31], Ortmann *et al*. [28], Mendonça *et al*. [32], Duque *et al*. [14], and Gazal *et al*. [13], also identified isoorientin using HPLC-UV/DAD, establishing it as a chemical and biological marker for the species.

Similarly to its isomer isoorientin, luteolin-8-*C*-glucoside, known as orientin (PubChem Code: 5281675), was also identified in the HE, based on signal 9 obtained by HPLC-UV/DAD (Figure 1), as detailed in Table 1. Fernandes *et al*. [15], Pacheco *et al*. [11], and Mathias and Oliveira [6] also identified and quantified orientin in extracts from *C. pachystachya* leaves, reporting concentrations ranging from 35.09 to 66.5 mg/g. Additional studies by Campos *et al*. [30], Pereira *et al*. [31], Ortmann *et al*. [28], Mendonça *et al*. [32], Duque *et al*. [14], and Gazal *et al*. [13] further confirmed the identification of orientin using HPLC-UV/DAD, attributing various biological activities to this compound.

From the spectra obtained for HE signal 12 (Figure 1), characteristic of a flavonoid from the flavonol class, and supported by previous studies [5-6,12], a chromatographic analysis was performed under the same conditions as the samples with quercetin-3-*O*-rutinoside, commonly known as rutin (PubChem Code: 5280805), as shown in Table 1. Rezende [33] identified and quantified rutin in the hydroethanolic extract of *C. pachystachya*, reporting a concentration of 6.40 μg/mg. Additionally, the presence of rutin was confirmed in both the ethanol-soluble fraction and the aqueous extract obtained from the leaves of *C. pachystachya* using HPLC-UV/DAD [5,12].

2.2 *ADMET profile assessment*

PreADMET®, an *in silico* computer simulation method, facilitates screening compounds with notable biological activity by predicting their pharmacokinetic, toxicological, and physicochemical properties. This approach helps reduce financial costs and minimizes the need for extensive *in vitro* and *in vivo* experimental analyses [29,34].

The first parameter analyzed was the percentage (%) of PPB (Plasma Protein Binding) of the substances identified in HE. As shown in Table 2, compounds 1, 8-9, and 12 presented values below 90 %, indicating weak interactions with plasma proteins. In contrast, compound 2 showed a value above 90 %, suggesting strong interactions with these macromolecules [35]. It is important to note that this parameter influences the biological activity, distribution, and efficacy of substances in the body. Only free substances, unbound to proteins, can be transported or diffused across cell membranes and interact with pharmacological targets [29].

Table 2. ADMET profile of the substances identified in the hydroethanolic extract of *Cecropia pachystachya*.

ADMET Profile	**Substances**				
	1	**2**	**8**	**9**	**12**
Plasma protein binding (PPB, %)	41.96	100.00	63.45	63.12	43.90
Blood-brain barrier (BBB) penetration	0.03	0.39	0.03	0.03	0.03
Skin permeability (logKp, cm/hour)	-3.89	-4.29	-4.69	-4.68	-4.66

Human intestinal absorption (HIA, %)	20.42	66.70	14.98	14.99	2.86
Permeability in CaCo-2 cells (nm/sec)	18.71	0.66	4.10	2.99	7.91
P-glycoprotein inhibition	Not	Not	Not	Not	Not
CYP2C19* inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
CYP2C9* inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
CYP2D6* inhibition	Not	Not	Not	Not	Not
CYP2D6** inhibition	Not	Not	Not	Not	Not
CYP3A4* inhibition	Inhibition	Inhibition	Inhibition	Inhibition	Inhibition
CYP3A4** inhibition	Weak	Weak	Weak	Weak	Weak
Ames Test	Mutation	Mutation	Non-mutation	Non-mutation	Non-mutation
Ames TA100 (+S9)	Negative	Negative	Negative	Negative	Negative
Ames TA100 (-S9)	Negative	Positive	Positive	Negative	Negative
Ames TA1535 (+S9)	Negative	Negative	Negative	Negative	Negative
Ames TA1535 (-S9)	Positive	Negative	Negative	Negative	Negative
Carcinogenicity in rats	Negative	Negative	Negative	Negative	Negative
Carcinogenicity in mice	Positive	Negative	Negative	Negative	Positive
Lipinski of Rule	Suitable	Suitable	Violated	Violated	Violated

Legend: 1 - Chlorogenic acid; 2 - Epicatechin; 8 - Isoorientin; 9 - Orientin; 12 - Rutin.
*Inhibitor; **Substrate.

