Bioprospection of *Tabebuia aurea* (Silva Manso) Benth. & Hook. f. ex S. Moore: chemical, biological and toxicity studies

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Abstract

Tabebuia aurea (Silva Manso) Benth. & Hook. f. The ex S.Moore (yellow ipe), belonging to the Bignoniaceae family, used in the popular for fever, inflammation and healing of skin wounds. The extract was prepared by maceration, using 70% ethanol. Through HPLC analysis, it was possible to identify substances, mainly phenolic, such as lapachol, present in Bignoniaceae. The phenolic content was 21.36 mg / Eag in the antioxidant activity, the effective concentration of 50% was 53.03 ± 1.14 µg / mL. The antimicrobial activity against *S.aureus*, *E. coli* and *C. albicans* was evaluated by microdilution in broth, which verified action against the tested microorganisms. Cell viability has been inhibited for tumor cells, although this has not been observed for normal cells. The LD50 against *A.aegypti* mosquito larvae was 3504.6 mg / L and there was no mortality in the concentration tested for the snail *B.glabrata*. Nontoxic or low toxicity for *A. salina* and *T. molitor*, respectively, and did not exhibit hemolytic action at concentrations of antibacterial effect. Given the above, it was concluded that the bark extract of the studied species has bioprospecting potential for the future development of antimicrobial products.
Key words: Antimicrobial, Antioxidant, Bioprospecting, Lapachol, T*abebuia aurea*, toxicity.

Introduction

One socio-cultural asset in which Brazil is rich is the use of medicinal plants for curative purposes. Combined with a worldwide trend for the use of products considered

to be natural, as well as the high cost of conventional therapy and the ease of access to such products, this makes traditional medicine an indispensable tool in the treatment of diseases and illnesses [1,2,3,4].

Validation studies involving medicinal plants are fundamental to ensure the safety, efficacy and quality of these products [5,6,7] In Primary Health Care, such studies have been a fundamentally important tool for proving efficacy, as well as reducing adverse reactions and side effects when the products are used in a plant drug, medicinal plant or phytotherapy form [8].

Evaluation of the bioprospecting potential of plant species has been growing in recent years and represents an important tool for the discovery of new therapeutic activities, as well as a means of substantiating uses previously found in popular medicine, one example of which is the species *Tabebuia aurea* (Silva Manso) Benthe. & Hook.f. ex Moore, popularly known in Portuguese as *craibeira, paratudo* and in English as yellow ipe [9].

The species under study, *Tabebuia aurea*, has different uses, ranging from ornamental, as due to belonging to the ipe family its flowers have an intense yellow color, distinguishing landscapes throughout Brazil at different times of the year, as well as economic purposes, as a provider of hardwood [10, 11]. It is also used in popular practice to treat various diseases such as influenza, inflammation and snake bites, as a natural antibiotic against microorganisms such as *S. aureus, Enterococcus faecalis, E. coli, C. albicans* [9]; and is also used in the treatment of diseases of hepatic and renal origin [12]; for restoring oral health [13]; for its properties against fever, diabetes, malaria, and for its abortifacient characteristics [14] .

Scientific studies have identified therapeutic properties, such as anticancer and anti-inflammatory activities [15,16], healing [17] properties; toxicity for vectors that transmit pathologies, such as the *Biomphalaria glabrata* snail, a transmitter of schistosomiasis [9] and larvae of the *Aedes aegypti* mosquito [18]; antioxidant and antifungal action [19] and photoprotection, with the development of cosmetic formulations [20] .

This variety of therapeutic properties can be attributed to its chemical composition, since species of the genus *Tabebuia* can feature secondary metabolites such as alkaloids, catechins, quinones, saponins and tannins [15]. Among the cited classes, that which deserves special mention is found among the naphthoquinones. A well-known representative of the ipe species is lapachol, which in addition to being a marker, is one

of the main agents responsible for the therapeutic properties of the genus [21].

Lapachol is considered one of the main quinones with therapeutic properties, and is a metabolite found in the barks of ipe species, representing around 3.7% [22]. Given its diverse properties and economic importance, there is a growing need to cultivate the species, encouraging the development of cultivation techniques, such as nitrogen fertilization, which in addition to favoring faster growth of the species, enriches the development of minerals and consequently the strengthening of the seedlings [23,24].

The present article therefore aimed to evaluate the biological activities of the species *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S.Moore, collected in the state of Maranhão, Brazil, as well as to determine its chemical profile and to evaluate *in vitro* toxicity, aiming to foster the scientific development and the bioprospecting potential of plant species of the Brazilian flora.

Methodology

Botanic identification

Flowering branches of the plant species *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore were collected in the neighborhood of Maracanã, Vila Esperança, in the city of São Luís, in the state of Maranhão, Brazil. Their exsiccate were prepared and sent to the Maranhão Herbarium of the Universidad Federal do Maranhão, in order to confirm their botanical identification, and were registered as MAR 9.647.

Preparation of extract

As the study pharmacogen, the stem barks of the *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore species were removed, dried and pulverized in a knife mill to give a semi-fine powder [25] . To obtain the extract, the maceration method was applied for 14 days using a 1:10 (w/v) hydromodule in 70% ethanol. Maceration occurred with frequent agitation, terminating with filtration. After extraction, the extract was concentrated in a rotary evaporator and refrigerated at 4ºC in an amber flask [26, 25, 27].

Chemical Analysis of the Extract

Analysis of extract by High Performance Liquid Chromatography Coupled to Ultraviolet

Detector (HPLC-UV)

For HPLC-UV analysis, the sample underwent solid phase extraction (SPE) using Phenomenex Strata C18 cartridges (500 mg stationary phase) which were previously activated with 5 mL MeOH and equilibrated with 5 mL of MeOH: H2O (1: 1, v / v). The compounds were eluted from the cartridges using 1 mL MeOH: H2O (1: 1, v / v) with a final volume of 5 mL. The sample was then filtered through a 0.22 µm PTFE filter and dried. The dried extract was diluted to 10 mg/ mL in HPLC solvent. Aliquots of 20 µL were injected directly into the HPLC-UV with a detection of 270 nm. A Shimadzu model HPLC system was used (ShimadzuCorp. Kyoto, Japan), consisting of a binary pump module and UV-VIS detector (SPA-10A). A Luna 5µm C18 100 A column was used (150 µm x 4.6 µm). The elution solvents used were A (Water and acetic acid at 2%) and B (methanol). Samples were eluted according to the following gradient: 5% to 60% B over 60 min. The flow rate was 1 mL / min. The column temperature was 20 °C and the sample injection volume was 20 µL. Data were processed using LC Solution software (Shimadzu).

Analysis of extract by LC-ESI-IT-MS and FIA-ESI-IT-MS^n

For the FIA-ESI-IT-MSn assay, 10 mg of the crude hydroalcoholic extract was dissolved in 1 mL MeOH: H2O (1: 1, v / v). The sample was filtered through a 0.22 µm PTFE filter, and 20 µL aliquots were injected into the LC-MS and directly into the FIA-ESI-IT-MS^n system.

The crude extract was analyzed by LC-MS in a Thermo Scientific® LCQ Fleet mass spectrometer. HPLC separation was performed using a Kinetex® C18 100 Å chromatographic column with 5µm pores and dimensions of 4.6 x 100 mm. The mobile phase consisted of water, 0.1% formic acid (A) and acetonitrile plus 0.1% formic acid (B), to which was added 0.1% formic acid in an exploratory gradient starting at 10% to 100% of B over 60 minutes at a flow rate of 1.0 mL/min.

The mass spectra were obtained on a Thermo Scientific® LCQ Fleet mass spectrometer, equipped with a direct sample insertion device via continuous flow injection analysis (FIA). The sample was electrospray ionized (ESI) and fragmentation was obtained in multiple stages (MS^n), in an ion-trap (IT) interface. The negative mode was chosen for the generation and analysis of all spectra. The experimental conditions were: capillary voltage -35 V, spray voltage -5000 V, capillary temperature at 350ºC,

carrier gas (N_2) and flow 60 (arbitrary units). The acquisition range was100-2000 *m/z*, with two or more scan events performed simultaneously on the spectrum.

Direct flow infusion of the sample was performed on a Thermo Scientific LTQ XL Iontrap analyzer equipped with a negative mode electrospray ionization (ESI) source (Thermo, San Jose, California, USA). A 280 °C stainless steel capillary tube, 5.00 kV spray voltage, 90 V capillary voltage, a -100 V tube lens and a flow rate of 5 µL min-1 were used. The full scan analysis was recorded in the 100-1000 m/z range. Multiple stage fragmentation (ESI-MSn) was performed using the collision-induced dissociation (ICD) method against helium for ion activation. The first event was a full scan mass spectrum to acquire ion data in this m/z range. The second scan event was an MS / MS experiment performed using a data dependent scan of the [M + H] molecules of the compounds of interest with a collision energy of 30% and an activation time of 30 ms. The product ions were then subjected to further fragmentation under the same conditions until no further fragments were observed. The different compounds of the hydroalcoholic extract were identified by comparing their retention times, UV spectra and fragments obtained from Mass Spectrometry with literature.

Evaluation of antioxidant capacity

Sequestering of DPPH radical•

Antioxidant activity was evaluated according to the procedure described by [69] with modifications. The DPPH• methanolic solution (25 g/mL) was prepared on the day of the experiment and kept in an amber flask. Aliquots of 10 µL of the methanol-diluted extract (final concentration 1-1000µg / mL) were added to 190µL of the DPPH solution and absorbance were measured every minute until reading stabilization (plateau) was observed for 30 minutes at 515nm. The inhibition percentage was calculated according to the formula:

$$\% \text{ Inhibition} = \left[\frac{\text{A control} - \text{A sample}}{\text{A control}}\right] \times 100$$

A = absorbance

The IC_{50} values (effective concentration for 50% inhibition of the preformed radical) were determined by nonlinear regression. Results were expressed as mean ± standard error of means with experiments performed in triplicate.

Total polyphenol grades

Total polyphenol levels were determined using Folin-Ciocalteau reagent and 20% sodium carbonate by spectrophotometry (UV-Vis Lambda 35 spectrophotometer, Pekin Elmer) at 760nm after 2h of reaction. Results were expressed as gallic acid equivalent (%), calculated from a standard gallic acid curve (1 to 30µg/mL) [28].

Analysis of biological activity

Antimicrobial activity

Antimicrobial activity was tested *in vitro* by bioassays using the microdilution technique in 96-well plate broth. The protocol is described by the Clinical and Laboratory Standards Institute [29]. The hydroalcoholic extract used was 100 mg/mL

Selection of bacterial strains

The bacterial samples were taken from the Clinical Microbiology Laboratory of the Universidad Federal do Maranhão. Standard microorganisms ATCC (American Type Culture Collection) were used for the tests. The bacterial samples were:

- *Staphylococcus aureus* ATCC 25923
- *Escherichia coli* ATCC 25922
- *Candida albicans* ATCC 90028

Preparation of microbial suspensions

The microorganisms were initially reactivated from their original cultures and kept in BHI (Brain Heart Infusion) medium at 35°C for 24h. Subsequently, the samples were cultured in Nutrient Agar plates at 35°C for 24 hours and fungi for 48h. Isolated colonies were then resuspended in 3mL of sterile saline solution (0.9% NaCl) until a turbidity equivalent to 0.5 on the McFarland scale was achieved (1.5 x 10^8 UFC/mL).

Minimum Inhibitory Concentration (MIC)

MIC was determined by the microdilution technique according to the broth dilution methodology proposed by the National Committee for Clinical Laboratory Standard (CLSI, 2013). For this, the colonies were resuspended in physiological solution (0.9%) until a 0.5 McFarland scale turbidity equivalent was achieved and the 96-well sterile

plates were prepared with 150µL BHI broth and 50µL extract, followed by serial dilutions. Each bacterial innocuous was then transferred to the wells and brought to the oven at 35 °C for 24h for bacterium and 48h for fungus. Following the incubation period, the 0.1% resazurin developer was added and the reading was taken after 4 hours of incubation. Ethanol 70% was used as a negative control and Chloramphenicol 0.02 mg/mL (bacteria) Nystatin 100,000 IU/mL (fungus) was used as a positive control. The MIC was the lowest concentration of the solution which inhibited microbial growth, and the tests were performed in triplicate.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The incubated plates were used to determine MIC in liquid medium to determine CBM and CFM. One filter paper disk of each well is inoculated in Müeller Hinton Agar plates and subsequently incubated in an environment at 35°C for 24h and fungi for 48h in Sabouraud Dextrose Agar. The MBCs and MFCs were considered the lowest solution concentration where there was no cell growth on the surface of the inoculated agar (99.9% microbial death).