Blood-brain barrier (BBB) penetration reflects the ability of a substance to traverse the endothelial cells of the central nervous system (CNS) from the peripheral blood, a critical factor in developing new products to minimize side effects and potential damage to the CNS [36]. All substances exhibited a low penetration rate (low PBH < 0.1), except for compound 2, which demonstrated moderate CNS penetration (moderate PBH = 0.1 to 2). No substance presented a high CNS uptake (high PBH > 2) [37].

Substance 2, identified as epicatechin, possesses a molecular mass of 290.27 g/mol, the smallest among the constituents of the HE. As noted by Banks [38], low molecular weight facilitates the transport of lipophilic molecules across the BBB. In the study conducted by Mendonça *et al*. [32], the aqueous extract of *C. pachystachya* demonstrated the ability to permeate the BBB, causing brain tissue damage at doses ranging from 500 to 2000 mg/kg.

Skin permeability (logKp, cm/hour) is a crucial parameter in the development of new cosmetics, assessing the ability of a substance to cross the stratum corneum from contact with the skin, whether intentional or accidental [39]. Good permeability promotes the passage of the substance from the corneal extract to other tissues, facilitating interaction with enzymes and other epithelial components. According to Bastos *et al*. [40] and Mourão *et al*. [29], values below 0.1 cm/h indicate high permeability, while values above 0.1 cm/h suggest low permeability. Based on Table 2, all the compounds studied exhibited high permeability (< 0.1 cm/h).

The percentage (%) of human intestinal absorption (HIA) is classified as the sum of bioavailability and absorption, evaluated from the proportion of excretion or cumulative excretion in urine, bile, and feces [41]. In PreADMET®, compounds are assessed at pH 7.0 and classified as poorly absorbed (0 to 20 %), moderately absorbed (20 to 70 %), and well absorbed (70 to 100 %). Substances 8, 9, and 12 exhibited values below 20 %, indicating low HIA, while compounds 1 and 2 showed values between 20 and 70 %, which are classified as moderate HIA. None of the evaluated substances demonstrated high HIA.

The permeability in Caco-2 cells (nm/sec) was also considered to predict the oral absorption of the substances. These cells, derived from human colon adenocarcinoma, have various drug transport pathways through the intestinal epithelium [40]. In PreADMET®, compounds are evaluated at pH 7.4 and classified as: a) low permeability (< 4 nm/sec); b) medium permeability (4 to 70 nm/sec); and c) high permeability (> 70 nm/sec) [42]. Thus, compounds 2 (0.66 nm/sec) and 9 (2.99 nm/sec) showed low permeability, while compounds 1, 8, and 12 exhibited intermediate permeability in Caco-2 cells.

P-glycoprotein (P-gp) inhibition was also evaluated, as shown in Table 2. P-gp has a molecular weight of 170 kDa and consists of two subunits with twelve transmembrane segments (TM), comprising 1280 amino acids. It is expressed in various tissues, including the kidneys, liver, colon, endometrium of the uterus, and endothelial cells of the blood-brain barrier [43]. In general, the function of P-gp is to prevent the entry of unusual substances into cells and facilitate their elimination, depending on their location in the body. This contributes to a reduction in the bioavailability of many drugs [40].

The results showed that none of the compounds analyzed inhibited P-gp, favoring the efflux of these substances in the body and, consequently, reducing their bioavailability. These compounds have structures with intermediate molecular weights, ranging from 289 g/mol (epicatechin) to 610 g/mol (rutin), which could contribute to the lack of P-gp inhibition.

In the present study, we chose to analyze the isoenzymes CYP2C19 and CYP2D6, which are important for drug metabolism as they metabolize many drugs and are subject to significant genetic polymorphism. Additionally, the isoenzyme CYP3A4, responsible for the oxidative metabolism of many substances such as benzodiazepines, calcium channel blockers, antiarrhythmics, antibiotics, and anticonvulsants, was also evaluated [44].