Antitumor activity

Culture of tumor and normal cell lines

This process took place in partnership with the Laboratory of Immunology Applied to Cancer of the Universidad Federal do Maranhão, under the coordination of Professor Ana Paula Silva Azevedo dos Santos. The cells used were cervical carcinoma strains (HELA) and human fibroblasts (GM0749). They were cultured in D-MEM (Gibco, BRL) medium supplemented with 1% antibiotic (Streptomycin, Penicillin) and antimycotic (Amphotericin) and 10% fetal bovine serum (Gibco, BRL). Cultures were picked every 5 days and kept in a greenhouse (Forma Scientific Inc., model 3159) at 37°C, 5% CO_2 and humidity controlled.

Evaluation of the biological activity of compounds against cells

Cells were plated at 100 µl/well (2x10 4 cells/mL) in 96-well flat-bottom plates treated with compounds at final concentrations of 1000 µg/mL, 500 µg/mL, 250 µg/mL,

125 µg/mL, 62.5 µg/mL 31.25 µg/mL, 15.625 µg/mL and 7.8125 µg/mL for MTT cell viability testing. The cells were kept in a greenhouse at 37 °C, with 5% CO2 and controlled humidity. At 24, 48 and 72 hours the cells were evaluated for growth inhibition by colorimetric micro-assay using 3(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide - MTT (Sigma, 5mg / mL). After the treatment periods, 10µL MTT was added for each 100µL of culture. The plates were kept in the greenhouse for three hours. After this period 150µL of the supernatant was removed from each well and 100µL of ethyl alcohol (P.A) was added, homogenizing the well until the complete dissolution of the salt crystals formed. The 96-well plate was read in a spectrophotometer (EPOCH-gen5) using a wavelength of 540nm. The results were analyzed by the absorbance of each well and the experiments were performed in triplicate. As negative control, only DMEM culture medium was used, and for positive control 20% DMSO was employed.

Molluscicidal activity assay

The *B. glabrata* molluscicidal activity test was developed according to World Health Organization standards. Wild snails were collected from the Sá Viana neighborhood (2º33'30.38 "S; 44º18'12.46" W), São Luís, Maranhão, Brazil, kept in a laboratory in dechlorinated aquariums and fed lettuce for identification of the species through morphological characteristics and verification of *S. mansoni* infection. Groups of ten adult *B. glabrata* snails (10-19 mm in diameter), free from *S. mansoni* infection, were exposed to extract solutions at concentrations of 0.5; 1; 3; 6; 12; 25; 50; 75 and 100 mg/L. Each concentration was evaluated in triplicate and a negative control with chlorinated water only and a positive control with 50 mg / L copper carbonate were used. The snails were kept in the tested solutions for 24 hours at room temperature. After this period, the mollusks were washed, transferred to 500 mL containers of chlorinated water and fed with lettuce leaves, remaining under observation for 96 hours to evaluate mortality. This was indicated by discoloration of the conch, total retraction of the cephalopodal mass into the conch, absence of muscle contraction, hemorrhages and deterioration of the cephalopodal mass [30].

Larvicidal Bioassay

The larvicidal activity bioassay was developed according to the methodology

proposed by the World Health Organization (WHO). For the bioassay, stock solutions containing the extract, mineral water and 0.01% dimethylsulfoxide (DMSO) solution were prepared, from which aliquots were taken to prepare 20 mL solutions in six concentrations, ranging from 50 to 1000 mg/l. Ten larvae were added to each solution. Mortality was verified 24 hours after the start of the test. The negative control consisted of 0.01% DMSO solution and the positive control of 1 mg/L temephos solution; the bioassay was performed in triplicate and was repeated three times on different days [30].

Toxicity assessment

Hemolytic activity

The test was carried out according to the [31] method. Blood was collected and a 4mL aliquot was washed three times with saline (0.9%) by centrifugation at 3000rpm for 5min, with the supernatant discarded. The erythrocytes sedimented in the tube were diluted in saline until a 1% suspension was obtained. A 0.5mL volume of this cell suspension was mixed with 0.5mL of solutions containing concentrations of 1000 to 5µg/mL of EBH. The mixtures were incubated at 37ºC, with continuous shaking, for 60min. The solutions were then centrifuged at 3000 rpm for 5 minutes. The absorbance of the supernatant was measured at 540nm. Red blood cell suspensions plus saline and distilled water were used as the minimum and maximum hemolytic control, respectively. To eliminate the interference of EBH in the absorbance, control solutions (white) were prepared, without the addition of the red cell solution. All experiments were performed twice in triplicate. This protocol is authorized by the Ethics Committee on Research with Humans of the Universidad Federal Do Maranhão (Ceuma opinion number 1.732.522).

Toxicity against *Artemia salina* Lesh

Artemia salina eggs were used for the biological toxicity tests. Approximately 80 mg of the eggs were added to an aquarium containing 1L of synthetic saline solution (60g of sea salt/liter of distilled water). This system was saturated with oxygen gas with the aid of an air pump and artificially lit with a 100 W lamp for 24-48h, until the eggs hatched and the larvae (nauplio) migrated towards the more illuminated area, revealing positive phototropism. These were transferred to a beaker containing synthetic saline and kept in incubation for another 24 hours, under the same conditions of illumination and oxygenation, so that the larvae would develop to the metanauplius stage [32,33].

To assess the lethality of *A. salina*, a 20 mg extract was added to 20 µL of DMSO, and the volume was completed to 10 mL with artificial saline. This dilution was performed to obtain a stock solution of 10,000 µg/mL and with a concentration of 0.01% DMSO. Samples of 5.10, 50, 100, 500, 1000µL of this stock solution were transferred to flasks with 5 ml of final solution. Ten larvae in the metanauplius phase were transferred to each of the flasks. The negative control was 2 ml of synthetic saline and 20 µL of DMSO; positive control was performed with potassium dichromate (K_2Cr_2O7). After 24 hours of incubation, the live larvae were counted. Microcrustaceans that did not move during the observation, nor with the slight agitation of the flask, were considered dead [34]

Bioassay using *Tenebrio molitor* larvae

The *Tenebio molitor* larvae were separated, counted, weighed (~100 mg), disinfected with 70% alcohol, and ten larvae were distributed in experimental groups (test and control) in Petri dishes. Concentrations of 1000 to 5 µg/mL of the standardized extract from the bark of the *Tabebuia aurea* stem were prepared from saline solution (0.9%) and DMSO at 1%. With the aid of an insulin syringe, 10 µL of the extract was injected into the caudal region of the larvae in the second visible sternitis, in the ventral portion. The negative control was saline solution (0.9%) and 1% DMSO. The larvae remained at room temperature and the survival rate was observed in intervals of 24 h, for 5 days. To establish the death of the larvae, melanization and the response to physical stimuli were visually verified by touching them gently. The experiments were carried out twice in duplicate [35].

Results and discussion

According to [36,37] , bioprospecting can be considered as the knowledge of a given biological resource, aimed at exploring its biochemical properties, genetic heritage and/or derived substances (oils, gums, latex etc.) that can provide commercial use for the chemical, pharmaceutical, cosmetic and/or food industries. One example of this natural resource is the species *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore which has already been the subject of scientific studies, although species of the Maranhão flora have not been studied. In the present study, in addition to determining the chemical

profile of the hydroalcoholic extract, several important biological activities were evaluated, such as antimicrobial, antioxidant, molluscicidal and larvicidal properties, as well as a toxicity assessment.

Chemical analysis

The phytochemical analysis of the hydroalcoholic extract of the bark of *Tabebuia aurea* revealed the presence of a variety of chemical components, including lapachol, which is a characteristic naphthoquinone, among the representatives of the Bignoniaceae family, most precisely among the ipês, namely species of the *Tabebuia* genus [21,38,39] Figure 1 shows that lapachol had a retention time of 27.9 minutes. The identification was performed by HPLC-UV, using a commercial standard lapachol.

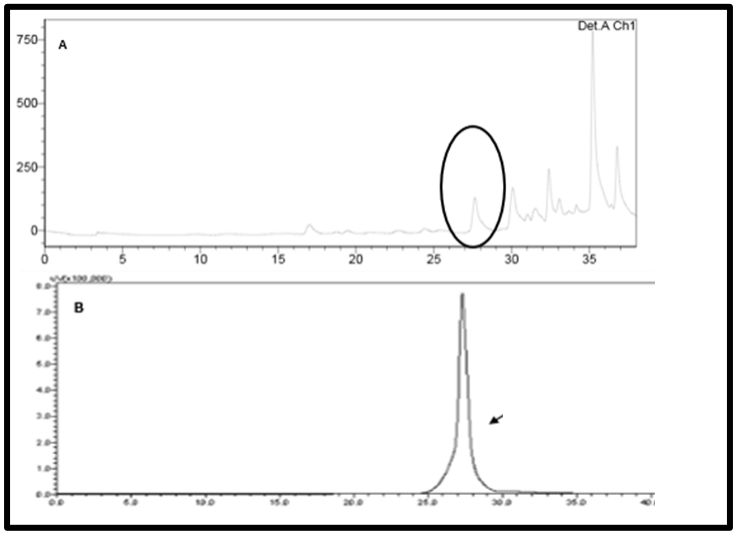


Figure 1: A. Identification of compounds present in the hydroethanolic extract of the barks of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore by High Performance Liquid Chromatography. **B.** Lapachol's Sigma-Aldrich standard identified through High Performance Liquid Chromatography.

Lapachol has already been identified in several species of *Tabebuia*. Studies carried out by [40] describe the presence of this naphthoquinone in different parts of the representatives of Bignoniaceae, as in the heartwood of species such as *Paratecoma peroba* (Record) Kuhlm. (sin. Tecoma peroba Record and Paratecoma diandra Kuhlm.), *Handroanthus impetiginosus* (Mart. ex DC.) Mattos; in the bark of *Handroanthus impetiginosa* (Mart. ex DC.) Mattos [sin.*Tabebuia impetiginosa* (Mart. ex DC.) Standl.] and in the trunk of *Handroanthus serratifolius* (Vahl) S.O. Grose [sin. *Tabebuia serratifolia* (Vahl) G. Nicholson and *Tecoma araliacea* (Cham.) DC.]; in the bark of

Tabebuia avellanedae [41].

In the chemical analysis, with the mass spectrometry data, the presence of lapachol was confirmed and five more compounds were identified, the peaks of which are shown in the chromatogram of figure 2 and table 1. These substances were isohamnetin-3-o-rutinoside, 1-hydroxy-4-methylanthaquinone, 3,7-dimethoxy-3-hydroxyflavone and caffeic acid (Figure 3), revealing that this extract was formed by phenolic substances.