According to Table 2, all the compounds analyzed inhibited the isoenzymes CYP2C19 and CYP2C9. No substance blocked CYP2D6, while all molecules inhibited CYP3A4*. In addition, the compounds interacted weakly with the isoenzyme CYP3A4**. These data are relevant since by inhibiting the expression of CYP450 isoenzymes, the substances can alter the metabolization profile and, consequently, the body's response. In addition, this information is important to predict the simultaneous action of these substances and drug interactions, to prevent possible side effects, and to promote dose adjustment for each drug [45].

The Ames test is a simple method to determine the *in silico* mutagenicity of a substance using several strains of *Salmonella typhimurium*, with mutations in genes involved in synthesizing the amino acid His, sensitive to mutation-inducing substances [29]. Therefore, the method evaluates the ability of the mutagenic substance to cause the growth of *S. typhimurium* colonies in His-free medium [40].

According to Table 2, compounds 1, 2, and 8 exhibited mutagenic effects, while the other substances were considered non-mutagenic. As demonstrated, compound 1 showed mutagenicity for strain TA1535 (-S9), while compounds 2 and 8 for strain TA100 (-S9). These data agree with the work of Mourão *et al*. [29], who also detected mutagenicity for 5-CQA and epicatechin concerning the strains TA1535 (-S9) and TA100 (-S9). It should be noted that, despite the sensitivity of the Ames test, as it is an *in silico* method, other complementary assays should be performed to exclude or confirm the genotoxicity of the chemical constituents of HE.

It is important to note that Mendonça *et al*. [32] found that the aqueous extract of *C. pachystachya* leaves did not exhibit mutagenic activity in *in vitro* tests with the strains TA100 and TA1535, both with (+S9) and without metabolic activation (-S9), as demonstrated in the present work for orientin and rutin. However, Pereira *et al*. [31] observed genotoxic effects in V79 lung fibroblast cells when using *in vitro* tests with the aqueous extract of *C. pachystachya*, attributing this negative effect to the high concentration of chlorogenic acid in the extract. Nevertheless, *in vivo* acute toxicity assays showed that the extract can be classified as safe, as no DNA damage was observed after 28 days of administration in rats. This likely occurred due to the metabolization of the substances in the liver, making them more hydrophilic and less toxic, allowing for more efficient excretion.

Carcinogenicity refers to the ability of a substance to promote the development of carcinoma, meaning its potential to foster the emergence of cancer cells in an organism. Carcinogenicity studies are typically conducted in experimental models that closely resemble humans, such as mice and rats. In this context, PreADMET® predicts *in silico* carcinogenicity outcomes based on data from the National Toxicology Program (NTP) and the Food and Drug Administration (FDA) of the United States, which are derived from two-year in vivo carcinogenicity tests in mice and rats [40].

As shown in Table 2, compounds 1 and 12 exhibited carcinogenic effects in mice, while none of the substances analyzed demonstrated carcinogenicity in rats. However, it is important to note that, as this is a non-clinical study, the results presented here do not guarantee complete pharmacological and toxicological safety. Additional trials are necessary to complement these findings.

Lipinski's rule, also known as the "*rule of five*" (RO5), assesses the physicochemical properties of a substance to evaluate its aqueous solubility and intestinal permeability, which are essential for good oral bioavailability [46]. The RO5 is intended to guide decisions regarding chemical modifications, helping to modify or reduce the number of compounds with undesirable physicochemical properties [47].

In general, it considers the following properties: (a) molecular weight less than 500 Da; (b) octanol/water partition coefficient (log P) greater than 5; (c) have no more than 5 hydrogen bond donors (groups OH and NH, for example); and (d) have no more than 10 hydrogen bond acceptors (namely N and O). All the numbers involved in RO5 are multiples of 5, which gives rise to the rule's name [29].