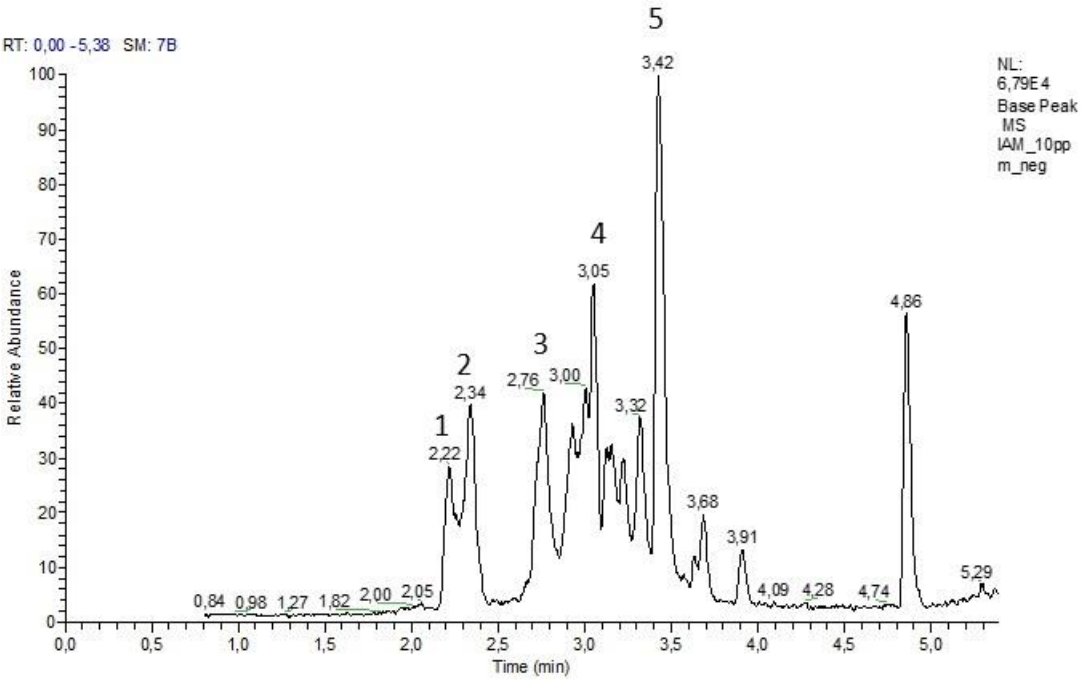


Figure 2: LC-ESI-IT-MS analysis, negative mode, of the hydroethanolic extract of the barks of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore

Table 1: Substancies identified in the hydroethanolic extract of the barks of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore

	M-H	MSN	SUBSTÂNCIA
1	179	132;90	Caffeic acid
2	623	487;301	Isohamnetin-3-O-rutinoside
3	236	220;205	1-Hidroxy-4-methylanthaquinona
4	297	265;211	3,7-Dimethoxy-3-hidroxyflavone
5	241	227;	Lapachol

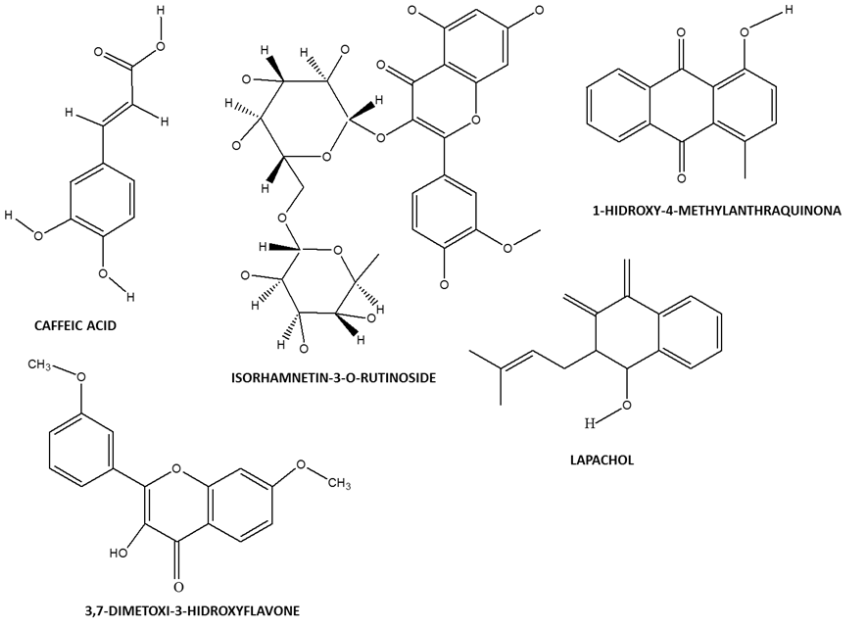


Figure 3: Molecular structure of the substances identified in the hydroethanolic extract of the barks of Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore in the standardized extract the phenolic nature. Source: Structures designed with the aid of the ChemDraw® program

These data on the presence of such substances were confirmed in the analysis of the content of phenolic compounds, which demonstrated a concentration of 21.36 mg EAG/g extract. In this test, gallic acid was used as a standard and the result expressed in relation to this compound. This result corroborates those obtained by [42], who evaluated the content of phenolic compounds in crude ethanolic extracts obtained from the barks of this species, and obtained a result of 20.4 ± 0.1 mg EAG/g extract. Other results have also been found for other species of the genus, such as *Tabebuia heptaphylla* (purple ipe), with a result of 26.72 ± 1.95 EAG/g extract [43].

Phenolic substances form a large class of secondary metabolites that are characterized by having at least one aromatic ring and are linked to hydroxyl groups. Several subclasses of secondary metabolites are considered phenolic, such as flavonoids, phenylpropanoids, tannins, naphthoquinones and anthraquinones, among others [44].

In general, phenolic compounds can be classified into phenols, phenolic acids and phenylacetic acids, cinnamic acids, coumarins, isocoumarins and chromones; lignans; ten groups of flavonoids; lignins; tannins; benzophenones, xanthones and stilbenes; quinones; betacyanins [45], which corroborates the metabolites (flavonoids and anthraquinones) previously found in the species under study [46]. The presence of phenolic compounds can be associated with a variety of properties, which can vary from food adjuvants to therapeutic properties, such as antioxidant, antifungal, anti-

inflammatory, healing and anti-cancer qualities and the prevention of cardiovascular diseases, among others [47,48] .

In the hydroalcoholic extract of the bark of the stem of *T. aurea*, a naphthoquinone, anthraquinone, two flavonoids and a derivative of phenolic acid were identified. The quinones form a group of secondary metabolites that present a 6-membered cyclic ring and two carbonyls at the extremities, in positions 1,2 (ortho) or 1,4 (para). Quinones can be divided, according to their fundamental structures, into benzoquinones, naphthoquinones and anthracene derivatives. Benzoquinones are not aromatic, but naphthoquinones and anthracene derivatives are. Lapachol is the most important naphthoquinone extracted from plants and is notable for having several pharmacological activities [49].