According to Table 2, only compounds 1 and 2 did not violate RO5, presenting adequate results according to Lipinski's rule, with good absorption and permeability. The other substances violated RO5, demonstrating a higher probability of presenting problems in oral absorption. These substances have a high molecular weight, such as compounds 8-9, and 12, with values above 500 Da. All compounds that violated RO5 are made up of more than five hydrogen bond donors, due to the presence of hydroxyls and more than ten hydrogen bond acceptors, due to oxygen atoms in their structures. Finally, it was found that the log P was less than 5 in these compounds.

Despite the widespread application of the rule for developing new molecules with pharmacological activity, controversies remain regarding its parameters. Many drugs, such as macrolide antibiotics, violate these parameters yet remain effective when administered orally [48]. According to Lipinski and Reaume [49], RO5 has been questioned due to its rigid parameters, which fail to account for the specific characteristics of each therapeutic target. This limitation highlights the necessity of not discarding substances prematurely, allowing for proper testing before conclusions are drawn.

In addition, it should be noted that substances that did not show good oral absorption, such as 8-9 and 12, could be used in other absorption routes, such as topical. This fact is also justified by the high permeability exhibited by these compounds, as shown in Table 2. Studies with plant extracts of Embaúba {15-17] have demonstrated its promising potential in topical applications, and this data is relevant to justify the results found in the present study.

3. Materials and Methods

3.1 *Plant material*

Embaúba leaves were collected in October 2017 in Juiz de Fora, Minas Gerais, Brazil. The exsiccata was deposited in the Leopoldo Krieger Herbarium of the Federal University of Juiz de Fora (UFJF) under registration CESJ 46591. The Genetic Heritage Management Council (CGEN/SISGEN) authorized access to the botanical material under registration number A7E0A0C.

3.2 *Preparation of hydroethanolic extract*

To obtain the HE, the dried and crushed leaves were subjected to decoction (1:10 m/v) in an extractive solution of ethanol and water (75:25 v/v) for 30.0 min at a temperature of

60.0 °C. The plant material was filtered and concentrated in a rotary evaporator at reduced pressure until the ethanolic solvent was completely removed. The plant material was subjected to the freeze-drying process to eliminate moisture. After this process, the HE was stored in a glass bottle and adequately sealed in the freezer at -4.0 ºC.

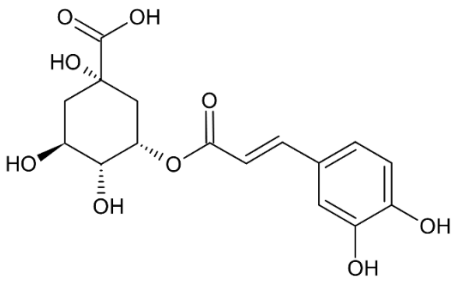
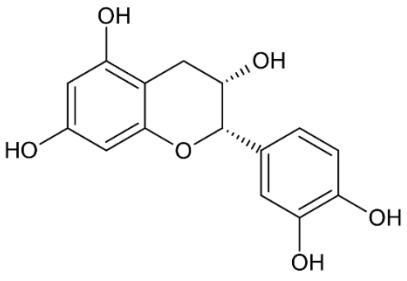
3.3 *Chemical characterization*