Anthracene is a structure composed of 3 six-membered rings, two of which are aromatic. When carbonyls are present in the intermediate ring, these compounds are called anthraquinones [50]. Some anthraquinones are important for therapy, such as those found in the bark of the stem of *Rhamnus purshiana*, which is used as a laxative [51] (SANTOS, 2019). *Aloe vera* has anthraquinones with a toxic action, which prevents the oral use of the leaf of this species in pregnancy, as well as its use against cancer cells, since the anthraquinone aloe-emodin can reduce mitoses by inducing apoptosis [52]. In the extract under study, an anthraquinone, 1-hydroxy-4-methyl-anthraquinone, was identified, which according to literature has antimicrobial activity against *S.aures* [53].

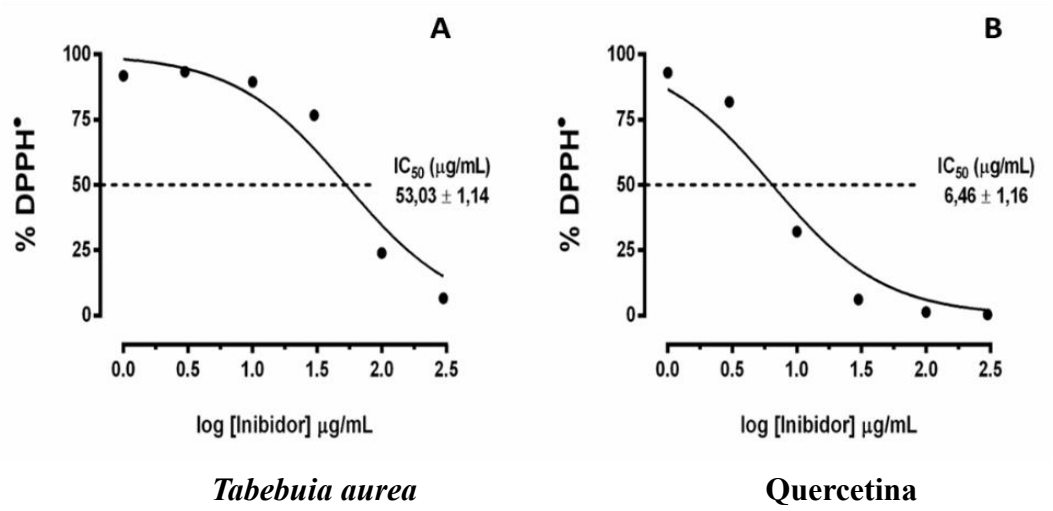
Another important class of phenolic compounds are flavonoids, which have a fundamental chain formed by 2 aromatic rings and an intermediate 3-carbon chain that may or may not be cyclic. These compounds are abbreviated to C6C3C6 and have a mixed biosynthesis, being formed by the shikimate and malonyl acetate pathways. Flavonoids have a very diverse structural variety, and it is more common for the 3-carbon chain to be cyclized to form a pyran [54]. This type of flavonoid was identified in the studied extract, 3'7-dimethoxy-3-hydroxyflavone, which has a fundamental phenylbenzopyran core, in addition to a carbonyl on carbon 4 and a double bond between C2-C3 (Figure 3).

The other phenolic identified was caffeic acid, which is a compound derived from phenolic acid, called C6C1 compounds, as they have an aromatic ring and a side carbon with a carboxylic group [47,55] (SOARES, 2002; SOARES, 2013). Several species exhibit caffeic acid such as *Rosmarinus officinalis* L. *Morus nigra* L [56, 57] .

Like flavonoids, flavones are compounds that possess carbon 4 in carbonyls with antioxidant activity. In this study, it was observed that the concentration responsible for

inhibiting 50% of the preformed DPPH radical was calculated as 53.03±1.14 µg/mL using as a reference the quercetin standard (6.46±1.16 µg/mL) (Graph 1) which is lower than that found by [42] 213±19.8µg/mL. In studies by [58], using stem barks of the *Tabebuia pallida* species, of the same genus, a result of 100 ± 4.66 µg/mL was obtained. This fact may be based on temperature, water availability, nutrients, macro and micronutrients, ultraviolet radiation, among other factors that may interfere with the production of secondary metabolites in the vegetable [59].

The presence of phenolic compounds in plant species infers antimicrobial activity, which corroborates the results found for the species *Tabebuia aurea* [46].



Graph 1: A. Concentration responsible for inhibiting 50% of the preformed radical in the hydroethanolic extract of the barks of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore. B. Concentration responsible for inhibiting 50% of the radical preformed in the Quercetin pattern.

Biological Activity

Evaluation of antimicrobial activity

Biological activities for the crude extract of the species *T.aurea* were evaluated, including the assessment of antimicrobial activity for microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans.* A minimum inhibitory concentration of 12.5 mg/mL and minimum bactericidal concentration of 25 mg/mL was obtained for the microorganisms *S.aureus* and *E.coli*, respectively. For *C.albicans*, a minimum inhibitory concentration of 25 mg/mL and a minimum fungicidal concentration of 25 mg/mL was found. The extract can therefore be considered a promising bactericidal agent, since the MIC/MBC ratio obtained was two, and compounds with results less than or equal to four can be considered bactericidal agents, supporting the bioprospecting potential of the species [60,35] .

The antibacterial activity is based on the presence of compounds such as caffeic acid, which acts against the bacteria *S.aureus* and *E.coli*, as well as being an adjuvant in cosmetics, promoting antioxidant activity, as the resonance of the aromatic ring eliminates free radicals [61]. This fact is relevant as both tested bacteria, even though of different morphological natures, presented similar results, as can be seen in Table 2.

Table 2: Evaluation of the antimicrobial activity of the hydroethanolic extract of the barks of Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore.

Microorganismos	CIM	CBM	CFM	CBM/CIM
Gram-postiva				
Staphylococcus aureus	12,5	25	NA	2
Gram-negativa				
Escherichia coli	12,5	25	NA	2
Fungo				
Candida albicans	25	NA	25	NA

CIM= CBM= CFM= CBM/CIM= NA= Não aplicado ao microrganismo; As concentrações do extrato foram testadas em mg/mL.