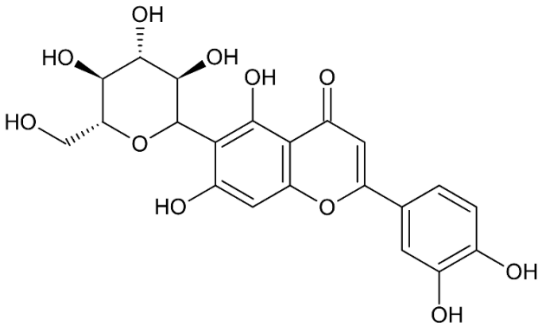
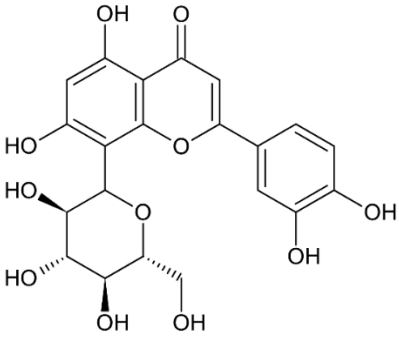
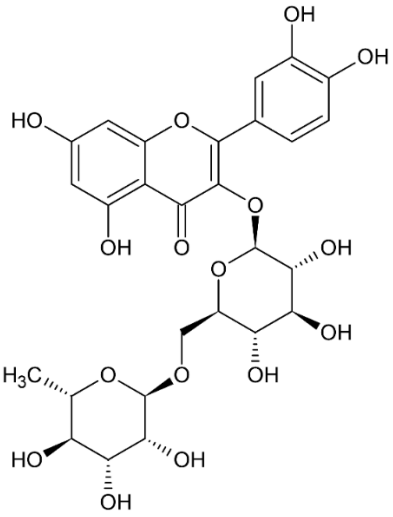
The characterization of the chemical constituents of the HE was carried out according to Freitas *et al*. [16] and Machado *et al*. [5], with some modifications. The sample was submitted to high-performance liquid chromatography with ultraviolet detector and diode array (CLAE-UV/DAD), performed in the Agilent 1200 equipment (Agilent Technologies, California, USA), consisting of a quaternary pump, ultraviolet detector and diode array and automatic injector. An Eclipse XDB C18 backup phase column (Kromasil, Bohus, Sweden) was used, with the following dimensions: 150 mm x 4.6 mm x 5.0 μm. The samples were prepared at 1 mg/mL, filtered on a 0.45 μm membrane and solubilized in the initial mobile phase, consisting of water (A) and acetonitrile (B) (95:05 v/v). The elution gradient consisted of 5 % of B (0-3.0 min.) and 15 % of B (3.0-30.0 min.). The ultraviolet spectrum was determined using a scan between 190 and 400 nm. The wavelengths used in the detection were 330 and 350 nm. For the identification of chlorogenic acid, epicatechin, isoorientin/orientin and rutin, an analysis of the retention time and co-elution of the reference substance with the HE was performed.

3.4 *ADMET profile assessment*

To evaluate the ADMET profile of the chemical constituents of the HE, the online software PreADMET® [Prediction of Absorption, Distribution, Metabolization, Excretion and Toxicity (<https://preadmet.webservice.bmdrc.org/>) was used, according to the work of Mourão *et al*. [29]. This *in silico* tool is based on the relationship between structure and activity through the pharmacokinetic and pharmacodynamic properties of substances, bringing together results present in several databases [40]. The structural formulas of the compounds identified in HE (Table 3) were elaborated in the ACD/ChemSketch software and later submitted to PreADMET®.

Table 3. Information on the compounds identified in the hydroethanolic extract of *Cecropia pachystachya*.

Nomenclature	PubChem code	Molecular weight (g/mol)	Structural formula
Chlorogenic acid (5-CQA)	5280633	354.31	
Epicatechin	72276	290.27	

Isoorientin	114776	448.40	
Orientin	5281675	448.40	
Rutin	5280805	610.50	

The pharmacokinetic, toxicological and physicochemical parameters evaluated were: plasma protein binding (PPB); penetration of the blood-brain barrier (BBB); skin permeability; human intestinal absorption (HIA); permeability in CaCo-2 cells; P-glycoprotein (P-gp) inhibition; inhibition to cytochrome P450 isoenzymes (CYP2C19, CYP2C9, CYP2D6, and CYP3A4); Ames test [strains TA100 (-S9), TA100 (+S9), TA1535 (-S9) and TA1535 (+S9)]; carcinogenicity in mice and rats; and Lipinski's rule. It was standardized that all the values obtained in the quantitative prediction were displayed with two decimal places.

4. Conclusions

The HE of Embaúba leaves exhibits a diverse chemical composition, highlighting the presence of chlorogenic acid, epicatechin, isoorientin/orientin, and rutin. Additionally, the ADMET profile analysis indicates that the compounds present in the HE have promising therapeutic potential, with favorable characteristics such as high skin permeability and

low toxicity. However, the interaction of these compounds with metabolic enzymes like CYP3A4 and CYP2C19 could influence their bioavailability and may require consideration in developing pharmaceutical products. Therefore, the *in silico* evaluations conducted are crucial for predicting pharmacokinetic parameters, ensuring the feasibility of developing new drugs, and optimizing time, costs, and minimizing failures throughout the research and development process.

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