Other compounds found in the crude extract of *T.aurea*, such as Isorhamnetin-3-O-rutinoside, known synthetically as narcissin, were tested in isolation against microorganisms such as *Escherichia coli* and *Acinetobacter baumannii*, both gram-negative, with greater effectiveness than the antibiotic ertapenem. There are also reports that the compound can be used as a cosmetic adjuvant, in order to improve absorption [62]. 1-Hydroxy-4-methylantharquinone also exhibited antibacterial action against the microorganism *S.aureus*, increasing the bactericidal potential of the species [53].

The antimicrobial activity of the species is therefore based on the presence of its chemical constituents, as four of the five identified constituents have been reported as exhibiting bactericidal action against the tested bacteria, which corroborates the results found.

Assessment of cell viability

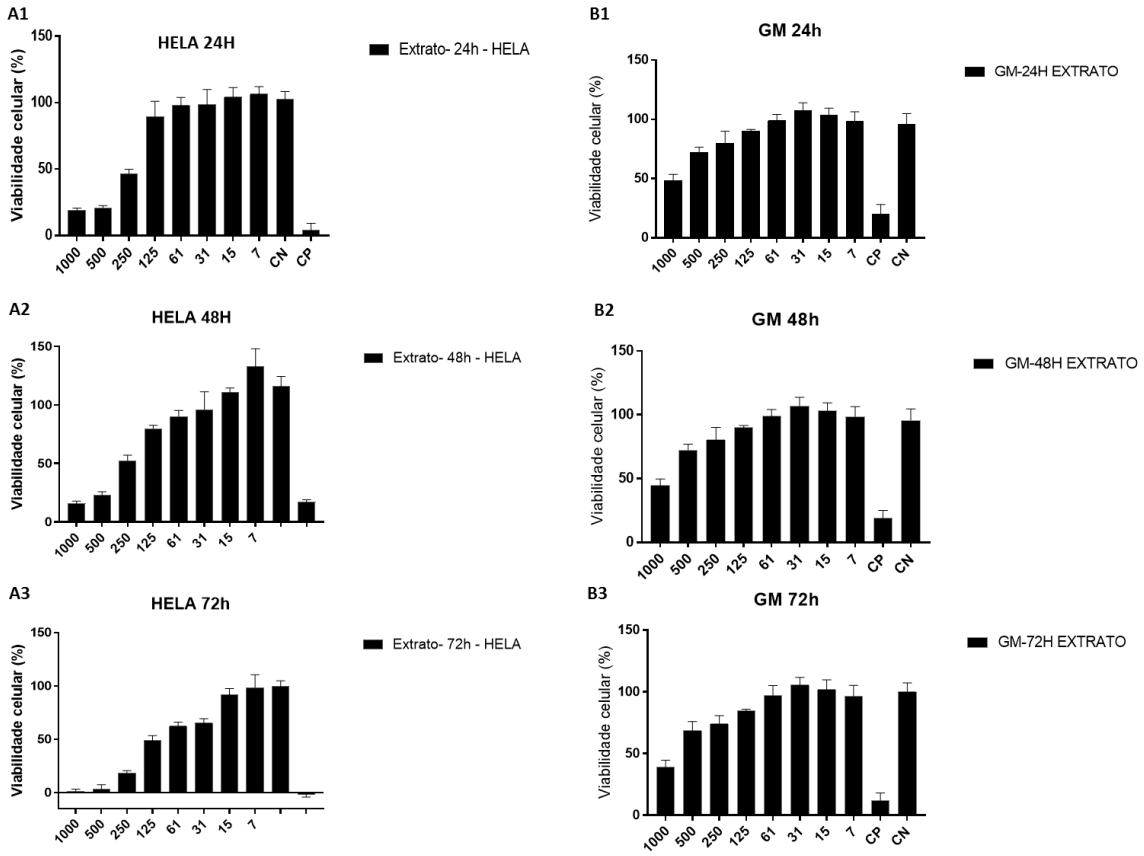
The extract of the species studied reduced the viability of the tested cell lines. This can be seen in Graph 2, where the lowest concentration of the extract was 250 µg/mL, capable of inhibiting the growth of the cervical carcinoma lineage (HELA) by about 50% at the evaluated times (24 to 72 hours), which infers its ability to inhibit cell proliferation.

The cell viability of the extract can be evaluated against normal cells such as human fibroblasts (GM0749), where no significant toxic effects were observed, reinforcing the safety of the compound and emphasizing its ability to inhibit cell proliferation, promoting its antitumor action, as can be seen in graph 2. The evaluation of the cytotoxic or cytostatic effect of the compounds was carried out through colorimetric micro assay using MTT [3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide], with the same conditions and concentrations being considered for both tumor cells and normal cells.

Cell viability studies against normal cells have already been carried out for species of the genus, such as *Tabebuia impetiginosa*, where no presence of cytotoxicity was detected at a concentration of 1000 µg/mL [9]; and studies have also been found for the species *T.aurea* with murine macrophages J774, where the ethanol extracts tested did not reveal inhibition of cell variability [63]. Such results corroborate those found after the evaluation of viability against the species *T.aurea*.

In the extract, it was observed that the presence of the inhibition of cell viability for the tumor cell (HELA), where the same did not occur for the normal cell (GM), with the IC_{50} remaining constant at the evaluated times, as can be seen in Graph 3. The IC_{50} can be seen in Table 3.

Graph 2A. Cytotoxic effect of the ethanol extract of Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore against human HELA cells after 24, 48 and 72 hours of incubation. The evaluation was done through the colorimetric microassay using MTT. The compound DMSO (2%) was used as a positive control. n = 3; T-test or ANOVA ** P <0.01. 2B.Cytotoxic effect of the ethanol extract of Tabebuia aurea (Silva Manso) Benth. & Hook.f. ex S. Moore against human GM cells after 24, 48 and 72 hours of incubation. The evaluation was done through the colorimetric microassay using MTT. The compound DMSO (2%) was used as a positive control. n = 3; T-test or ANOVA ** P <0.01.



[64], in studies carried out for the species *T. impetiginosa*, identified the absence of toxicity in normal cells for the extract from the stem bark, obtaining an IC_{50} greater than the maximum concentration tested, corroborating the results found for the extract of the stem species studied.

Graph 3. A. Minimum inhibitory concentration of the ethanol extract of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore against human HELA cells after 24, 48 and 72 hours of incubation. B. Minimum inhibitory concentration of the ethanol extract of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore against human HELA cells after 24, 48 and 72 hours of incubation

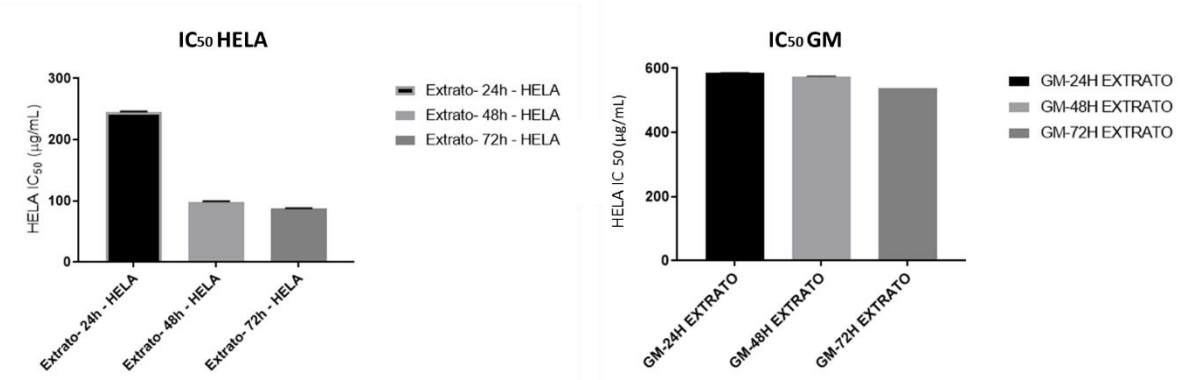


Table 3: Minimum inhibitory concentration of the ethanol extract of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore against human HELA and GM cells after 24, 48 and 72 hours of incubation

	IC_{50}(±DP)	*p*valor
HeLa	585,40	<0,0001
GM	245,00	<0,0001

Evaluation of larvicidal and molluscicidal activity

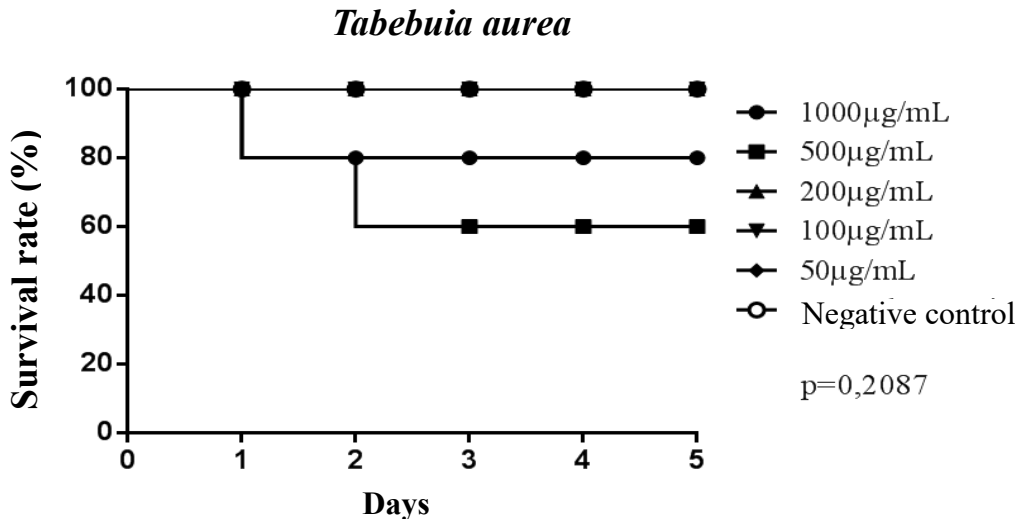
For the extract under study, larvicidal activity was tested against the larvae of the *Aedes aegypti* mosquito, and an LD_{50} of 3504.6 mg/LA was observed, corroborating the results found for crude extracts from the barks of *Tabebuia elliptica* and *Tabebuia rosea-alba* (white ipe) [65]. Studies carried out for the species *Tabebuia caraiba* also detected mortality after 24 hours at a concentration of 500 ppm [18]. For molluscicidal activity, there was no mortality in at least 90% of snails at a concentration of 100 ppm, which is the maximum concentration acceptable by the WHO [66].

Toxicity assessment

For the toxicity assessment, *Artemia salina* microcrustace an assay was performed, which found an LC_{50} of 1255.5 µg/mL, which is considered non-toxic, according to the parameters established by [32]Meyer (1982). Other studies undertaken for the species *T.aurea* showed a LC_{50} of 815.4 µg/mL and 4608 µg/mL, also classified as non-toxic [67,68] . Other tests and toxicity were carried out, such as the evaluation of

hemolytic activity, which found an LC_{50} of 488.1 µg/mL as well as for toxicity against the *Tenebrio molitor* larva, where a larva survival rate of 80% was found, with the crude extract considered to be of low toxicity [35]; as the test reliability is based on the p-value, with p> 0.05. In the analysis performed using the statistical program Prisma 8, the *p* value was calculated as 0.2087, bringing veracity to the test, as can be seen in Graph 4.

Graph 4: Toxicity analysis of the hydroethanolic extract of the barks of *Tabebuia aurea* (Silva Manso) Benth. & Hook.f. ex S. Moore, for the larvae of the insect Tenebrio molitor, in different days and concentrations.



Conclusion

Based on the above, it can be concluded that the crude extract of the species *T.aurea* is a promising bactericidal agent, based on the characteristics of the constituents identified, together with the absence of toxicity found in the tests performed, thus promoting the bioprospecting potential of the species, as well as identifying it as a promising agent for the development of herbal medicines.

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